

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: P1.-SANOFI-AVENTIS.-Solicitud de Patente de Invención en Fase Nacional PCT para "DERIVADOS DE 5-(PIRIDIN-3-IL)-1-AZABICICLO [3.2.1] OCTANO, SU PREPARACIÓN Y SU APLICACIÓN EN TERAPÉUTICA".-Clase C07D 471/08.-Expediente número 04-062.934.-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 07 de Marzo de 2008 y a mi memorial de plazo de 04 de Abril de 2008.

2. Con el fin de dar respuesta a las objeciones expuestas por el señor Examinador en su Concepto Técnico No. **330**, en relación con la claridad de la descripción y de las reivindicaciones, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos, por medio de los cuales se superan completamente dichas objeciones.

3. **MODIFICACIONES VOLUNTARIAS**

Antes de entrar a analizar las objeciones presentadas por el señor Examinador en contra de la solicitud, me permito manifestar que es deseo del solicitante presentar un nuevo capítulo reivindicatorio que consta de **5 reivindicaciones**. Este nuevo capítulo reivindicatorio se basa completamente en el capítulo reivindicatorio originalmente presentado y tiene la estructura que se presenta a continuación:

Nueva reivindicación 1: Esta reivindicación corresponde a la reivindicación 1 originalmente presentada en la cual se han realizado importantes modificaciones con el fin de superar las objeciones expuestas por el señor Examinador. Dichas modificaciones serán expuestas en el punto 5 del presente memorial.

Nueva reivindicación 2: Esta reivindicación corresponde a la reivindicación 2 originalmente presentada en la cual se han realizado importantes modificaciones con el fin de superar las objeciones expuestas por el señor Examinador. Dichas modificaciones serán expuestas en el punto 5 del presente memorial.

Nueva reivindicación 3: Esta reivindicación corresponde a la reivindicación 5 actualmente presentada sobre la cual no se ha realizado ninguna modificación.

Nueva reivindicación 4: Esta reivindicación corresponde a la reivindicación 3 actualmente presentada en la cual se ha modificado su dependencia, de tal forma que ahora es dependiente de las nuevas reivindicaciones 1 a 3.

Nueva reivindicación 5: Esta reivindicación corresponde a la reivindicación 4 actualmente presentada en la cual se ha modificado su dependencia, de tal forma que ahora es dependiente de las nuevas reivindicaciones 1 a 3.

4. **CLARIDAD DEL CAPÍTULO DESCRIPTIVO**

El señor Examinador en su concepto técnico No **330** manifiesta que la descripción de la solicitud bajo estudio carece de claridad asegurando que:

- (i) *"La solicitud en su descripción no contiene la tecnología anterior conocida por el solicitante la cual es útil para la comprensión y el examen de la invención"*

Sobre este particular, el solicitante se permite aclarar que Los documentos del estado de la técnica han mencionado previamente compuestos que exhiben una alta afinidad hacia los receptores nicotínicos. Entre estos documentos, WO00/34279 se refiere a derivados de 1,4-diazabicyclo[3.2.2]nonano, y el documento WO00/34284 se ocupa de derivados de 2,5-diazabicyclo[2.2.1]heptano. El documento US 5,817,679 revela derivados de 7-aza-bicyclo[2.2.1]heptano y hepteno. Los inventores han demostrado que los derivados de 1-azabicyclo[3.2.1]octano presentan una alta afinidad hacia los receptores nicotínicos".

Adjunto a este memorial adjunto como anexo un nuevo capítulo reivindicatorio del cual se han modificado las páginas 2, 3 y 4 con respecto al originalmente presentado debido a la inclusión de esta mención al estado del arte previo a la invención. A este respecto considero de gran importancia señalar, que **la inclusión de la información que se suministra no**

amplía de manera alguna el alcance de la invención tal y como fue originalmente presentada, toda vez que la información allí contenida se limita simplemente a brindar al lector información de las anterioridades conocidas en el estado del arte por medio de la citación de documentos.

Adicionalmente, el señor Examinador manifiesta que:

- (ii) *"El solicitante expresa que las CI_{50} de los compuestos de la invención más afines se sitúan entre 0.1 y 10 μM , pero no indica cuáles fueron los compuestos que presentaron dichos valores".*

En respuesta a la anterior objeción, me permito de manera respetuosa dirigir la atención del señor Examinador a la tabla que se adjunta como anexo al presente memorial, en la cual aparecen algunos de los compuestos de la presente solicitud que exhiben los valores de IC_{50} frente a los receptores nicotínicos que tienen la subunidad $\alpha_4\beta_2$ y α_7 mencionados en la descripción. En dicha tabla los compuestos están identificados con números, los cuales se han asignado de conformidad con la numeración hecha en el cuadro de la página 14 del capítulo descriptivo, y los valores allí presentados evidencian que las IC_{50} de los compuestos de la presente invención se sitúan entre 0.01 y 10 μM .

Finalmente, asegura el señor Examinador que:

- (iii) *"El solicitante indica en el folio 112 que los resultados de las pruebas sugieren la utilización de los compuestos en el tratamiento o la prevención de los desórdenes relacionados con disfunción de los receptores nicotínicos. Sin embargo el solicitante no entrega resultados que permitan establecer dicha afirmación, puesto que el hecho de que un compuesto posea afinidad por un receptor no es suficiente evidencia para determinar que dicha afinidad conlleve a su vez una actividad terapéutica específica".*

En respuesta a la afirmación anterior, considero importante señalar que en el estado de la técnica es ampliamente conocida la relación existente entre los receptores nicotínicos y las enfermedades citadas en la presente solicitud tales como Alzheimer, Parkinson, Down o Korsakiff. Tan es así, que en el estado de la técnica se encuentran numerosas publicaciones en las cuales se demuestra la relación anteriormente mencionada. Algunos de dichas publicaciones han sido adjuntados al presente memorial como anexo con el fin de ofrecer una rápida referencia de las mismas al señor Examinador, demostrando que la afirmación objetada está completamente sustentada

en las publicaciones existentes en el estado de la técnica, razón por la cual la persona versada en la materia podría comprender fácilmente las enseñanzas de la presente solicitud.

Teniendo en cuenta lo anterior, me permito manifestar que incluir datos que enseñen la relación entre los receptores nicotínicos y las enfermedades citadas no resta claridad al capítulo descriptivo de la presente solicitud, toda vez que esta relación es ampliamente conocida por la persona versada en la materia y se encuentra sustentada en varias publicaciones existentes en el estado de la técnica.

5. **CLARIDAD DEL CAPÍTULO REIVINDICATORIO**

Ahora bien, el señor Examinador ha objetado la claridad del capítulo reivindicatorio asegurando que:

"Las reivindicaciones 1 y 2 enseñan derivados de la fórmula (I) en los cuales R corresponde a un elevado número de sustituyentes con características funcionales muy diferentes entre si; puesto que la descripción no contiene información que permita determinar que esta amplia variedad de derivados de fórmula (I) sean todas una solución al problema técnico planteado, se entiende que estas reivindicaciones no están por lo tanto cumpliendo con lo establecido en el artículo 30."

En respuesta a la anterior objeción, me permito manifestar que es deseo del solicitante limitar las reivindicaciones 1 y 2 a los compuestos específicos enseñados en la descripción de la presente solicitud, tal como se indica a continuación:

Reivindicación 1: En esta reivindicación ha sido modificada con el fin de limitar la definición del sustituyente "R" mediante la eliminación del grupo "cicloalquilo (C3-C6)", y eliminar de los posibles sustituyentes del grupo fenilo los grupos "amino", "dialquilamino (C1-C3)", "trifluorometoxi", "ciano", "hidroxi", "morfolin-4-ilo", "pirrolidin-1-ilo", "azetidin-1-ilo", "azepin-1-ilo", "pirazinilo", "fenoxazinilo", "dibenzotienilo", "trifluorometoxi", "trifluorometilo", "nitro", "ciano", "amino", "dialquilamino (C1-C3)" y "cicloalquilamino (C1-C8)",

Reivindicación 2: Esta reivindicación ha sido modificada con el fin de eliminar de la definición del sustituyente "R" los grupos "amino", "ciano" e "hidroxi".

De conformidad con lo anterior, es claro que la objeción presentada por el señor Examinador en contra de la claridad de estas reivindicaciones queda plenamente superada, toda vez que los compuestos ahora reivindicados se refieren específicamente a los compuestos enseñados en el capítulo descriptivo de la presente solicitud.

Finalmente, me permito manifestar que **las modificaciones realizadas al capítulo reivindicatorio no constituyen una ampliación de la materia tal y como fue originalmente presentada**, toda vez que, simplemente se refieren a la limitación de la definición de un sustituyente específico con el fin de superar la objeción presentada por el señor Examinador, lo que evidentemente corresponde a una limitación de la materia protegida.

6. **CONCLUSIÓN**

Queda entonces claro que las objeciones relativas a la claridad de la descripción de la presente solicitud fueron superadas mediante la introducción de un párrafo destinado a mencionar el estado de la técnica, la presentación de los valores de IC₅₀ de los compuestos reivindicados y la presentación de publicaciones que demuestran la relación de los receptores nicotínicos con algunas enfermedades citadas en la descripción.

Finalmente quedó claro que las objeciones presentadas en contra de la claridad de las reivindicaciones fueron plenamente superadas mediante la limitación

7. Anexo me permito presentar:

- (a) Recibo número 08-55,596, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de las modificaciones mencionadas;
- (b) Copia del nuevo Capítulo Descriptivo, en donde se han modificado las páginas 2, 3 y 4 con información relacionada con el estado de la técnica;
- (c) Copia del nuevo Capítulo Reivindicatorio, **que reemplaza al actualmente presentado**, en el cual se han realizado las modificaciones expuestas anteriormente;
- (d) Tabla de valores de IC₅₀ de los compuestos reivindicados; y
- (e) Publicaciones que demuestran la relación que existe entre los receptores nicotínicos y las enfermedades mencionadas en la descripción.

LLOREDA & CIA. S. A.

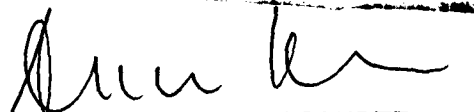
8. En vista de todo lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las argumentaciones presentadas y de las modificaciones realizadas y conceder el privilegio de patente solicitado.

Señor Superintendente,


ALICIA LLOREDA RICAURTE
T.P. 53.215

NOTARIA
SEXTA
BOGOTÁ D.C.

NOTARIA S E X T A DE BOGOTA
RECONOCIMIENTO Y PRESENTACION PERSONAL
Bogotá, D.C.
Ante mi PABLO MENDEZ BARAJAS
NOTARIO SEXTO (E) DEL CIRCULO DE BOGOTA
Comparecieron: *Alicia Lloreda Ricaurte*
quien (en) exhibió (en) la(s) C.C.
CC 39.690.713
TP 53.215
y declaró (aron) que la(s) firma(s) que aparece(n) en el presente documento es(son) la(s) suya(s) y que el contenido del mismo es cierto. En constancia se firma esta diligencia.


ALICIA LLOREDA RICAURTE
C.C. No. 39.690.713 de Usaquén

NOTARIA SEXTA
PABLO MENDEZ
BARAJAS
Notario Sexto E

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 900176089-2

RECIBO OFICIAL DE CAJA : 08 - 55,596
FECHA : JULIO 11 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
LLOPESA Y CIA S. A	CONSIGNACION	BANCO DE BOGOTA	062754387	1859807	11/07/2008	6,040,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEMAS COMERCIALES	103,000.00
		TOTAL :	\$ 103,000.00

SON: CIENTO TRES MIL PESOS

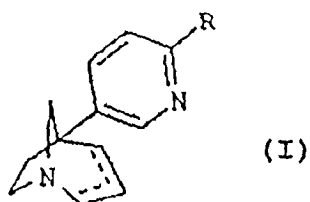
RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No.

DERIVADOS DE 5-(PIRIDIN-3-IL)-1-AZABICICLO[3.2.1]OCTANO, SU
PREPARACIÓN Y SU APLICACIÓN EN TERAPÉUTICA.

RESUMEN OBJETO Y FINALIDAD DE LA INVENCION

Compuestos que responden a la fórmula general (I):



en la cual R representa o bien un átomo de halógeno o un grupo fenilo substituido por uno o varios grupos elegidos entre los átomos de halógenos y los grupos alquilo (C_1-C_6), alcoxi (C_1-C_6), nitro, amino, trifluorometilo, ciano, hidroxilo, acetilo o metilendioxi, o bien un grupo piridinilo, o bien un grupo tienilo, o bien un grupo indolilo, o un grupo pirimidinilo eventualmente substituido por uno o varios grupos alcoxi (C_1-C_6). Por otra parte, entre los dos enlaces carbono-carbono representados por ---, uno es simple y el otro puede ser simple o doble. Los compuestos de la invención son ligandos de los receptores nicotínicos.

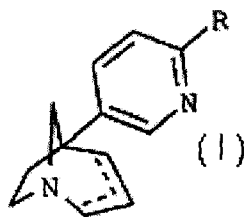
**DERIVADOS DE 5-(PIRIDIN-3-IL)-1-AZABICICLO
[3.2.1]OCTANO, SU PREPARACIÓN Y SU APLICACIÓN EN
TERAPÉUTICA**

Los documentos del estado de la técnica han mencionado previamente compuestos que exhiben una alta afinidad hacia los receptores nicotínicos. Entre estos documentos, WO00/34279 se refiere a derivados de 1,4-diazabicyclo[3.2.2]nonano, y WO00/34284 se ocupa de derivados de 2,5-diazabicyclo[2.2.1]heptano. El documento US 5,817,679 revela derivados de 7-aza-bicyclo[2.2.1]heptano y hepteno.

Los inventores han demostrado que los derivados de 1-azabicyclo[3.2.1]octano presentan una alta afinidad hacia los receptores nicotínicos.

La presente invención tiene por objetivo compuestos que son ligandos de los receptores nicotínicos, y que son útiles en el tratamiento o la prevención de los desordenes relacionados con una disfunción de los receptores nicotínicos, en especial al nivel del sistema nervioso central.

Los compuestos de la presente invención corresponden a la fórmula general (I):



En la cual R representa un átomo de halógeno o un grupo cicloalquilo (C_3-C_6) o un grupo fenilo substituido por uno o varios grupos elegidos entre un átomo de halógeno, o un grupo alquilo (C_1-C_6), alcoxi (C_1-C_6), nitro, amino, dialquilamino (C_1-C_3), trifluorometilo, trifluorometoxi, ciano, hidroxilo, acetilo o metilendioxo, o un grupo piperidinilo, o morfolin-4-ilo, o pirrolidin-1-ilo, o azetidín-1-ilo, o azepín-1-ilo, o piridinilo, o quinolinilo, o tienilo, o pirazinilo, o furilo, o benzofurilo, o benzotienilo, o indolilo, o pirimidinilo o isoxazolilo, o fenoxazinilo, o fenoxatiinilo, o dibenzotienilo, o dibenzofurilo, o pirrolilo o naftilo, y cada uno de esos grupos puede eventualmente ser substituido por uno o varios grupos elegidos entre los átomos de halógenos, los grupos alquilo (C_1-C_6), alcoxi (C_1-C_6), trifluorometoxi, trifluorometilo, nitro, ciano, hidroxilo, amino, dialquilamino (C_1-C_3) o cicloalquilamino (C_1-C_8).

Entre los dos enlaces carbono-carbono representados por ---, uno es simple y el otro puede ser simple o doble. Por otra parte, el átomo de carbono en posición 5 es asimétrico, de forma que los compuestos pueden existir en forma de dos enantiómeros o mezclas de esos últimos.

Los compuestos de la invención pueden existir en estado de bases o de sales derivadas de adición con ácidos.

Un subconjunto de compuestos preferidos es el de los compuestos de fórmula general (I) en la cual R representa o bien un átomo de halógeno o un grupo fenilo substituido por uno o varios grupos elegidos entre los átomos de halógenos y los grupos alquilo (C_1-C_6), alcoxi (C_1-C_6), nitro, amino, trifluorometilo, ciano, hidroxilo, acetilo o metilendioxo, o bien un grupo piridinilo, o bien un grupo tienilo, o bien un grupo indolilo, o bien un grupo pirimidinilo

eventualmente substituido por uno o varios grupos alcoxi (C_1-C_6).

Los compuestos de fórmula general (I) pueden prepararse por un procedimiento ilustrado por el esquema siguiente. Se hace reaccionar el 3-oxo-1,4-azabicyclo[2.2.2]octano, de fórmula (II), con un derivado de piridina de fórmula general (III), en la cual R es tal como se ha definido anteriormente y W representa un átomo de halógeno. De esta forma, se puede efectuar una reacción de condensación entre el 3-oxo-1-azabicyclo[2.2.2]octano y el derivado litiado de los compuestos de fórmula general (III) obtenidos por intercambio halógeno-metal con un derivado alquil litio. Se obtienen compuestos de fórmula general (IV) que, cuando se tratan en medio ácido por calor, conducen a los compuestos de fórmula general (I) en la cual uno de los dos enlaces carbono-carbono representados por --- es doble. La hidrogenación catalítica del doble enlace conduce a los compuestos de fórmula general (I), donde todos los enlaces del ciclo azabicyclooctano son saturados.

El 3-oxo-1-azabicyclo[2.2.2] octano está disponible en el comercio.

Los compuestos de fórmula general (III) están disponibles en el comercio o pueden accederse a ellos por métodos descriptos en la literatura.

general (I). De esta forma, los compuestos de fórmula general (I) en la cual R representa un grupo arilo, por ejemplo, pueden prepararse a partir de los compuestos correspondientes, en cuya fórmula R representa un átomo de halógeno, de acuerdo con cualquier método conocido, tal como un acoplamiento de tipo Suzuki en presencia de un ácido borónico y de un catalizador de paladio, por ejemplo tetrakis trifenilfosfina paladio, o un acoplamiento de Stille con los reactivos apropiados.

Los ejemplos que siguen a continuación, ilustran la preparación de algunos compuestos de la invención. Los microanálisis elementales, y los espectros I.R. y R.M.N. confirman las estructuras de los compuestos obtenidos.

Los números indicados entre paréntesis en los títulos de los ejemplos corresponden a aquellos de la primera columna del cuadro que se presenta más adelante.

En los nombres de los compuestos el guión "-" forma parte de la palabra, y el guión "_" solo sirve para el corte de fin de línea; hay que suprimirlo a falta de corte, y no debe ser reemplazado ni por un guión normal, ni por un espacio.

Ejemplo 1 (Compuesto N°1).

Bromhidrato de 5-(2-fenilpiridin-5-il)-1-azabicyclo[3.2.1]oct-3-eno (2:1).

1.1. 5-Bromo-2-fenil-piridina.

Se introduce sucesivamente en un balón tricol de 500 ml , 30 g (0,127 mol) de 2,5-dibromopiridina en suspensión en 100 ml de tolueno, 15,4 g (0,127 mol) de ácido fenilborónico, 4,4 g (0,0038 mol) de tetrakis-(trifenilfosfina)-paladio, 90 ml de una solución acuosa 2M de carbonato de sodio y 4 ml de etanol, y se calienta la mezcla a 90°C durante 22 h.

Se la decanta, se lava la fase orgánica con 100 ml de agua, se la seca, se la concentra bajo presión reducida y se purifica el residuo por cromatografía sobre columna de gel de sílice, y se procede a la elución con una mezcla 30/70 de ciclohexano y diclorometano.

Se obtienen 22,4 g de cristales.

Punto de fusión: 69-72°C.

1.2. 3-hidroxi-3-(2-fenilpiridin-5-il)-1-azabicyclo[2.2.2]octano.

Se introduce en un balón tricol de 100 ml 2,5 g (0,0107 mol) de 5-bromo-2-fenil-piridina en solución en 40 ml de éter etílico y se enfría el medio reaccionante a -60°C para agregar, gota a gota en 10 min, 5,6 ml (0,0139 mol) de una solución de n-butil litio de 2,5 M en hexano, y se mantiene a temperatura a -70°C durante 1 h.

Se agrega 1,34 g (0,0107 mol) de 1-azabicyclo[2.2.2]octano-3-ona en solución en 20 ml de

tetrahidrofurano en 10 min, se agita la mezcla durante 30 min a -70°C , luego durante 4 h a temperatura ambiente.

Se hidroliza el medio reaccionante por adición de 100 ml de metanol y se lo concentra bajo presión reducida. Se retoma el residuo por medio de 100 ml de una solución acuosa saturada de cloruro de amonio y se extrae la fase acuosa con cloroformo. Se secan las fases orgánicas, se las concentra bajo presión reducida y se purifica el residuo por cromatografía sobre columna de gel de sílice, procediendo a la elución con una mezcla 90/10/1 de cloroformo, metanol y amoníaco.

Se obtiene 0,8 g de cristales.

Punto de fusión: 214°C .

1.3. Bromhidrato de 5-(2-fenilpiridin-5-il)-1-azabicyclo[3.2.1]oct-3-eno (2:1).

Se introduce en un balón tricol de 25 ml 0,8 g (2,85 mmoles) de 3-hidroxí-3-(2-fenilpiridin-5-il)-1-azabicyclo[2.2.2]octano, luego 10 ml de ácido metanosulfónico y se calienta la mezcla a 180°C durante 24 h.

Se la vierte sobre hielo, se alcaliniza por adición de una solución acuosa concentrada de hidróxido de sodio, se extrae la fase acuosa por medio de cloroformo, se seca la fase orgánica y se la concentra bajo presión reducida. Se purifica el residuo por cromatografía sobre columna de gel

de sílice, y se procede a la elución con una mezcla 98/2/0,2 de cloroformo, metanol y amoníaco.

Se obtiene 0,25 g de producto cuyo dibromhidrato se hace por adición de una solución de ácido bromhídrico 5,7 M en ácido acético.

Se obtiene 0,22 g de dibromhidrato.

Punto de fusión: 273-274°C.

Ejemplo 2 (Compuesto N° 2).

Bromhidrato de 5-(2-fenilpiridin-5-il)-1-azabicyclo[3.2.1]octano (2:1).

En un frasco de Parr de 250 ml se introduce 0,14 g (0,33 mmol) de dibromhidrato de 5-(2-fenilpiridin-5-il)-1-azabicyclo[3.2.1]oct-3-eno en solución en 20 ml de metanol y se agrega 0,14 g de paladio al 10% adsorbido sobre carbón. Se somete luego el medio reaccionante a una presión de 0,35 Mpa de hidrógeno bajo agitación durante 5 h.

Se recoge el catalizador por filtración sobre tierra de infusorios y se concentra el solvente bajo presión reducida.

Se obtiene 0,058 g de producto.

Punto de fusión: 272-277°C.

Ejemplo 3 (Compuesto N° 8).

Etanodioato de 5-[2-(3-metilfenil)piridin-5-il)-1-azabicyclo [3.2.1] oct-3-eno (1:1).

3.1. 3-Hidroxi-3-(2-bromopiridin-5-il)-1-azabicyclo [2.2.2] octano.

Se introducen en un balón tricol de 2000 ml 27,6 g (0,116 mol) de 2,5-dibromopiridina en 1000 ml de éter etílico, se enfria el medio reaccionante a -67° C y se agregan 56 ml (0,140 mol) de una solución de 2,5 M de butil litio en hexano, gota a gota en 10 min. Se deja agitar el medio a -67° C durante 45 min antes de agregar 14,5 g (0,116 mol) de 1-azabicyclo [2.2.2] octano-3-ona en solución en 150 ml de éter etílico en 45 min se deja agitar a -67° C durante 3 h.

Se agregan 300 ml de una solución acuosa saturada de cloruro de amonio, luego 200 ml de una solución acuosa concentrada de hidróxido de sodio, se extrae la fase acuosa con cloroformo, se secan las fases orgánicas y se las concentra bajo presión reducida. Se purifica el residuo por cromatografía sobre columna de gel de sílice, y se procede a la elución con una mezcla 95/5/0,5, luego 80/15/1,5 de cloroformo, metanol y amoníaco.

Se obtienen 19,7 g de producto en forma de sólido amorfo.

3.2. 5-(2-bromopiridin-5-il)-1-azabicyclo [3.2.1] oct-3-eno

En un balón tricol de 100 ml se introducen 9,4 g (0,033 mol) de 3-hidroxi-3-(2-bromopiridin-5-il)-1-azabicyclo[2.2.2]octano y 35 ml de ácido sulfúrico concentrado y se calienta la mezcla a 190°C durante 1h45.

Se la enfría, se la vierte sobre 400 ml de una solución acuosa helada de hidróxido de sodio, se extrae la fase acuosa con cloroformo, se secan las fases orgánicas y se las evapora bajo presión reducida. Se purifica el residuo por cromatografía sobre columna de gel de sílice, y se procede a la elución con una mezcla 90/10/1 de cloroformo, metanol y amoníaco.

Se obtienen 3,9 g de producto en forma de sólido amarillo pálido.

Punto de fusión: 73-75°C.

3.3. Etanodioato de 5-[2-(3-metilfenil)piridin-5-il)-1-azabicyclo[3.2.1]oct-3-eno (1:1).

En un tubo de 10 ml se introduce sucesivamente 0,2 g (0,75 mmol) de 5-(2-bromopiridin-5-il)-1-azabicyclo[3.2.1]oct-3-eno, 3 ml de tolueno, 0,7 ml de una solución acuosa 2M de carbonato de sodio, 0,147 g (1,05 mmol) de ácido 3-metilbencenoborónico, 0,042 g (0,04 mmol) de tetrakis(trifenilfosfino)paladio y 0,7 ml de etanol, y se calienta la mezcla a 100°C durante 15 h.

Se elimina la fase acuosa por decantación y se extrae el bruto sobre columna de resina Dowex® por lavados

sucesivos con metanol, luego cloroformo antes de proceder a la elución por una solución de amoníaco. Se purifica el residuo por cromatografía sobre columna de gel de sílice y se eluciona con una mezcla 90/10/1 de cloroformo, metanol y amoníaco. Se obtiene así 0,167 g de producto en forma de aceite, que se pone en solución en 2 ml de alcohol isopropílico para hacer un etanodioato por adición de 0,051 g (0,057 mmol) de ácido etanodioico en solución en alcohol isopropílico. Se obtiene 0,188 g de producto cristalizado.

Pf: 173-174°C.

Ejemplo 4 (Compuesto N° 26).

Bromhidrato de 5-[2-(3-fluorofenil)piridin-5-il)-1-azabicyclo[3.2.1]octano 2:1.

En un frasco de Parr de 250 ml se introduce 0,18 g (0,51 mmol) de etanodioato de 5-[2-(3-fluorofenil)piridin-5-il)-1-azabicyclo[3.2.1]oct-3-eno en solución en 20 ml de metanol, se agrega 0,36 g de paladio al 10% adsorbido sobre carbón y se somete el medio reaccionante a una presión de 0,42 Mpa de hidrógeno bajo agitación a 45°C durante 6 h. Se recoge el catalizador por filtración sobre tierra de infusorios®, se concentra el filtrado bajo presión reducida, se retoma el residuo en 10 ml de una solución acuosa de hidróxido de sodio N y se extrae la fase acuosa por cloroformo y se purifica el bruto por cromatografía

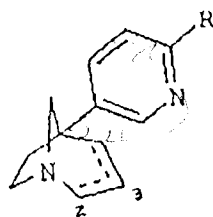
sobre columna de gel de sílice, procediendo a la elución con una mezcla 80/20/2 de cloroformo, metanol y amoníaco. Se obtiene 0,085 g de producto cuyo dibromhidrato se hace por adición de 0,107 ml de una solución de ácido bromhídrico al 33% en ácido acético.

Se obtiene 0,097 g de cristales.

Punto de fusión: 98-100°C.

El cuadro siguiente ilustra las estructuras químicas y las propiedades físicas de algunos compuestos de la invención. En la columna "R", "(+)" designa el enantiómero dextrógiro y "(-)" el enantiómero levógiro; los compuestos sin mención en esta columna son racematos. En la columna "= ", el número indicado corresponde a la posición del doble enlace en el caso de un 1-azabíciclo-octeno, y "-" designa un heterociclo saturado. En la columna "Sal", "-" designa un compuesto en estado de base, "HBr" designa un bromhidrato y "ox." designa un oxalato. Las relaciones molares ácido:base se indican enfrente. En la columna "F(°C)", "(d)" designa un punto de fusión con descomposición.

Cuadro



(I)

N°	R	=	Sal	F(°C)
1	C ₆ H ₅	3	HBr 2:1	273-274
2	C ₆ H ₅	-	HBr 2:1	272-277
3	C ₆ H ₅	2	HBr 2:1	297-305
4	2,4-(OCH ₃) ₂ -5-pirimidinil	2	HBr 2:1	340 (d)
5	3,4-(OCH ₃) ₂ -C ₆ H ₃	3	HBr 2:1	261-262
6	3,4-(OCH ₃) ₂ -C ₆ H ₃	-	HBr 2:1	234-236
7	2-F-C ₆ H ₄	3	ox. 1:1	157-158
8	3-CH ₃ -C ₆ H ₄	3	ox. 1:1	173-174
9	3-F-C ₆ H ₄	3	ox. 1:1	163-164
10	3-NO ₂ -C ₆ H ₄	3	ox. 1:1	183-184
11	3-CF ₃ -C ₆ H ₄	3	ox. 1:1	156-157
12	4-CH ₃ -C ₆ H ₄	3	ox. 1:1	213-215
13	3-Tienil	3	ox. 1:1	189-190
14	3,4-OCH ₂ O-C ₆ H ₃	3	ox. 1:1	201-202
15	4-Cl-C ₆ H ₄	3	ox. 1:1	201-203
16	3-CH ₃ CO-C ₆ H ₄	3	ox. 1:1	155-156

N°	R	=	Sal	F (°C)
17	3-Piridinil	3	ox. 1:1	183-184
18	5-Indolil	3	ox. 1:1	253-254
19	4-CH ₃ O-C ₆ H ₄	3	ox. 1:1	205-207
20	3,5-(CH ₃) ₂ -C ₆ H ₃	3	ox. 1:1	192-193
21	4-Piridinil	3	ox. 1:1	172-174
22	4-CH ₃ O-C ₆ H ₄	-	HBr 2:1	246-247
23	4-CH ₃ -C ₆ H ₄	-	HBr 2:1	295-297
24	3-CH ₃ -C ₆ H ₄	-	HBr 2:1	284-287
25	3,5-(CH ₃) ₂ -C ₆ H ₃	-	HBr 2:1	250-254
26	3-F-C ₆ H ₄	-	HBr 2:1	98-100
27	3-Tienil	-	HBr 2:1	193-196
28	3,4-OCH ₂ O-C ₆ H ₃	-	HBr 2:1	260-263
29	2-F-C ₆ H ₄	-	HBr 2:1	266-269
30	3-Piridinil	-	HBr 3:1	256-260
31	4-Piridinil	-	HBr 2:1	249-253
32	3-NO ₂ -C ₆ H ₄	-	HBr 3:1	264-267
33	3-CF ₃ -C ₆ H ₄	-	HBr 2:1	218-221
34	Br	3	HBr 2:1	234-236
35	Br	2	HBr 2:1	>350
36	4-Piperidinil	-	HBr 3:1	289-292
37	3-Piperidinil	-	HBr 3:1	261-265

N°	R	=	Sal	F (°C)
38	4-CH ₃ O-C ₆ H ₄ (+)	-	-	125-129
39	4-CH ₃ O-C ₆ H ₄ (-)	-	-	125-129
40	3-F-C ₆ H ₄ (+)	-	-	68-70
41	3-F-C ₆ H ₄ (-)	-	-	68-70
42	2-Tienil	-	HBr 2:1	251 (d)
43	2-Tienil	3	HBr 2:1	246-247
44	5-CH ₃ -2-tienil	3	HBr 2:1	237-238
45	5-CH ₃ -2-tienil	-	HBr 2:1	210-211
46	5-Cl-2-tienil	-	HBr 2:1	248-250
47	5-Cl-2-tienil	3	HBr 2:1	258-259
48	2-Furil	3	HBr 2:1	262-264
49	2-Furil	-	HBr 2:1	182 (d)
50	5-Indolil	-	ox. 1:1	268-269
51	2-Benzofuril	3	-	145-146
52	2-Benzofuril	-	HBr 2:1	303-305
53	2-Pirrolil	3	HBr 2:1	265-266
54	2-Pirrolil	-	ox. 1:1	95-97
55	2-Benzotienil	3	-	165-166
56	2-Benzotienil	-	HBr 2:1	311-313
57	3-Furil	3	HBr 2:1	291-294
58	3-Furil	-	HBr 2:1	313-315

N°	R	=	Sal	F(°C)
59	4-OH-3-piridinil	-	HBr 2:1	268-270
60	3,5-(CH ₃) ₂ -1,2-oxazol-4-il	-	-	116-117
61	3,5-(CH ₃) ₂ -1,2-oxazol-4-il	3	HBr 2:1	250-252
62	2,4-(CH ₃ O) ₂ -pirimidin-5-il	-	ox. 1:1	70-72
63	4-CH ₃ -2-tienil	-	HBr 2:1	336-338
64	4-CH ₃ -2-tienil	3	HBr 2:1	284-285
65	1-Dibenzofuril	-	HBr 2:1	188-189
66	1-Dibenzofuril	3	HBr 2:1	302-304
67	1-Fenoxatiinil	3	HBr 2:1	292-293
68	1-Fenoxatiinil	-	HBr 1:1	200-203
69	8-Quinoleinil	-	HBr 2:1	260-208
70	8-Quinoleinil	3	HBr 2:1	309-310
71	3-Benzotienil	3	HBr 2:1	222-223
72	3-Benzotienil	-	ox. 1:1	80-82

Los compuestos de la presente invención han sido estudiados en lo que hace a su afinidad frente a los receptores nicotínicos que contienen la subunidad $\alpha_4\beta_2$ de acuerdo con los métodos descriptos por Anderson y Arneric en *Eur. J. Pharmacol.* (1994), 253, 261 y por Hall et coll. en *Brain Res.* (1993), 600, 127.

Se decapitan ratas macho Sprague Dawley de 150 a 200 g, se extrae rápidamente la totalidad del cerebro, se lo

homogeneiza en 15 volúmenes de una solución de sacarosa 0,32 M a 4°C, luego se lo centrifuga a 1000 G durante 10 min. Se elimina el culote y se centrifuga el sobrenadante a 2000 G durante 20 min a 4°C. Se recupera el culote y se lo homogeneiza con la ayuda de un triturador Polytron™ en 15 volúmenes de agua bidestilada a 4°C, luego se lo centrifuga a 8000 G durante 20 min. Se elimina el culote y se centrifuga el sobrenadante y la capa de piel ("buffy coat") a 40000 G durante 20 min. Se recupera el culote, se lo vuelve a poner en suspensión en 15 ml de agua bidestilada a 4°C y se lo centrifuga una vez más a 40000 G durante 20 min., antes de conservarlo a -80°C.

El día de la experiencia, se descongela lentamente el tejido y se lo pone en suspensión en 3 volúmenes de tampón. Se incuban 150 µl de esta suspensión membranaria a 4°C durante 120 min en presencia de 100 µl de [³H]-cistisina en 1 nM en un volumen final de 500 µl de tampón, en presencia o en ausencia del compuesto a probar. Se detiene la reacción por filtración sobre filtros Whatman GF/C™ previamente tratados durante 3 horas con polietilenimina se enjuagan los filtros dos veces con 5 ml de tampón a 4°C, y se mide la radioactividad retenida en el filtro por centellografía líquida. Se determina el enlace no específico en presencia de (-)-nicotina 10 µM; el enlace no específico representa 75 a 85% del enlace total recuperado sobre el filtro. Para cada concentración de compuesto estudiado, se determina el porcentaje de inhibición del

enlace específico de [^3H]-cistisina, luego se calcula la CI_{50} , concentración de compuesto que inhibe 50% del enlace específico.

Las CI_{50} de los compuestos de la invención más afines, se sitúan entre 0,01 y 10 μM .

Los compuestos de la invención también han sido estudiados en lo que hace a su afinidad frente a receptores nicotínicos que contiene la subunidad α_7 , de acuerdo con los métodos descriptos por Marks y Collins en *J. Pharmacol. Exp. Ther.* (1982), **22**, 564 y por Marks et coll. en *Mol. Pharmacol.* 1986, **30**, 427.

Se decapitan ratas macho OFA de 150 a 200 g, se extrae rápidamente la totalidad del cerebro, se lo homogeneiza con la ayuda de un triturador PolytronTM en 15 volúmenes de una solución de sacarosa de 0,32 M a 4°C, luego se lo centrifuga a 1000 G durante 10 min. Se elimina el culote y se centrifuga el sobrenadante a 8000 G durante 20 min a 4°C. Se recupera el culote y se lo homogeneiza con la ayuda de un triturador PolytronTM en 15 volúmenes de agua bidestilada a 4°C, luego se lo centrifuga a 8000 G durante 20 min. Se elimina el culote y se centrifuga el sobrenadante y la capa de piel ("buffy coat") a 40000 G durante 20 min. Se recupera el culote, se lo vuelve a poner en suspensión en 15 ml de agua bidestilada a 4°C y se lo centrifuga una vez más a 40000 G durante 20 min., antes de conservarlo a -80°C.

El día de la experiencia, se descongela lentamente el tejido y se lo pone en suspensión en 5 volúmenes de tampón. Se preincuba 150 μ l de esta suspensión membranaria a 37°C durante 30 min. a oscuras, en presencia o en ausencia del compuesto a probar. Luego se incuban las membranas durante 60 min. a 37°C, a oscuras, en presencia de 50 μ l de [^{3} H]- α -bungarotoxina 1 nM en un volumen final de 250 μ l de tampón HEPES 20 mM, polietilenimina 0,05%. Se detiene la reacción por filtración sobre filtros Whatman GF/C™ previamente tratados durante 3 horas con polietilenimina al 0,05%. Se enjuagan los filtros dos veces con 5 ml de tampón a 4°C, y se mide la radioactividad retenida sobre cada filtro por centellografía líquida. Se determina el enlace no específico en presencia de α -bungarotoxina 1 μ M final; el enlace no específico representa aproximadamente 60% del enlace total recuperado sobre el filtro. Para cada concentración de compuesto estudiado, se determina el porcentaje de inhibición del enlace específico de [^{3} H]- α -bungarotoxina, luego se calcula la CI_{50} , concentración de compuesto que inhibe 50% del enlace específico.

Las CI_{50} de los compuestos de la invención más afines, se sitúan entre 0,005 y 20 μ M.

Los resultados que preceden muestran que los compuestos de la invención son ligandos para los receptores nicotínicos. Algunos son selectivos para los receptores que contienen las subunidades α_7 y otros son mixtos para los receptores de tipo $\alpha_4\beta_2$ y α_7 .

Los resultados de las pruebas sugieren la utilización de los compuestos en el tratamiento o la prevención de los desórdenes relacionados con una disfunción de los receptores nicotínicos, en especial al nivel del sistema nervioso central.

Esos desórdenes comprenden las alteraciones cognitivas, más específicamente mnésicas, pero también atencionales, relacionadas con la enfermedad de Alzheimer, el envejecimiento patológico (Age Associated Memory Impairment, AAMI), el síndrome Parkinsoniano, la trisomía 21 (Down's syndrome), el síndrome alcohólico de Korsakoff, las demencias vasculares (multi-infarct dementia, MDI).

Los compuestos de la invención podrían también ser útiles en el tratamiento de los trastornos motores observados en la enfermedad de Parkinson u otras enfermedades neurológicas tales como corea de Huntington, el síndrome de Tourette, la disquinesia tardía y la hiperquinesia.

Los compuestos de la invención pueden también constituir un tratamiento curativo o sintomático de los accidentes cerebrovasculares y de los episodios hipóxicos cerebrales, así como de las patologías neurodegenerativas crónicas tales como la enfermedad de Alzheimer y la enfermedad de Parkinson. Pueden ser utilizados en los casos de patologías psiquiátricas: esquizofrenia, depresión,

ansiedad, ataques de pánico, comportamientos compulsivos y obsesivos.

Pueden prevenir los síntomas debidos a la abstinencia de tabaco, de alcohol, de diferentes sustancias que inducen una dependencia, tales como la cocaína, LSD, cannabis, benzodiazepinas.

De esta forma, la presente invención también tiene por objeto composiciones farmacéuticas que contienen una dosis eficaz de al menos un compuesto conforme a la invención, en estado de base o de sal o de solvato farmacéuticamente aceptable, y dado el caso, mezclado con excipientes convenientes.

Dichos excipientes se eligen de acuerdo con la forma farmacéutica y el modo de administración deseado.

Las composiciones farmacéuticas conformes a la invención, puede entonces destinarse a la administración oral, sublingual, subcutánea, intramuscular, intravenosa, tópica, intratraqueal, intranasal, transdérmica, rectal, intraocular.

Las formas unitarias de administración pueden ser, por ejemplo, comprimidos, cápsulas de gelatina, gránulos, polvos, soluciones o suspensiones orales o inyectables, parches transdérmicos ("patch"), supositorios. Para la administración tópica se pueden prever pomadas, lociones y colirios. Dichas formas unitarias se dosifican para permitir una administración diaria de 0,01 a 20 mg de

principio activo por kg de peso corporal, de acuerdo con la forma galénica.

Para preparar comprimidos se agrega al principio activo, micronizado o no, un vehículo farmacéutico que puede estar compuesto por diluyentes, como por ejemplo lactosa, celulosa microcristalina, almidón, y adyuvantes de formulación tales como aglutinantes, (polivinilpirrolidona, hidroxipropilmetilcelulosa, etc), agentes de deslizamiento tales como sílice, lubricantes como estearato de magnesio, ácido esteárico, tribehenato de glicerol, estearilfumarato de sodio. También pueden agregarse agentes humectantes o tensioactivos tales como laurilsulfato de sodio.

Las técnicas de realización pueden ser compresión directa, granulación seca, granulación húmeda o la fusión en caliente.

Los comprimidos pueden estar desnudos, grageados, por ejemplo con sacarosa, o recubiertos con diversos polímeros u otras materias apropiadas. Pueden ser concebidos para permitir una liberación rápida, retardada o prolongada del principio activo gracias a matrices polímeras o a polímeros específicos utilizados en el recubrimiento.

Para preparar cápsulas de gelatina, se mezcla el principio activo con vehículos farmacéuticos secos (simple mezcla, granulación seca o húmeda, o fusión en caliente), líquidos o semisólidos. Las cápsulas de gelatina pueden ser duras o blandas, peliculadas o no, para que tengan una

actividad rápida, prolongada o retardada (por ejemplo para una forma entérica).

Una composición en forma de jarabe o de elixir o para la administración en forma de gotas, puede contener el principio activo conjuntamente con un edulcorante, preferentemente acalórico, metilparaben o propilparaben como antiséptico, un agente saborizante y un colorante.

Los polvos y gránulos dispersables en agua pueden contener el principio activo mezclado con agentes de dispersión o agentes humectantes, o agentes dispersantes como polivinilpirrolidona, como así también con edulcorantes y agentes correctores del sabor.

Para la administración rectal, se recurre a supositorios preparados con aglutinantes que funden a la temperatura rectal, por ejemplo manteca de cacao o polietilenglicoles.

Para una administración parenteral, se utilizan suspensiones acuosas, soluciones salinas isotónicas o soluciones estériles inyectables que contienen agentes de dispersión y/o humectantes farmacológicamente compatibles, por ejemplo propilenglicol o butilenglicol.

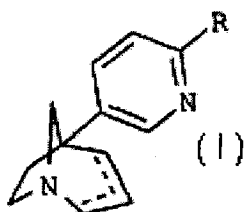
El principio activo también puede ser formulado en forma de microcápsulas, eventualmente con uno o varios soportes o aditivos, o bien con una matriz polímera o con una ciclodextrina (parches transdérmicos, formas de liberación prolongada).

Las composiciones tópicas conformes a la invención, comprenden un medio compatible con la piel. Pueden presentarse principalmente en forma de soluciones acuosas, alcohólicas o hidroalcohólicas, geles, emulsiones agua en aceite o aceite en agua, que tienen el aspecto de una crema o de un gel, microemulsiones, aerosoles, o también en forma de dispersiones vesiculares que contienen lípidos iónicos y/o no iónicos. Esas formas galénicas se preparan de acuerdo con los métodos usuales de los ámbitos considerados.

Por último, las composiciones farmacéuticas conformes a la invención pueden contener, junto con un compuesto de fórmula general (I), otros principios activos que pueden ser útiles en el tratamiento de los trastornos y enfermedades indicados precedentemente.

Reivindicaciones

1. Compuesto en forma de enantiómero puro o en forma o en forma de mezcla de enantiómeros, que responde a la fórmula general (I):



en la cual R representa un átomo de halógeno o un grupo fenilo substituido por uno o más grupos elegidos entre un átomo de halógeno, o un grupo alquilo (C_1-C_6), alcoxi (C_1-C_6), nitro, trifluorometilo, acetilo o un grupo metilendioxi, o un grupo piperinidilo, o piridinilo, o quinolinilo, o tienilo, o furilo, o benzofurilo, o benzotienilo, o indolilo, o pirimidinilo o isoxazolilo, o fenoxatiinilo, o dibenzofurilo, o pirrolilo, y donde cada uno de estos grupos puede eventualmente ser substituido por uno o varios grupos elegidos entre los átomos de halógenos, los grupos alquilo (C_1-C_6), alcoxi (C_1-C_6), hidroxilo, y donde, los dos enlaces carbono-carbono representados por ---, uno es simple y el otro puede ser simple o doble, en la forma de base o de una sal derivada de adición con un ácido;

siendo excluidos los siguientes compuestos:

- 5-(2-bromopiridin-5-il)-1-azabicyclo[3.2.1]oct-3-ano
- 5-(2-cloropiridin-5-il)-1-azabicyclo[3.2.1]oct-3-ano
- 5-(2-fluoropiridin-5-il)-1-azabicyclo[3.2.1]oct-3-ano.

2. Compuesto de acuerdo con la reivindicación 1, caracterizado porque R representa o bien un átomo de halógeno o un grupo fenilo substituido por uno o mas grupos elegidos entre los átomos de halógenos y los grupos alquilo (C_1-C_6), alcoxi (C_1-C_6), nitro, trifluorometilo, acetilo, o metilendioxi, o representa un grupo piridinilo o un grupo tienilo o un grupo indolilo o un grupo pirimidinilo opcionalmente sustituido por uno o más grupos alcoxi (C_1-C_6).

ANEXO 1

Valores de IC₅₀ para los compuestos reivindicados

N° del compuesto en el cuadro pag 14 de la descripción	α_7 CI50 μ M	$\alpha_4\beta_2$ CI50 μ M
1	0,126	
2	0,03	
3	0,074	
4	5,24	0,428
5	10	1,153
6	0,619	3
7	0,8	3
8	0,313	3
9	0,193	3
10	0,375	3
11	1,249	3

N° del compuesto en el cuadro pag 14 de la descripción	α_7 CI50 μM	$\alpha_4\beta_2$ CI50 μM
12	0,349	3
13	0,079	3
14	0,396	1,831
15	0,939	3
16	0,103	3
17	0,662	3
18	0,01	1,489
19	0,533	3
20	10	3
21	0,277	0,027
22	0,056	3
23	0,12	3

204

N° del compuesto en el cuadro pag 14 de la descripción	α_7 CI50 μ M	$\alpha_4\beta_2$ CI50 μ M
24	0,074	3
25	10	3
26	0,016	3
27	0,017	3
28	0,17	3
29	0,09	0,293
30	0,087	0,286
31	0,05	0,072
32	0,055	0,11
33	0,39	0,519
36	1	3
37	3	3

Nicotinic receptors – littérature

Alzheimer

<1>

UI - 16787838

ST - PubMed-not-MEDLINE

AU - Nordberg A

FA - Nordberg, A

IN - Karolinska Institutet, NEUROTEC, Division of Molecular
Neuropharmacology, Geriatric Clinic, Huddinge Hospital B84, S-141 86

Huddinge, Sweden. Agneta.Nordberg@neurotec.ki.se TI - Neuroprotection in
Alzheimer's disease - new strategies for treatment.

SO - Neurotoxicity Research. 2(2-3):157-65, 2000.

AB - Alzheimer's disease is the most common dementia disorder
characterized by multiple pathological changes in the brain leading to a
progressive memory loss and other cognitive symptoms producing occupational
and social disabilities. Although a great deal of progress has been made in
recent years in further understanding the genetic aberrations and patho-
physiological processes of Alzheimer's disease there is still no cure of
the disease. The transmitter replacement therapy is so far the most
explored therapy. Three cholinesterase inhibitors have so far been approved
and presently in clinical use in many countries. Although the
cholinesterase inhibitors generally appear to produce symptomatic effects
with palliative effect on existing cognitive disturbances recent data
suggest that they also may have effect on progression of the disease
including possible neuroprotective effects. Possible interactions between
Abeta and cholinergic neurotransmission may exist. Treatment of!

cells with Abeta causes decreased cholinergic activity. Pretreatment of
PC12 cells with cholinesterase inhibitors such as tacrine and donepezil in
clinical relevant concentrations can attenuate Abeta (25-35) toxicity
through mechanisms which may be mediated via nicotinic receptors. Estrogen
has been shown to protect against Abeta toxicity in different cell lines
and also to reduce the formation of Abeta. Its mechanism for the
neuroprotective effect is however not fully clarified. A potentiation of
the clinical effect of cholinesterase inhibitors in Alzheimer patients has
been given together with estrogen. Experimental data suggest that the
neuroprotective effect of estrogen as studied in PC12 cells was mediated at
least partly via the alpha(7) nicotinic receptor. Treatment with Abeta in
nanomolar concentrations for 7 days in PC12 cells significantly decreased
the number of nicotinic receptor binding sites and mRNA levels. The effects
by Abeta on nicotinic receptors are pre!

vented by nicotine pretreatment. The finding suggests a possible link
between Abeta and nicotinic receptor deficits in Alzheimer patients in the
early course of the disease.

PT - Journal Article

<2>

UI - 17992264

ST - MEDLINE

AU - Nizri E

AU - Wirguin I

AU - Brenner T

FA - Nizri, Eran

FA - Wirguin, Itzhak

FA - Brenner, Talma

IN - Department of Neurology, Agnes Ginges Center for Human Neurogenetic,
Hadassah University Hospital, Hebrew University Medical School, Jerusalem,
Israel.

TI - The role of cholinergic balance perturbation in neurological diseases.

[Review] [64 refs] SO - Drug News & Perspectives. 20(7):421-9, 2007 Sep.

AB - The maintenance of a balanced cholinergic homeostasis is crucial for the function of the central nervous system, peripheral nervous system and the neuromuscular junction. However, it appears that the cholinergic system is not restricted to neurons and synapses but may also involve immune reactions. In the present review we reassess the role of the cholinergic balance in myasthenia gravis and Alzheimer's disease which for a long time are known to be associated with cholinergic transmission perturbation. We have included neuroinflammation, particularly multiple sclerosis in this group of neurological disorders in light of the relatively new studies involving the immune cholinergic system. In all the aforementioned disorders, treatment with acetylcholinesterase inhibitors can attenuate inflammation. This is performed by increasing the acetylcholine (ACh) concentration near immune cells and making it available for interaction with alpha(7) nicotinic ACh receptor, expressed!

on these cells. This outcome is additional to the effect of acetylcholinesterase inhibitors on neurons and synapses. [References: 64]

PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Review

<3>

UI - 16472157

ST - MEDLINE

AU - D'Andrea MR

AU - Nagele RG

FA - D'Andrea, Michael R

FA - Nagele, Robert G

IN - Johnson & Johnson Pharmaceutical Research and Development, Drug Discovery, Spring House, Pennsylvania, 19477, USA. mdandrea@prdus.jnj.com

TI - Targeting the alpha 7 nicotinic acetylcholine receptor to reduce amyloid accumulation in Alzheimer's disease pyramidal neurons. [Review] [82 refs] SO - Current Pharmaceutical Design. 12(6):677-84, 2006.

AB - Although there is still no known effective preventative treatment or cure for Alzheimer's disease (AD), the development of new drugs that target pathological features that appear early in the course of this disease and alleviate some of the early cognitive and memory symptoms is a laudable goal that may be one step closer. To date, the acetylcholinesterase inhibitors have been the most widely used AD drugs and have been somewhat successful in slowing loss of cognition. In the last few years, a number of studies have demonstrated that amyloid beta (1-42) (Abeta42), the predominant Abeta peptide species in amyloid plaques, first accumulates in vulnerable neurons prior to plaque formation. Recently, we have shown that many (if not most) amyloid plaques in the entorhinal cortex of AD brains are actually the lysis remnants of degenerated, Abeta42-overburdened neurons. Furthermore, the most vulnerable neurons appear to be those that abundantly express the alpha7 nicotinic ac!

etylcholine receptor (alpha7nAChR), and internalization of Abeta42 appears to be facilitated by the high-affinity binding of Abeta42 to the alpha7nAChR on neuronal cell surfaces, followed by endocytosis of the resulting complex and its accumulation within the lysosomal compartment. This mechanism provides a reasonable explanation for the selective vulnerability of cholinergic and cholinceptive neurons in AD brains and for the fact that Abeta42 is the dominant Abeta peptide species in both intraneuronal accumulations and amyloid plaques. In view of the pathophysiological consequences of Abeta42 binding to alpha7nAChR on neuronal surfaces that stem from excessive intraneuronal Abeta42 accumulation, the alpha7nAChR could be an important therapeutic target for treatment of AD. In addition, it further emphasizes the potential merits of new and effective therapeutic strategies pointed towards the goal of lowering of Abeta42 levels in the blood and cerebrospinal fluid as well as !

blocking Abeta42 in the blood from penetrating the blood-brain barrier and entering into the brain parenchyma. [References: 82] PT - Journal Article PT - Review

<4>

UI - 12814128

ST - MEDLINE

AU - Bourin M

AU - Ripoll N

AU - Dailly E

FA - Bourin, Michel

FA - Ripoll, Nadege

FA - Dailly, Eric

IN - Research Group Neurobiology of Anxiety and Depression, Faculty of Medicine, Nantes, France. mbourin@sante.univ-nantes.fr TI - Nicotinic receptors and Alzheimer's disease. [Review] [112 refs] SO - Current Medical Research & Opinion. 19(3):169-77, 2003.

AB - Nicotinic receptors (NRs) belong to the group of polymeric receptors of the cell membrane and are key elements of cholinergic transmission. Numerous subtypes of NRs exist with the alpha 4 beta 2 and alpha 7 types being encountered most frequently. Deficiencies in NRs seem to play a role in Alzheimer's disease, which is characterised by accumulation of senile plaques, mainly composed of beta-amyloid peptide (beta A). Although the aetiology of this disease is unknown, different pathogenesis hypotheses implicating alpha 7 NRs have been proposed, with the receptors exerting a direct or indirect action on the mechanism of beta A toxicity. Allosteric modulators of NRs, such as the cholinesterase inhibitor galantamine, that facilitate the action of acetylcholine on these receptors may provide therapeutic benefits in the areas of cognition, attention and antineurodegenerative activity. [References: 112] PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Review

Schizophrenia

<1>

UI - 15100704

ST - MEDLINE

AU - De Luca V

AU - Wong AH

AU - Muller DJ

AU - Wong GW

AU - Tyndale RF

AU - Kennedy JL

FA - De Luca, Vincenzo

FA - Wong, Albert H C

FA - Muller, Daniel J

FA - Wong, Gregory W H

FA - Tyndale, Rachel F

FA - Kennedy, James L

IN - Neurogenetics Section, Clarke Site, Centre for Addiction and Mental Health, Department of Psychiatry, Toronto, Ontario, Canada.

TI - Evidence of association between smoking and alpha7 nicotinic receptor subunit gene in schizophrenia patients.

SO - Neuropsychopharmacology. 29(8):1522-6, 2004 Aug.

AB - A number of studies have suggested that the alpha-7-nicotinic receptor D15S1360 polymorphism is associated with schizophrenia and a deficiency in the normal inhibition of the P50 auditory-evoked response. Schizophrenia patients and some of their unaffected relatives show a failure of inhibition in their 50-ms response to the second of a pair of

tones. Biochemical studies have suggested that the alpha7 nicotinic acetylcholine receptor is involved in this sensory gating deficit. Furthermore, high-dose nicotine transiently normalizes the abnormality in P50 inhibition in schizophrenic patients and in their relatives. Schizophrenic patients are unusually heavy smokers, and up to 85% of hospitalized schizophrenic patients smoke. This rate is three times higher than the general population, and may represent an attempt to self-medicate through this pathophysiologic mechanism. Despite schizophrenics' extremely heavy nicotine use, nicotinic receptor genes have not been previously

investigated in relation to smoking in schizophrenia. In this study, we hypothesized that the D15S1360 marker is associated with smoking in patients with schizophrenia. We found an association between the homozygous 113 bp allele and smoking risk ($\chi^2=10.37$, 3 df, $p=0.015$). Although this novel finding requires replication, it suggests that further study into the relationship between schizophrenia and nicotine system genes is warranted.

PT - Journal Article

<2>

UI - 15265251

ST - MEDLINE

AU - Ripoll N

AU - Bronnec M

AU - Bourin M

FA - Ripoll, Nadege

FA - Bronnec, Marie

FA - Bourin, Michel

IN - Neurobiology of Anxiety and Depression Research Group, Faculty of Medicine, University of Nantes, France.

TI - Nicotinic receptors and schizophrenia. [Review] [303 refs] SO - Current Medical Research & Opinion. 20(7):1057-74, 2004 Jul.

AB - The incidence of smoking is very high in non-schizophrenic subjects presenting various psychiatric disorders (35 to 54%). However, the incidence of smoking is extremely high in schizophrenic patients: 80% to 90%, versus 25% to 30% of the general population. Various studies have demonstrated that the use of tobacco transiently restores the schizophrenic patient's cognitive and sensory deficits. Smoking cessation also appears to exacerbate the symptoms of the disease. Post-mortem binding studies have revealed a disturbance of nicotinic receptor expression, affecting the alpha(7) and alpha(4)beta(2) subunits, in various cerebral areas. Genetic linkage studies have also shown that the alpha(7) subunit is involved in schizophrenia. This review assesses the involvement of the nicotinic system in schizophrenia and suggests ways in which this system may participate in the pathophysiology of this disease. [References: 303] PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Review

<3>

UI - 15197106

ST - MEDLINE

AU - Suemaru K

AU - Yasuda K

AU - Umeda K

AU - Araki H

AU - Shibata K

AU - Choshi T

AU - Hibino S

AU - Gomita Y

FA - Suemaru, Katsuya

FA - Yasuda, Kayo

FA - Umeda, Kenta

FA - Araki, Hiroaki

FA - Shibata, Kazuhiko

FA - Choshi, Tominari
 FA - Hibino, Satoshi
 FA - Gomita, Yutaka
 IN - Division of Pharmacy, Ehime University Hospital, Shitsukawa, Shigenobu-cho, Onsen-gun, Ehime 791-0295, Japan. suemaru@m.ehime-u.ac.jp TI
 - Nicotine blocks apomorphine-induced disruption of prepulse inhibition of the acoustic startle in rats: possible involvement of central nicotinic alpha7 receptors.
 SO - British Journal of Pharmacology. 142(5):843-50, 2004 Jul.
 AB - Nicotine has been reported to normalize deficits in auditory sensory gating in the cases of schizophrenia, suggesting an involvement of nicotinic acetylcholine receptors in attentional abnormalities. However, the mechanism remains unclear. The present study investigated the effects of nicotine on the disruption of prepulse inhibition (PPI) of the acoustic startle response induced by apomorphine or phencyclidine in rats. Over the dose range tested, nicotine (0.05-1 mg kg⁻¹, s.c.) did not disrupt PPI. Neither methyllycaconitine (0.5-5 mg kg⁻¹, s.c.), an alpha(7) nicotinic receptor antagonist, nor dihydro-beta-erythroidine (0.5-2 mg kg⁻¹, s.c.), an alpha(4)beta(2) nicotinic receptor antagonist, had any effect on PPI. Nicotine (0.01-0.2 mg kg⁻¹, s.c.) dose-dependently reversed the disruption of PPI induced by apomorphine (1 mg kg⁻¹, s.c.), but had no effect on the disruption of PPI induced by phencyclidine (2 mg kg⁻¹, s.c.). The reversal of apomorphine-induced !
 PPI disruption by nicotine (0.2 mg kg⁻¹) was eliminated by mecamylamine (1 mg kg⁻¹, i.p.), but not by hexamethonium (10 mg kg⁻¹, i.p.), indicating the involvement of central nicotinic receptors. The antagonistic action of nicotine on apomorphine-induced PPI disruption was dose-dependently blocked by methyllycaconitine (1 and 2 mg kg⁻¹, s.c.). However, dihydro-beta-erythroidine (1 and 2 mg kg⁻¹, s.c.) had no effect. These results suggest that nicotine reverses the disruption of apomorphine-induced PPI through central alpha(7) nicotinic receptors.
 PT - Journal Article
 PT - Research Support, Non-U.S. Gov't

<4>

UI - 15205879
 ST - MEDLINE
 AU - Martin LF
 AU - Kem WR
 AU - Freedman R
 FA - Martin, Laura F
 FA - Kem, William R
 FA - Freedman, Robert
 IN - Department of Psychiatry, Department of Veterans Affairs and University of Colorado Health Sciences Center, 4200 East Ninth Avenue, C268-71, Denver, CO 80262, USA. Laura.martin@uchsc.edu TI - Alpha-7 nicotinic receptor agonists: potential new candidates for the treatment of schizophrenia. [Review] [164 refs] SO - Psychopharmacology. 174(1):54-64, 2004 Jun.
 AB - RATIONALE AND OBJECTIVE: Auditory sensory gating, a biological measurement of the ability to suppress the evoked response to the second of two auditory stimuli, is diminished in people with schizophrenia. Deficits in sensory gating are associated with attentional impairment, and may contribute to cognitive symptoms and perceptual disturbances. This inhibitory process, which involves the alpha(7) nicotinic receptor mediated release of gamma-aminobutyric acid (GABA) by hippocampal interneurons, represents a potential new target for therapeutic intervention in schizophrenia. METHOD: This paper will review several lines of evidence implicating the nicotinic-cholinergic, and specifically, the alpha(7) nicotinic receptor system in the pathology of schizophrenia and the evidence that alpha(7) nicotinic receptor agonists may ameliorate some of

these deficits. RESULTS: Impaired auditory sensory gating has been linked to the alpha(7) nicotinic receptor gene on the chromosome 15q!

14 locus. Single nucleotide polymorphisms of the promoter region of this gene are more frequent in people with schizophrenia. Although nicotine can acutely reverse diminished auditory sensory gating in people with schizophrenia, this effect is lost on a chronic basis due to receptor desensitization. Clozapine is able to reverse auditory sensory gating impairment, probably through an alpha(7) nicotinic receptor mechanism, in both humans and animal models with repeated dosing. The alpha(7) nicotinic agonist 3-2,4 dimethoxybenzylidene anabaseine (DMXBA) can also enhance auditory sensory gating in animal models. DMXBA is well tolerated in humans and improves several cognitive measures. CONCLUSION: Alpha-7 nicotinic receptor agonists appear to be reasonable candidates for the treatment of cognitive and perceptual disturbances in schizophrenia. [References: 164]

PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Research Support, U.S. Gov't, Non-P.H.S.

PT - Research Support, U.S. Gov't, P.H.S.

PT - Review

<5>

UI - 17192894

ST - MEDLINE

AU - Martin LF

AU - Leonard S

AU - Hall MH

AU - Tregellas JR

AU - Freedman R

AU - Olincy A

FA - Martin, Laura F

FA - Leonard, Sherry

FA - Hall, Mei-Hua

FA - Tregellas, Jason R

FA - Freedman, Robert

FA - Olincy, Ann

IN - Department of Psychiatry, Denver Veterans Affairs Medical Center, Denver, Colorado 80262, USA. laura.martin@uchsc.edu TI - Sensory gating and alpha-7 nicotinic receptor gene allelic variants in schizoaffective disorder, bipolar type.

SO - American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: the Official Publication of the International Society of Psychiatric Genetics. 144(5):611-4, 2007 Jul 5.

AB - OBJECTIVES: Single nucleotide allelic variants in the promoter region of the chromosome 15 alpha-7 acetylcholine nicotinic receptor gene (CHRNA7) are associated with both schizophrenia and the P50 auditory evoked potential sensory gating deficit. The purpose of this study was to determine if CHRNA7 promoter allelic variants are also associated with abnormal P50 ratios in persons with schizoaffective disorder, bipolar type. METHODS: P50 auditory evoked potentials were recorded in a paired stimulus paradigm in 17 subjects with schizoaffective disorder, bipolar type. The P50 test to conditioning ratio was used as the measure of sensory gating. Mutation screening of the CHRNA7 promoter region was performed on the subjects' DNA samples. Comparisons to previously obtained data from persons with schizophrenia and controls were made. RESULTS: Subjects with schizophrenia, regardless of allele status, had an abnormal mean P50 ratio. Subjects with schizoaffective disorder, bipola!

r type and a variant allele had an abnormal mean P50 ratio, whereas those schizoaffective subjects with the common alleles had a normal mean P50 ratio. Normal control subjects had a normal mean ratio, but controls with variant alleles had higher P50 ratios. CONCLUSIONS: In persons with bipolar type schizoaffective disorder, CHRNA7 promoter region allelic variants are linked to the capacity to inhibit the P50 auditory evoked potential and

thus are associated with a type of illness genetically and biologically more similar to schizophrenia.

PT - Journal Article

PT - Research Support, N.I.H., Extramural PT - Research Support, U.S. Gov't, Non-P.H.S.

<6>

UI - 17455307

ST - MEDLINE

AU - Hansen HH

AU - Timmermann DB

AU - Peters D

AU - Walters C

AU - Damaj MI

AU - Mikkelsen JD

FA - Hansen, Henrik H

FA - Timmermann, Daniel B

FA - Peters, Dan

FA - Walters, Carrie

FA - Damaj, M Imad

FA - Mikkelsen, Jens D

IN - Department of Translational Neurobiology, NeuroSearch A/S, Ballerup, Denmark. heh@neuroresearch.dk TI - Alpha-7 nicotinic acetylcholine receptor agonists selectively activate limbic regions of the rat forebrain: an effect similar to antipsychotics.

SO - Journal of Neuroscience Research. 85(8):1810-8, 2007 Jun.

AB - It is considered that activation of nicotinic alpha7 receptors (alpha7 nAChR) is useful for the treatment of cognitive disturbances in schizophrenia and Alzheimer's disease. Recently, selective alpha7 nAChR agonists have been discovered and are used to validate the alpha7 nAChR as a drug target for the treatment of cognitive disturbances in schizophrenia. One important feature shared by all known antipsychotics is their capacity to induce expression of the neuronal immediate-early gene c-fos in the limbic forebrain. Using two novel and selective alpha7 nAChR agonists, PNU-282987 and SSR180711, we investigated their ability to induce c-Fos expression in the limbic forebrain with particular emphasis on the same regions reported to be activated by antipsychotics. Both alpha7 nAChR agonists increased c-Fos dose-dependently in the prefrontal cortex and the shell of nucleus accumbens, while leaving the core of nucleus accumbens and the dorsolateral striatum unaffected. The a!

ccumbal and cortical effect of SSR180711 was blocked completely by pre-administration of the alpha7 nAChR antagonist methyllycaconitine. Also, SSR180711 displayed no c-Fos-inducing effect in alpha7 nAChR knock-out mice. In conclusion, these results show that selective pharmacologic stimulation of alpha7 nAChR function results in activation of forebrain regions similar to conventional antipsychotics. Copyright (c) 2007 Wiley-Liss, Inc.

PT - Journal Article

PT - Research Support, N.I.H., Extramural

<7>

UI - 16469942

ST - MEDLINE

AU - Potter D

AU - Summerfelt A

AU - Gold J

AU - Buchanan RW

FA - Potter, David

FA - Summerfelt, Ann

FA - Gold, James

FA - Buchanan, Robert W

IN - University of Maryland School of Medicine, Department of Psychiatry, Baltimore, Maryland, USA.

TI - Review of clinical correlates of P50 sensory gating abnormalities in patients with schizophrenia. [Review] [58 refs] SO - Schizophrenia Bulletin. 32(4):692-700, 2006 Oct.

AB - A large percentage of patients with schizophrenia are characterized by an abnormality in P50 sensory gating. This abnormality has been shown to be genetically linked to the alpha-7 nicotinic receptor and is transiently reversed by acute nicotine administration. These observations have led to the development of pharmacological treatments designed to improve sensory gating. However, if normalization of P50 gating abnormalities is to guide drug development, then it becomes important to delineate the clinical correlates of enhanced P50 gating. We conducted a review of all available articles through March 2005 that have examined this issue. We found that, despite the prominent role that P50 abnormalities have played in our understanding of schizophrenia, there is a relative dearth of data examining P50 clinical correlates. There is evidence suggestive of an association between P50 and measures of attention, and multiple studies have failed to document a cross-sectional or longitudinal relationship between P50 and positive, negative, or other symptoms. These results suggest that considerably more work needs to be done to understand and validate the clinical significance of this impairment. [References: 58] PT - Journal Article PT - Research Support, N.I.H., Extramural PT - Review

ongitudinal relationship between P50 and positive, negative, or other symptoms. These results suggest that considerably more work needs to be done to understand and validate the clinical significance of this impairment. [References: 58] PT - Journal Article PT - Research Support, N.I.H., Extramural PT - Review

<8>

UI - 12759805

ST - MEDLINE

AU - O'Neill HC

AU - Rieger K

AU - Kem WR

AU - Stevens KE

FA - O'Neill, Heidi C

FA - Rieger, Kate

FA - Kem, William R

FA - Stevens, Karen E

IN - Department of Psychiatry C268-71, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262, USA.

TI - DMXB, an alpha7 nicotinic agonist, normalizes auditory gating in isolation-reared rats.

SO - Psychopharmacology. 169(3-4):332-9, 2003 Sep.

AB - RATIONALE: Impaired auditory gating is common in schizophrenic patients. Evidence suggests that this deficit is related to a reduced number of alpha(7) nicotinic receptors and therefore treatment with alpha(7) nicotinic agonists may improve this condition. 3-(2,4)-Dimethoxybenzylidene anabaseine (DMXB; also known as GTS-21) is such an agonist and has shown efficacy in mice both orally and intraperitoneally. OBJECTIVE: Rats reared in social isolation post weaning have demonstrated a deficit in auditory gating similar to that seen in schizophrenia patients. The current study determined the effects of DMXB on auditory gating in awake, freely moving rats, comparing a group born and raised in-house and reared in isolation post-weaning (isolation reared) with a group shipped from the supplier as adults and housed in groups prior to surgery (controls). METHODS: Ten unmedicated, baseline recordings were obtained following surgical implantation of a recording electrode. All control group rats and the isolation-reared rats that showed deficient gating at baseline were treated with 1.0, 3.33, 10 or 33 mg/kg DMXB, IP, to determine the drug's impact on auditory gating. RESULTS: Isolation-reared rats had significantly improved auditory gating at the 3.33, 10 and 33 mg/kg doses, while control rats had a significant impairment in their auditory gating at the 33 mg/kg dose. CONCLUSIONS: DMXB improved the

control group rats and the isolation-reared rats that showed deficient gating at baseline were treated with 1.0, 3.33, 10 or 33 mg/kg DMXB, IP, to determine the drug's impact on auditory gating. RESULTS: Isolation-reared rats had significantly improved auditory gating at the 3.33, 10 and 33 mg/kg doses, while control rats had a significant impairment in their auditory gating at the 33 mg/kg dose. CONCLUSIONS: DMXB improved the

auditory gating deficit seen in isolation-reared rats. As previously observed in another model, the change was produced through a decrease in the test amplitude in isolation-reared animals. Control animals had a significant reduction in conditioning amplitude at the high dose, which produced the loss of auditory gating. The results in the isolation-reared rats are in concert with previous studies which found similar improvement in auditory gating following administration of DMXB to DBA mice, the only differences being in the duration of the effect.

PT - Comparative Study

PT - Journal Article

PT - Research Support, U.S. Gov't, Non-P.H.S.

PT - Research Support, U.S. Gov't, P.H.S.

<9>

UI - 12685995

ST - MEDLINE

AU - Freedman R

AU - Olincy A

AU - Ross RG

AU - Waldo MC

AU - Stevens KE

AU - Adler LE

AU - Leonard S

FA - Freedman, Robert

FA - Olincy, Ann

FA - Ross, Randall G

FA - Waldo, Merilyne C

FA - Stevens, Karen E

FA - Adler, Lawrence E

FA - Leonard, Sherry

IN - Departments of Psychiatry and Pharmacology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, C-268-71, Denver, CO 80262, USA. robert.freedman@UCHSC.edu TI - The genetics of sensory gating deficits in schizophrenia. [Review] [64 refs] SO - Current Psychiatry Reports. 5(2):155-61, 2003 Jun.

AB - Sensory gating abnormalities are an early clinical symptom of schizophrenia, and are characterized by a decrease in the brain's normal ability to inhibit the response to unimportant stimuli. Patients appear hypervigilant and have difficulty focusing their attention. A neurobiologic mechanism involved in these difficulties is nicotinic cholinergic modulation of inhibitory neuronal activity in the hippocampus. One measure of sensory gating abnormalities, diminished inhibition of the P50 evoked response to repeated auditory stimuli, has been linked to the chromosome 15q14 locus of the alpha-7-nicotinic receptor gene. This site is one of several that have shown evidence for linkage to schizophrenia, as well as to bipolar disorder, across several studies. Polymorphisms in the core promoter of the gene are associated with schizophrenia and also with diminished inhibition of the P50 response. These genetic data may identify a new pathophysiologic target for drug discovery. [R!]

References: 64]

PT - Journal Article

PT - Review

<10>

UI - 12625025

ST - MEDLINE

AU - Williams M

FA - Williams, Michael

IN - Department of Molecular Pharmacology, Feinberg School of Medicine, Northwestern University, 303 East Chicago Ave, Chicago, IL 60611-3008, USA. mazarinel643@aol.com TI - Genome-based drug discovery: prioritizing

disease-susceptibility/disease-associated genes as novel drug targets for schizophrenia. [Review] [55 refs] SO - Current Opinion in Investigational Drugs. 4(1):31-6, 2003 Jan.

AB - The search for new classes of antipsychotics based on novel targets identified from linkage/linkage association in diseased cohorts and microarray approaches using tissue from affected individuals is a high priority in central nervous system research. Genes linked to schizophrenia, a disease affecting 1% of the population, have been identified on nearly every chromosome of the human genome leading to a diverse choice of targets for validation. Interestingly, while the majority of currently used antipsychotic medications act by blocking dopamine receptors, there have been few genetic studies implicating the dopamine receptor family in disease etiology. Recently, four genes have been identified that encode dysbindin, neuroregulin, D-amino acid oxidase and G72, respectively, that support previous studies suggesting that schizophrenia may result from a hypofunction of glutamatergic neurotransmission. Linkage and microarray studies have similarly supported studies implicati!

ng the alpha 7 neuronal nicotinic receptor in the etiology of schizophrenia. Microarray studies using brain tissue from schizophrenic patients have shown changes in gene expression that number in the thousands, involving a number of proteins related to synaptic structure and function (PSYN gene group) and cellular metabolism. The majority of these proteins are not traditional drug discovery targets, nor are their functional roles in schizophrenia obvious, providing a challenge to validate them from the drug target identification/drug discovery perspective. The current state-of-the-art in genome-based approaches to schizophrenia, target discovery highlights a need for a multidisciplinary, integrative, null hypothesis-based approach to sort through these novel genes as drug targets.

[References: 55] PT - Journal Article PT - Review

<11>

UI - 12436426

ST - MEDLINE

AU - Levin ED

FA - Levin, Edward D

IN - Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Neurobehavioral Research Laboratory, Box 3412, Durham, North Carolina 27710, USA. edlevin@duke.edu TI - Nicotinic receptor subtypes and cognitive function. [Review] [60 refs] SO - Journal of Neurobiology. 53(4):633-40, 2002 Dec.

AB - Nicotinic receptor systems are involved in a wide variety of behavioral functions including cognitive function. Nicotinic medications may provide beneficial treatment for cognitive dysfunction such as Alzheimer's disease, schizophrenia, and attention deficit hyperactivity disorder (ADHD). Nicotine has been shown to improve attentional performance in all of these disorders. Better efficacy with fewer side effects might be achieved with novel nicotinic ligands selective for particular nicotinic subtypes. To develop these novel selective nicotinic ligands it is important to use animal models to determine the critical neurobehavioral bases for nicotinic involvement in cognitive function. Nicotine-induced cognitive improvement in rats is most consistently seen in working memory tasks. We have found that both acute and chronic nicotine administration significantly improves working memory performance of rats in the radial-arm maze. The pharmacologic and anatomic mechanisms fo!

r this effect have been examined in our laboratory in a series of local drug infusion studies. Both alpha 4 beta 2 and alpha 7 nicotinic receptors in the ventral hippocampus and basolateral amygdala are involved in working memory function. Working memory impairments were caused by local infusion of either alpha 4 beta 2 or alpha 7 antagonists. Ventral hippocampal alpha 4 beta 2 blockade-induced working memory deficits are reversed by chronic systemic nicotine treatment, while ventral hippocampal alpha 7 blockade-

induced working memory deficits were not found to be reversed by the same nicotine regimen. Interestingly, alpha 4 beta 2 and alpha 7 induced deficits were not found to be additive in either the ventral hippocampus or the basolateral amygdala. In fact, in the amygdala, alpha 7 antagonist cotreatment actually reversed the working memory impairment caused by alpha 4 beta 2 antagonist administration. These studies of the neural nicotinic mechanisms underlying cognitive!

function are key for opening avenues for development of safe and effective nicotinic treatments for cognitive dysfunction. Copyright 2002 Wiley Periodicals, Inc. [References: 60] PT - Journal Article PT - Research Support, U.S. Gov't, P.H.S.
PT - Review

<12>

UI - 12399955
ST - MEDLINE
AU - Raux G
AU - Bonnet-Brilhault F
AU - Louchart S
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AU - Levillain D
AU - Allio G
AU - Haouzir S
AU - Petit M
AU - Martinez M
AU - Frebourg T
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FA - Allio, G
FA - Haouzir, S
FA - Petit, M
FA - Martinez, M
FA - Frebourg, T
FA - Thibaut, F
FA - Campion, D
IN - INSERM EMI 9906, IRFMP, Faculte de Medecine et de Pharmacie, Rouen, France.
TI - The -2 bp deletion in exon 6 of the 'alpha 7-like' nicotinic receptor subunit gene is a risk factor for the P50 sensory gating deficit.
SO - Molecular Psychiatry. 7(9):1006-11, 2002.
AB - Abnormality in the P50 auditory-evoked potential gating is an endophenotype associated with schizophrenia. Biochemical and genetic studies have suggested that the alpha 7 nicotinic acetylcholine receptor (nAChR) is involved in this sensory gating deficit. Two related alpha 7 genes (CHRNA7 and CHRNA7-like gene) resulting from a partial duplication (from exon 5 to exon 10) are present in the human genome. Two types of genetic variation, a large deletion and a -2 base-pair deletion in exon 6 resulting in a truncation of the open reading frame, affect specifically the CHRNA7-like gene. We developed a simple multiplex PCR assay on genomic DNA, allowing the quantification of the number of exons 6 and the distinction of all possible exon 6 genotypes. Genotyping of 70 schizophrenic patients and 77 controls showed that carrying at least one -2 bp deletion of exon 6 did not constitute a risk factor for schizophrenia. In contrast, the distribution of genotypes differed significantly!

tly between subjects with normal and abnormal P50 ratios, with an over-representation of genotypes carrying at least one -2 bp deletion of exon 6 among subjects exhibiting an abnormal P50 ratio. We thus conclude that the -2 bp deletion within the CHRNA7-like gene is a risk factor for P50 sensory gating deficit. Interestingly, most of the effect came from the non schizophrenic group, which may suggest that in schizophrenic patients other risk factors account for the large proportion of subjects exhibiting an abnormal P50 ratio.

PT - Journal Article

PT - Research Support, Non-U.S. Gov't

<13>

UI - 12193840

ST - MEDLINE

AU - Thaker GK

FA - Thaker, Gunvant K

IN - Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, P.O. Box 21247, Baltimore 21228, USA.

TI - Current progress in schizophrenia research: sensory gating deficit in schizophrenia: is the nicotinic alpha-7 receptor implicated?.

SO - Journal of Nervous & Mental Disease. 190(8):550-1, 2002 Aug.

PT - Journal Article

PT - Research Support, U.S. Gov't, P.H.S.

<14>

UI - 12061141

ST - MEDLINE

AU - Suemaru K

AU - Araki H

AU - Gomita Y

FA - Suemaru, Katsuya

FA - Araki, Hiroaki

FA - Gomita, Yutaka

IN - Department of Hospital Pharmacy, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. suemaru@cc.okayama-u.ac.jp
TI - [Involvement of neuronal nicotinic receptor in psychiatric disorders]. [Review] [32 refs] [Japanese]
SO - Nippon Yakurigaku Zasshi - Folia Pharmacologica Japonica. 119(5):295-300, 2002 May.

AB - Neuronal nicotinic acetylcholine receptors (nAChR) are a family of ligand-gated ion channels that have a pentameric structure composed of five membrane spanning subunits. Recent progress in clinical and neurochemical studies have shown that neuronal nAChR are involved in some psychiatric disorders such as schizophrenia, depression, and anxiety via its stimulating effect of multiple neurotransmitters. It has been suggested that the high prevalence of smoking in the patients with psychiatric disorders is an attempt to alleviate some psychiatric symptoms using the central stimulatory effect of nicotine (a self-medication effort) or to alleviate the exacerbated symptoms by nicotine withdrawal. Moreover, recent studies with mutant mice lacking specific nAChR subunits and animal models of psychiatric disorders have indicated the psychopharmacological role of individual nAChR subunits in psychiatric disorders. Thus, it is suggested that alpha 7 nAChR is involved in the attent!

ion deficit of schizophrenic patients and that alpha 4 beta 2 nAChR is related to nicotine dependence or the withdrawal symptoms. [References: 32]

PT - English Abstract PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Review

<15>

UI - 11803533

ST - MEDLINE

AU - Freedman R
 AU - Leonard S
 AU - Olincy A
 AU - Kaufmann CA
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 FA - Leonard, S
 FA - Olincy, A
 FA - Kaufmann, C A
 FA - Malaspina, D
 FA - Cloninger, C R
 FA - Svrakic, D
 FA - Faraone, S V
 FA - Tsuang, M T

IN - Department of Psychiatry, Denver VA Medical Center and University of Colorado Health Sciences Center, Denver, Colorado 80262, USA.

robert.freedman@uchsc.edu TI - Evidence for the multigenic inheritance of schizophrenia.

SO - American Journal of Medical Genetics. 105(8):794-800, 2001 Dec 8.

AB - Schizophrenia is assumed to have complex inheritance because of its high prevalence and sporadic familial transmission. Findings of linkage on different chromosomes in various studies corroborate this assumption. It is not known whether these findings represent heterogeneous inheritance, in which various ethnic groups inherit illness through different major gene effects, or multigenic inheritance, in which affected individuals inherit several common genetic abnormalities. This study therefore examined inheritance of schizophrenia at different genetic loci in a nationally collected European American and African American sample. Seventy-seven families were previously genotyped at 458 markers for the NIMH Schizophrenia Genetics Initiative. Initial genetic analysis tested a dominant model, with schizophrenia and schizoaffective disorder, depressed type, as the affected phenotype. The families showed one genome-wide significant linkage ($Z = 3.97$) at chromosome 15q14, which !

maps within 1 cM of a previous linkage at the alpha 7-nicotinic receptor gene. Chromosome 10p13 showed suggestive linkage ($Z = 2.40$). Six others (6q21, 9q32, 13q32, 15q24, 17p12, 20q13) were positive, with few differences between the two ethnic groups. The probability of each family transmitting schizophrenia through two genes is greater than expected from the combination of the independent segregation of each gene. Two trait-locus linkage analysis supports a model in which genetic alleles associated with schizophrenia are relatively common in the general population and affected individuals inherit risk for illness through at least two different loci. Copyright 2001 Wiley-Liss, Inc.

PT - Journal Article

<16>

UI - 11803513
 ST - MEDLINE
 AU - Xu J
 AU - Pato MT
 AU - Torre CD
 AU - Medeiros H
 AU - Carvalho C
 AU - Basile VS
 AU - Bauer A
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 FA - Coelho, I
 FA - Ferreira, C P
 FA - Azevedo, M H
 FA - Macciardi, F
 FA - Kennedy, J L
 FA - Pato, C N

IN - Behavioral Health Care Line, VA Western New York Healthcare System and Department of Psychiatry, State University of New York at Buffalo, Buffalo, New York 14214, USA.

TI - Evidence for linkage disequilibrium between the alpha 7-nicotinic receptor gene (CHRNA7) locus and schizophrenia in Azorean families.[see comment].

CM - Comment in: Am J Med Genet. 2001 Dec 8;105(8):655-7; PMID: 11803510

SO - American Journal of Medical Genetics. 105(8):669-74, 2001 Dec 8.

AB - Recent studies have suggested that the alpha 7-nicotinic receptor gene (CHRNA7) may play a role in the pathogenesis of schizophrenia. The alpha 7-nicotinic receptor gene (CHRNA7) is involved in P50 auditory sensory gating deficits, and the genomic locus for this gene lies in the chromosome 15q13-14 regions. The human gene is partially duplicated (exons 5-10) with four novel upstream exons. The marker D15S1360 has been shown to be significantly linked with the phenotype of abnormal P50 suppression in schizophrenia families. The marker L76630 is 3 kb in the 3' direction from the last exon of the CHRNA7 gene and is located in the duplicated region. The function of the two L76630 copies is unknown. We genotyped three polymorphic markers D15S1360, D15S165, and L76630 that are localized in a genomic fragment containing the CHRNA7 in 31 Azorean schizophrenia families/trios (including 41 schizophrenia individuals and 97 unaffected families members). An overall analysis utilizing

ng the family-based association test revealed significant linkage disequilibrium between L76630 and schizophrenia ($P = 0.0004$). Using the extended transmission disequilibrium test and limiting the analysis to one triad per family, transmission disequilibrium of D15S1360 was near significance ($P = 0.078$). The 15q13 region overlaps with the location of two well-known genomically imprinted disorders: Angelman syndrome and Prader-Willi syndrome. Therefore, we investigated maternal and paternal meioses. We found significant transmission disequilibrium for D15S1360 through paternal transmission ($P = 0.0006$) in our schizophrenia families. The L76630 marker showed a significant disequilibrium in maternal transmissions ($P = 0.028$). No parent-of-origin effect was found in D15S165. Overall, our results suggest that the CHRNA7 may play a role in schizophrenia in these families. A parent of origin effect may be present and requires further study.

PT - Journal Article
PT - Research Support, Non-U.S. Gov't
PT - Research Support, U.S. Gov't, P.H.S.

<17>

UI - 11150893
ST - MEDLINE
AU - Lai IC
AU - Hong CJ
AU - Tsai SJ
FA - Lai, I C
FA - Hong, C J
FA - Tsai, S J
IN - Department of Psychiatry, Veterans General Hospital-Taipei, Taipei, Taiwan, ROC.
TI - Association study of a nicotinic receptor variant with schizophrenic disorders.
SO - Neuropsychobiology. 43(1):15-8, 2001 Jan.
AB - Nicotine acetylcholine receptors (nAChRs) are implicated in the pathogenesis of schizophrenia because the prevalence of smoking among schizophrenic patients is extraordinarily high, and nicotine has been demonstrated to improve some psychophysiological dysfunction in schizophrenics. In addition, recent studies have suggested linkage of the alpha(7) nAChR gene region in families of schizophrenics. In a population-based association study, we tested the hypothesis that the allelic variant, with a 2-bp deletion, of the human alpha(7) nAChR gene confers susceptibility to schizophrenic disorders. We genotyped alpha(7) nAChR in 146 patients with schizophrenic disorders and 151 controls. The results showed no significant difference in genotype or allele frequencies between schizophrenic patients and control subjects. This suggests that alpha(7) nAChR 2-bp deletion plays no major role in the pathogenesis of schizophrenic disorders. Other nAChR variants in schizophrenic disorder! s may need further investigation. Copyright 2001 S. Karger AG, Basel PT - Journal Article PT - Research Support, Non-U.S. Gov't

<18>

UI - 10893497
ST - MEDLINE
AU - Riley BP
AU - Makoff A
AU - Mogudi-Carter M
AU - Jenkins T
AU - Williamson R
AU - Collier D
AU - Murray R
FA - Riley, B P
FA - Makoff, A
FA - Mogudi-Carter, M
FA - Jenkins, T
FA - Williamson, R
FA - Collier, D
FA - Murray, R
IN - Departments of Psychological Medicine and Neuroscience, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK. B.Riley@iop.kcl.ac.uk TI - Haplotype transmission disequilibrium and evidence for linkage of the CHR7A7 gene region to schizophrenia in Southern African Bantu families.
SO - American Journal of Medical Genetics. 96(2):196-201, 2000 Apr 3.
AB - Recent reports have strongly linked markers near the alpha-7 nicotinic cholinergic receptor subunit gene on human chromosome 15q13-q14 to a sensory gating deficit common in schizophrenics, and have shown positive though non-significant results linking this region to the primary

phenotype of schizophrenia in a sample of North American families. We therefore tested for linkage between markers in this region of chromosome 15q and schizophrenia in a sample of 15 multiply affected and 5 single case families with schizophrenia drawn from the Bantu-speaking black population of South Africa. An initial replication using markers from the original study gave an affected-only LOD score maximum of 1.08 under a recessive model at $\Theta=0.00$ for D15S1360, a dinucleotide polymorphism found on the same YAC as the alpha-7 receptor gene. Nonparametric affected-only multipoint analysis gave a Z-score of 1.29, $P=0.098$, for D15S1360, and $Z=1.45$, $p=0.075$ for D15S118. We then increased the !

resolution of the map with an extended set of 20 markers. Again, two peaks were observed, with NPL scores of 1.81, $p=0.037$, at D15S1043 and 1.79 at D15S1360 and 1.80 at D15S1010, both $p=0.037$. Transmission disequilibrium testing of data from D15S1360 gave an allele-wise and genotype-wise $\chi^2(2)$ of 6.59, 2 df, $p=0.037$. Haplotype transmission disequilibrium testing using a restricted allele and haplotype set from D15S1043 and D15S1360 gave a global $\chi^2(2)$ of 10.647, 4 df, $P=0.007$, and a maximum $\chi^2(2)$ of 6.567, 1 df, $P=0.004$ for excess transmission of the 1.2 haplotype into affected offspring. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 96:196-201, 2000. Copyright 2000 Wiley-Liss, Inc.

PT - Journal Article

PT - Research Support, Non-U.S. Gov't

<19>

UI - 10771019

ST - MEDLINE

AU - Leonard S

AU - Breese C

AU - Adams C

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AU - Olincy A

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FA - Leonard, S

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FA - Gault, J

FA - Stevens, K

FA - Lee, M

FA - Adler, L

FA - Olincy, A

FA - Ross, R

FA - Freedman, R

IN - Department of Psychiatry, University of Colorado Health Sciences Center, Denver, CO 80262, USA. sherry.leonard@uchsc.edu TI - Smoking and schizophrenia: abnormal nicotinic receptor expression.

SO - European Journal of Pharmacology. 393(1-3):237-42, 2000 Mar 30.

AB - Biological and genetic evidence suggests a role for the neuronal nicotinic receptors in the neuropathophysiology of schizophrenia. Nicotine normalizes an auditory evoked potential deficit seen in subjects who suffer from the disease. Nicotinic receptors with both high and low affinity for nicotine are decreased in postmortem brain of schizophrenics compared to control subjects. The chromosomal locus of the human alpha-7 gene (15q14) is linked to the gating deficit with a lod of 5.3, and antagonists of the alpha-7 receptor (alpha-bungarotoxin and methyllycaconitine) induce a loss of gating in rodents. We have cloned the human alpha-7 gene and found it to

be partially duplicated proximal to the full-length gene. The duplication is expressed in both the brain and in peripheral blood cells of normal subjects, but is missing in some schizophrenic subjects. The results of these studies suggest the presence of abnormal expression and function of the neuronal nicotinic recepto!

r gene family in schizophrenia.

PT - Journal Article

<20>

UI - 10439438

ST - MEDLINE

AU - Agulhon C

AU - Abitbol M

AU - Bertrand D

AU - Malafosse A

FA - Agulhon, C

FA - Abitbol, M

FA - Bertrand, D

FA - Malafosse, A

IN - Departement de Psychiatrie, Hopitaux Universitaires de Geneve (HUG), Chene-Bourg, Switzerland.

TI - Localization of mRNA for CHRNA7 in human fetal brain.

SO - Neuroreport. 10(11):2223-7, 1999 Aug 2.

AB - The aim of this study was to determine the regional distribution in situ of the mRNA for the alpha 7 subunit of the neuronal nicotinic acetylcholine receptor in human fetal brain. We found high levels of alpha 7 gene expression in nuclei that receive sensory information, such as those of the neocortex and hippocampus, the thalamic nuclei, the reticular thalamic nucleus, the pontine nuclei and the superior olive complex. These data support a possible regulatory function for alpha 7-containing receptors in sensory processing, which may be involved in the pathological physiology of schizophrenia and autism. Early alpha 7 gene expression is also consistent with a morphogenetic role for alpha 7 receptors in central nervous system development.

PT - Journal Article

PT - Research Support, Non-U.S. Gov't

<21>

UI - 10394470

ST - MEDLINE

AU - Adler LE

AU - Freedman R

AU - Ross RG

AU - Olincy A

AU - Waldo MC

FA - Adler, L E

FA - Freedman, R

FA - Ross, R G

FA - Olincy, A

FA - Waldo, M C

IN - Department of Psychiatry, University of Colorado Health Sciences Center, Denver 80262, USA.

TI - Elementary phenotypes in the neurobiological and genetic study of schizophrenia. [Review] [139 refs] SO - Biological Psychiatry. 46(1):8-18, 1999 Jul 1.

AB - This review describes the strategy of using elementary phenotypes for neurobiological and genetic linkage studies of schizophrenia. The review concentrates on practical aspects of selecting the phenotype and then understanding the confounds in its measurement and interpretation. Examples from the authors' studies of deficits in P50 inhibition and smooth pursuit eye movement dysfunction are presented. These two phenotypes share

considerable similarity in their neurobiology, including a similar response to nicotine. They also appear to co-segregate with the genetic risk for schizophrenia as autosomal co-dominant phenotypes. Although most schizophrenic patients inherit these abnormalities unilineally, i.e., from one parent, apparent bilineal inheritance produces a more severe illness, observed clinically as childhood-onset schizophrenia. The initial study showing linkage of the P50 deficit to the chromosome 15q14 locus of the alpha 7-nicotinic acetylcholine receptor is an !

example of the potential usefulness of these phenotypes for combined genetic and neurobiological study of schizophrenia. [References: 139] PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Review

<22>

UI - 9789829

ST - MEDLINE

AU - Albuquerque EX

AU - Pereira EF

AU - Braga MF

AU - Alkondon M

FA - Albuquerque, E X

FA - Pereira, E F

FA - Braga, M F

FA - Alkondon, M

IN - Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore 21201, USA.

TI - Contribution of nicotinic receptors to the function of synapses in the central nervous system: the action of choline as a selective agonist of alpha 7 receptors.

SO - Journal of Physiology, Paris. 92(3-4):309-16, 1998 Jun-Aug.

AB - The alpha 7-nicotinic receptor (nAChR)-selective agonist choline and nAChR-subtype-selective antagonists led to the discovery that activation of both alpha 7 and alpha 4 beta 2 nAChRs located in CA1 interneurons in slices taken from the rat hippocampus facilitates the tetrodotoxin (TTX)-sensitive release of gamma-aminobutyric acid (GABA). Experiments carried out in cultured hippocampal neurons not only confirmed that preterminal alpha 7 and alpha 4 beta 2 nAChRs modulate the TTX-sensitive release of GABA, but also demonstrated that evoked release of GABA is reduced by rapid exposure of the neurons to acetylcholine (ACh, 10 microM-1 mM) in the presence of the muscarinic receptor antagonist atropine (1 microM). This effect of ACh, which is fully reversible and concentration-dependent, is partially blocked by superfusion of the cultured neurons with external solution containing either the alpha 7-nAChR-selective antagonist methyllycaconitine (MLA, 1 nM) or the alpha 4 bet!

a 2-nAChR-selective antagonist dihydro-beta-erythroidine (DH beta E, 100 nM). A complete blockade of ACh-induced reduction of evoked release of GABA was achieved only when the neurons were perfused with external solution containing both MLA and DH beta E, suggesting that activation of both alpha 7 and alpha 4 beta 2 nAChRs modulates the evoked release of GABA from hippocampal neurons. Such mechanisms may account for the apparent involvement of nAChRs in the psychological effects of tobacco smoking, in brain disorders (e.g., schizophrenia and epilepsy), and in physiological processes, including cognition and nociception.

PT - In Vitro

PT - Journal Article

PT - Research Support, U.S. Gov't, P.H.S.

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UI - 9012828

ST - MEDLINE

AU - Freedman R

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FA - Adams, C
FA - Patterson, D
FA - Adler, L E
FA - Kruglyak, L
FA - Leonard, S
FA - Byerley, W
IN - Department of Psychiatry, University of Colorado School of Medicine, Denver, USA.
TI - Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus.
SO - Proceedings of the National Academy of Sciences of the United States of America. 94(2):587-92, 1997 Jan 21.
AB - Inheritance of a defect in a neuronal mechanism that regulates response to auditory stimuli was studied in nine families with multiple cases of schizophrenia. The defect, a decrease in the normal inhibition of the P50 auditory-evoked response to the second of paired stimuli, is associated with attentional disturbances in schizophrenia. Decreased P50 inhibition occurs not only in most schizophrenics, but also in many of their nonschizophrenic relatives, in a distribution consistent with inherited vulnerability for the illness. Neurobiological investigations in both humans and animal models indicated that decreased function of the alpha 7-nicotinic cholinergic receptor could underlie the physiological defect. In the present study, a genome-wide linkage analysis, assuming

autosomal dominant transmission, showed that the defect is linked [maximum logarithm of the odds (lod) score = 5.3 with zero recombination] to a dinucleotide polymorphism at chromosome 15q13-14, the site!

of the alpha 7-nicotinic receptor. Despite many schizophrenics' extremely heavy nicotine use, nicotinic receptors were not previously thought to be involved in schizophrenia. The linkage data thus provide unique new evidence that the alpha 7-nicotinic receptor gene may be responsible for the inheritance of a pathophysiological aspect of the illness.

PT - Journal Article

PT - Research Support, Non-U.S. Gov't

PT - Research Support, U.S. Gov't, Non-P.H.S.

PT - Research Support, U.S. Gov't, P.H.S.

<24>

UI - 8873294

ST - MEDLINE

AU - Leonard S

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AU - Griffith JM

AU - Miller C

AU - Myles-Worsley M

AU - Nagamoto HT

AU - Rollins Y

AU - Stevens KE

AU - Waldo M

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FA - Rollins, Y

FA - Stevens, K E

FA - Waldo, M

FA - Freedman, R

IN - Dept. of Pharmacology, University of Colorado Health Sciences Center, Denver 80262, USA.

TI - Nicotinic receptor function in schizophrenia. [Review] [101 refs] SO - Schizophrenia Bulletin. 22(3):431-45, 1996.

AB - Schizophrenia can be partially characterized by deficits in sensory processing. Biochemical, molecular, and genetic studies of one such endophenotype, the P50 auditory-evoked potential gating deficit, suggest that one of the neuronal nicotinic receptors, the alpha 7 nicotinic receptor, may function in an inhibitory neuronal pathway involved in this phenotype. The P50 deficit is normalized in nongating subjects by nicotine. Although most schizophrenia patients are heavy smokers, the effects of nicotine may be transient, as alpha 7 receptors are known to desensitize rapidly. In an animal model of the P50 gating deficit, antagonists of the alpha 7 nicotinic receptor block normal gating of the second of paired auditory stimuli. Regional localization of receptor expression includes

areas known to function in sensory filtering. An inhibitory mechanism, in the hippocampus, may involve nicotinic stimulation of gamma-aminobutyric acid (GABA)ergic interneurons, resulting in decreased

response to repetitive stimuli. Expression of the alpha 7 receptor is decreased in hippocampal brain tissue, dissected postmortem, from schizophrenia subjects. The P50 deficit is inherited in schizophrenia pedigrees, but it is not sufficient for disease development and thus represents a predisposition factor. Linkage analysis between the P50 deficit in multiplex schizophrenia pedigrees and deoxyribonucleic acid (DNA) markers throughout the genome yielded positive lod scores to DNA markers mapping to a region of chromosome 15 containing the alpha 7 nicotinic receptor gene. Elucidation of possible interactions of the P50 with other factors, known to be important in the etiology of the disease, is important in determining an overall pathobiology of schizophrenia. [References: 101] PT - Journal Article PT - Research Support, U.S. Gov't, Non-P.H.S.

PT - Research Support, U.S. Gov't, P.H.S.

PT - Review

Depression or anxiety

<1>

UI - 12061141

ST - MEDLINE

AU - Suemaru K

AU - Araki H

AU - Gomita Y

FA - Suemaru, Katsuya

FA - Araki, Hiroaki

FA - Gomita, Yutaka

IN - Department of Hospital Pharmacy, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. suemaru@cc.okayama-u.ac.jp TI

- [Involvement of neuronal nicotinic receptor in psychiatric disorders].

[Review] [32 refs] [Japanese] SO - Nippon Yakurigaku Zasshi - Folia Pharmacologica Japonica. 119(5):295-300, 2002 May.

AB - Neuronal nicotinic acetylcholine receptors (nAChR) are a family of ligand-gated ion channels that have a pentameric structure composed of five membrane spanning subunits. Recent progress in clinical and neurochemical studies have shown that neuronal nAChR are involved in some psychiatric disorders such as schizophrenia, depression, and anxiety via its stimulating effect of multiple neurotransmitters. It has been suggested that the high prevalence of smoking in the patients with psychiatric disorders is an attempt to alleviate some psychiatric symptoms using the central stimulatory effect of nicotine (a self-medication effort) or to alleviate the exacerbated symptoms by nicotine withdrawal. Moreover, recent studies with mutant mice lacking specific nAChR subunits and animal models of psychiatric disorders have indicated the psychopharmacological role of individual nAChR subunits in psychiatric disorders. Thus, it is suggested that alpha 7 nAChR is involved in the attention!

ion deficit of schizophrenic patients and that alpha 4 beta 2 nAChR is related to nicotine dependence or the withdrawal symptoms. [References: 32]

PT - English Abstract PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Review

<2>

UI - 9702746

ST - MEDLINE

AU - Maggi L

AU - Palma E

AU - Miledi R
 AU - Eusebi F
 FA - Maggi, L
 FA - Palma, E
 FA - Miledi, R
 FA - Eusebi, F
 IN - Centro Ricerca Sperimentale Istituto Regina Elena, Roma, Italy.
 TI - Effects of fluoxetine on wild and mutant neuronal alpha 7 nicotinic receptors.
 SO - Molecular Psychiatry. 3(4):350-5, 1998 Jul.
 AB - Fluoxetine is used in the treatment of a variety of clinical disorders including depression and obesity, and of cocaine detoxification or alcoholism. It is generally believed that fluoxetine exerts its clinical effects because it selectively blocks 5-hydroxytryptamine (5HT) reuptake into nerve terminals. In here we describe that fluoxetine antagonized the neuronal homomeric alpha 7 nicotinic acetylcholine receptors (nAChR) expressed in *Xenopus* oocytes, with an IC50 of 43 microM, when fluoxetine was coapplied with ACh, and of 1.6 microM when the oocytes were pretreated briefly with fluoxetine. A similar block occurred in oocytes expressing L247T alpha 7 mutant nAChR. Furthermore, blockage of mutant alpha 7 receptors appeared non-competitive and was stronger with cell membrane hyperpolarization. Cell-attached single channel recordings in oocytes expressing L247T alpha 7 mutant nAChR showed that the voltage-dependence of the blockage by fluoxetine could be due to a drastic decrease in channel opening frequency accompanied by marked channel flickering and reduced channel conductance. We conclude that fluoxetine behaves as a reversible blocker of both wild and mutant alpha 7 receptors; and that the Leu-247T mutation in the channel domain renders the blockage of alpha 7 nAChR by fluoxetine voltage-dependent. These effects of fluoxetine on alpha 7 receptors may be clinically important.
 PT - Journal Article
 PT - Research Support, Non-U.S. Gov't
 PT - Research Support, U.S. Gov't, P.H.S.

<3>

UI - 9164577
 ST - MEDLINE
 AU - Briggs CA
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 AU - Kim DJ
 AU - Marsh KC
 AU - O'Neill A
 AU - Prendergast MA
 AU - Ryther KB
 AU - Sullivan JP
 AU - Arneric SP
 FA - Briggs, C A
 FA - Anderson, D J
 FA - Brioni, J D
 FA - Buccafusco, J J

FA - Buckley, M J
FA - Campbell, J E
FA - Decker, M W
FA - Donnelly-Roberts, D
FA - Elliott, R L
FA - Gopalakrishnan, M
FA - Holladay, M W
FA - Hui, Y H
FA - Jackson, W J
FA - Kim, D J
FA - Marsh, K C
FA - O'Neill, A
FA - Prendergast, M A
FA - Ryther, K B
FA - Sullivan, J P
FA - Arneric, S P
IN - Abbott Laboratories, Abbott Park, Illinois 60064, USA.
clark.briggs@abbott.com TI - Functional characterization of the novel neuronal nicotinic acetylcholine receptor ligand GTS-21 in vitro and in vivo.
SO - Pharmacology, Biochemistry & Behavior. 57(1-2):231-41, 1997 May-Jun.
AB - (2.4)-Dimethoxybenzylidene anabaseine dihydrochloride (GTS-21), a compound that interacts with rat neuronal nicotinic acetylcholine receptors (nAChRs), was evaluated using human recombinant nAChRs in vitro and various pharmacokinetic and behavioral models in rodents, dogs and monkeys. GTS-21 bound to human alpha 4 beta 2 nAChR (K1-20 nM) 100-fold more potently than to human alpha 7 nAChR, and was 18- and 2-fold less potent than (-)-nicotine at human alpha 4 beta 2 and alpha 7 nAChR, respectively.
Functionally. GTS-21 stimulated [5H]dopamine release from rat striatal slices with an EC50 of 10 +/- 2 microm (250-fold less potent and 70% as efficacious as (-)-nicotine), an effect blocked by the nAChR antagonist dihydro-beta-erythroidine. However, GTS-21 did not stimulate human alpha 4 beta 2 nor human ganglionic nAChRs significantly. In vivo, GTS-21 had no adverse effect on dog blood pressure (< or = 2.5 micromol/kg i.v. bolus infusion), in marked contrast with (-)-nicotin!
e, GTS-21 (-62 micromol/kg.s.e.) also did not cross-discriminate significantly with (-)-nicotine in rats and did not reduce temperature or locomotion in mice. Neither was it active in the elevated plus maze anxiety model (0.19-6.2 micromol/kg.IP) in normal mice. However, GTS-21 did improve learning performance of monkeys in the delayed matching-to-sample task (32-130 nmol/kg.i.m.).
PT - In Vitro
PT - Journal Article

Other therapeutic indications

<1>
UI - 18033152
ST - MEDLINE
AU - Bennouna M
AU - Greene VB
AU - Defranoux L
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FA - Greene, V B
FA - Defranoux, L
IN - Interne de specialite, CHS de Sarreguemines, 3e secteur, 1, rue Calmette, 57206 Sarreguemines, France.
TI - [Cholinergic hypothesis in psychosis following traumatic brain injury and cholinergic hypothesis in schizophrenia: a link?]. [Review] [43 refs] [French]
SO - Encephale. 33(4 Pt 1):616-20, 2007 Sep.

AB - INTRODUCTION: While traumatic brain injury is a major public health issue, schizophrenia-like psychosis following traumatic brain injury is relatively rare and poorly studied. Yet the risk of developing schizophrenia-like psychosis after traumatic brain injury is 3 times more important than in the general population. LITERATURE FINDINGS: Risk factors associated with onset of psychosis after traumatic brain injury include: left hemispheric lesions, closed head injury and coma of duration superior to 24 hours. Most patients develop symptoms of psychosis after a moderate to severe traumatic brain injury and often have lesions of the frontal and temporal lobes. CHOLINERGIC HYPOTHESIS: ARGUMENTS: Neuropathologic, electrophysiological and pharmacologic evidence show that cognitive impairment including attention, memory and executive functioning impairment may be related with cholinergic dysfunction in patients with traumatic brain injury. The cholinergic hypothesis is also i!

ncriminated in the genesis of schizophrenia. The same biochemical disorders found in schizophrenia which imply many neurotransmitters are often present immediately after traumatic brain injury. However in chronic cognitive disorders secondary to traumatic brain injury, the cholinergic system alone seems to be specifically implied. This is due to the fragility of the cholinergic fibres and a chronic yet reversible reduction of the cholinergic reserves after traumatic brain injury. Cholinergic function can be studied by the P50 evoked response to paired auditory stimuli. While this is disturbed in patients presenting with cognitive impairment after traumatic brain injury its normalisation can be obtained after administration of an acetylcholine esterase inhibitor. In schizophrenic patients there is also an abnormal P50 evoked response due in part to a low number of alpha 7 nicotinic receptors which are implicated in sensory filtering in the frontal lobe. Moreover in schizophre!

nia, post-mortem studies show a negative correlation between the activity of acetylcholine transferase in the parietal cortex and the severity of the cognitive deficits, as well as a lesser density of the muscarinic M1 and M4 receptors in the frontal lobe. The lower concentration of M1 receptors in the frontal cortex is correlated with the severity of the positive symptoms. THERAPEUTICAL PERSPECTIVES: Antipsychotics have emerged as the first line treatment of psychotic disorders. In research, their ability for enhancing cognitive function could result in the increase of acetylcholine in the medial prefrontal cortex. Acetylcholinesterase inhibitors have been widely used for treatment of cognitive impairment in Alzheimer's disease. Galantamine could be interesting in schizophrenia and psychosis following traumatic brain injury because it has a dual mechanism of action: selective competitive inhibition of acetylcholinesterase and allosteric potentialisation of nicotinic receptor response. Therefore Galantamine remains active in nicotine addicted !

schizophrenic patients who may smoke as an auto treatment. Galantamine has shown efficacy in adjunction to Risperidone in one patient presenting with psychosis following traumatic brain injury and in 3 case reports of schizophrenic patients. CONCLUSION: Further systematic studies are needed to confirm this hypothesis. [References: 43] PT - English Abstract PT - Journal Article PT - Review

<2>

UI - 12436426

ST - MEDLINE

AU - Levin ED

FA - Levin, Edward D

IN - Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Neurobehavioral Research Laboratory, Box 3412, Durham, North Carolina 27710, USA. edlevin@duke.edu TI - Nicotinic receptor subtypes and cognitive function. [Review] [60 refs] SO - Journal of Neurobiology. 53(4):633-40, 2002 Dec.

AB - Nicotinic receptor systems are involved in a wide variety of behavioral functions including cognitive function. Nicotinic medications may provide beneficial treatment for cognitive dysfunction such as Alzheimer's disease, schizophrenia, and attention deficit hyperactivity disorder (ADHD). Nicotine has been shown to improve attentional performance in all of these disorders. Better efficacy with fewer side effects might be achieved with novel nicotinic ligands selective for particular nicotinic subtypes. To develop these novel selective nicotinic ligands it is important to use animal models to determine the critical neurobehavioral bases for nicotinic involvement in cognitive function. Nicotine-induced cognitive improvement in rats is most consistently seen in working memory tasks. We have found that both acute and chronic nicotine administration significantly improves working memory performance of rats in the radial-arm maze. The pharmacologic and anatomic mechanisms fo!

r this effect have been examined in our laboratory in a series of local drug infusion studies. Both alpha 4 beta 2 and alpha 7 nicotinic receptors in the ventral hippocampus and basolateral amygdala are involved in working memory function. Working memory impairments were caused by local infusion of either alpha 4 beta 2 or alpha 7 antagonists. Ventral hippocampal alpha 4 beta 2 blockade-induced working memory deficits are reversed by chronic systemic nicotine treatment, while ventral hippocampal alpha 7 blockade-induced working memory deficits were not found to be reversed by the same nicotine regimen. Interestingly, alpha 4 beta 2 and alpha 7 induced deficits were not found to be additive in either the ventral hippocampus or the basolateral amygdala. In fact, in the amygdala, alpha 7 antagonist cotreatment actually reversed the working memory impairment caused by alpha 4 beta 2 antagonist administration. These studies of the neural nicotinic mechanisms underlying cognitive!

function are key for opening avenues for development of safe and effective nicotinic treatments for cognitive dysfunction. Copyright 2002 Wiley Periodicals, Inc. [References: 60] PT - Journal Article PT - Research Support, U.S. Gov't, P.H.S.
PT - Review

<3>

UI - 12061141

ST - MEDLINE

AU - Suemaru K

AU - Araki H

AU - Gomita Y

FA - Suemaru, Katsuya

FA - Araki, Hiroaki

FA - Gomita, Yutaka

IN - Department of Hospital Pharmacy, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. suemaru@cc.okayama-u.ac.jp TI - [Involvement of neuronal nicotinic receptor in psychiatric disorders]. [Review] [32 refs] [Japanese] SO - Nippon Yakurigaku Zasshi - Folia Pharmacologica Japonica. 119(5):295-300, 2002 May.

AB - Neuronal nicotinic acetylcholine receptors (nAChR) are a family of ligand-gated ion channels that have a pentameric structure composed of five membrane spanning subunits. Recent progress in clinical and neurochemical studies have shown that neuronal nAChR are involved in some psychiatric disorders such as schizophrenia, depression, and anxiety via its stimulating effect of multiple neurotransmitters. It has been suggested that the high prevalence of smoking in the patients with psychiatric disorders is an attempt to alleviate some psychiatric symptoms using the central stimulatory effect of nicotine (a self-medication effort) or to alleviate the exacerbated symptoms by nicotine withdrawal. Moreover, recent studies with mutant mice lacking specific nAChR subunits and animal models of psychiatric disorders have indicated the psychopharmacological role of

individual nAChR subunits in psychiatric disorders. Thus, it is suggested that alpha 7 nAChR is involved in the attention!

ion deficit of schizophrenic patients and that alpha 4 beta 2 nAChR is related to nicotine dependence or the withdrawal symptoms. [References: 32]
 PT - English Abstract PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Review

<4>

UI - 11796154
 ST - MEDLINE
 AU - Leonard S
 AU - Adler LE
 AU - Benhammou K
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 AU - Olincy A
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 AU - Vianzon R
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 AU - Walton K
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 FA - Ross, R G
 FA - Stevens, K
 FA - Sullivan, B
 FA - Vianzon, R
 FA - Virnich, D E
 FA - Waldo, M
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IN - Department of Psychiatry, University of Colorado Health Sciences Center, Box C-268-71, 4200 East 9th Avenue, Denver, CO 80262, USA.
 sherry.leonard@uchsc.edu TI - Smoking and mental illness. [Review] [140 refs] SO - Pharmacology, Biochemistry & Behavior. 70(4):561-70, 2001 Dec.
 AB - Patients with mental illness have a higher incidence of smoking than the general population and are the major consumers of tobacco products. This population includes subjects with schizophrenia, manic depression, depression, posttraumatic stress disorder (PTSD), attention-deficit disorder (ADD), and several other less common diseases. Smoking cessation treatment in this group of patients is difficult, often leading to profound depression. Several recent findings suggest that increased smoking in the mentally ill may have an underlying biological etiology. The mental illness schizophrenia has been most thoroughly studied in this regard. Nicotine administration normalizes several sensory-processing deficits seen in this disease. Animal models of sensory deficits have been used to identify

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specific nicotinic receptor subunits that are involved in these brain pathways, indicating that the alpha 7 nicotinic receptor subunit may play a role. Genetic linkage in schizophrenic !

families also supports a role for the alpha 7 subunit with linkage at the alpha 7 locus on chromosome 15. Bipolar disorder has some phenotypes in common with schizophrenia and also exhibits genetic linkage to the alpha 7 locus, suggesting that these two disorders may share a gene defect. The alpha 7 receptor is decreased in expression in schizophrenia. [(3)H]-Nicotine binding studies in postmortem brain indicate that high-affinity nicotinic receptors may also be affected in schizophrenia. [References: 140] PT - Journal Article PT - Review

<5>

UI - 11796145

ST - MEDLINE

AU - Tribollet E

AU - Bertrand D

AU - Raggenbass M

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FA - Bertrand, D

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IN - Department of Physiology, University Medical Center, 1, rue Michel-Servet, CH-1211 Geneva 4, Switzerland.

TI - Role of neuronal nicotinic receptors in the transmission and processing of information in neurons of the central nervous system.

[Review] [64 refs] SO - Pharmacology, Biochemistry & Behavior. 70(4):457-66, 2001 Dec.

AB - The properties of nicotinic acetylcholine receptors (nAChRs) were studied following exogenous expression in a host system or using whole-cell recordings in brain slices, autoradiography and immunohistochemistry. When expressed in HEK-293 cells, alpha 4 beta 2 nAChRs displayed both a high and a low affinity component. The ratio of these two states was modified by chronic nicotine exposure, resulting in an enhanced sensitivity and a marked reduction in desensitization. Mutations in the gene coding for the alpha 4 subunit are responsible for a particular form of nocturnal epilepsy. When expressed in *Xenopus* oocytes, alpha 4 beta 2 nAChRs containing these mutations displayed distinct alterations in agonist affinity, desensitization and calcium permeability. Magnocellular endocrine neurons in the supraoptic (SO) nucleus of the hypothalamus were found to express functional alpha 7-containing nAChRs, which could play a role in regulating neurohypophysial peptide secretion. Fa!

cial (VII), hypoglossal (XII) and vagal (X) motoneurons of young rats responded to ACh by a fast inward current. The nAChRs present in VII and XII nuclei were of the non-alpha 7-containing type, whereas those present in the X nucleus contained the alpha 7 subunit. In Bcl-2 transgenic mice, facial nerve axotomy caused nAChRs downregulation by interfering negatively with the expression of the alpha 4 subunit. Binding sites corresponding to alpha 7-containing nAChRs were also detected in spinal motor nuclei and axotomy provoked a reduction of the binding. Together, these data indicate that long-term exposure to nicotine can promote neuroadaptive changes in nAChRs and that genetic alterations of neuronal nAChRs can result in transmissible neurological diseases. They also suggest that these receptors probably play a role in the central regulation of autonomic functions, as well as in motor control. [References: 64] PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Review

<6>

UI - 11233293

ST - MEDLINE

AU - Miyasato K

FA - Miyasato, K

IN - Department of Psychiatry, Saint Marianna University School of Medicine, 2-16-1, Sugao, Miyamae-ku, Kawasaki 216-8511, Japan. miyasato@marianna-u.ac.jp TI - [Recent advances in research on nicotine dependence and reward mechanism]. [Review] [41 refs] [Japanese] SO - Nippon Yakurigaku Zasshi - Folia Pharmacologica Japonica. 117(1):27-34, 2001 Jan.

AB - Tobacco/nicotine dependence involves both psychological and physiologic dependence on nicotine. A recent summary of the clinical aspects of nicotine dependence disorders is provided in the ICD-10 of the WHO. There is growing evidence suggesting that nicotine depends on dopamine (DA) for its reinforcing effects. Suppression of DA function using DA-receptor antagonist affects the intravenous self-administration of nicotine, the discriminative properties of nicotine, nicotine-induced intracranial self-stimulation and nicotine-conditioned place preference. The increase in extracellular DA in the nucleus accumbens (NAcc) is a major factor that mediates the self-administration of nicotine. Recently, alpha 7-nicotinic receptors in the ventral tegmental area (VTA) contribute to the acute effects of nicotine on the mesolimbic DA system, reinforcing effects of nicotine and withdrawal symptoms from nicotine. The action of glutamate (Glu) at N-methyl-D-aspartate (NMDA)-sensitive re-

ceptors within the VTA is required for nicotine to stimulate DA release in the NAcc. Molecular epidemiological studies have shown that smoking behavior is more strongly influenced by a combination of the serotonin transporter gene and the neuroticism than by either factor alone.

Clinically, bupropion, an antidepressant, has been licensed for use in USA to help patients stop smoking. The underlying mechanism is thought to be mediated by increasing the concentration of DA in the NAcc. [References: 41] PT - English Abstract PT - Journal Article PT - Review

<7>

UI - 9621396
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AU - O'Hara BF
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AU - Wiler SW
AU - Heller HC
AU - Kilduff TS
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FA - Edgar, D M
FA - Cao, V H
FA - Wiler, S W
FA - Heller, H C
FA - Kilduff, T S
FA - Miller, J D

IN - Department of Biological Sciences, Stanford University, CA 94305-5020, USA. bfo@leland.stanford.edu TI - Nicotine and nicotinic receptors in the circadian system. [Review] [92 refs] SO - Psychoneuroendocrinology. 23(2):161-73, 1998 Feb.

AB - Considerable data support a role for cholinergic influences on the circadian system. The extent to which these influences are mediated by nicotinic acetylcholine receptors (nAChRs) has been controversial, as have the specific actions of nicotine and acetylcholine in the suprachiasmatic nucleus (SCN) of the hypothalamus. In this article we review the existing literature and present new data supporting an important role for nAChRs in both the developing and adult SCN. Specifically, we present data showing that nicotine is capable of causing phase shifts in the circadian rhythms of rats. Like light and carbachol, nicotine appears to cause phase delays in the early subjective night and phase advances in the late subjective night. In the isolated SCN slice, however, only phase advances are seen,

and, surprisingly, nicotine appears to cause the inhibition rather than the excitation of neurons. Among nAChR subunit mRNAs, alpha 7 appears to be the most abundant subunit in the !

adult SCN, whereas in the perinatal period, the more typical nAChRs with higher affinity for nicotine predominate in the SCN. This developmental change in subunit expression may explain the dramatic sensitivity of the perinatal SCN to nicotine that we have previously observed. The effects of nicotine on the SCN may contribute to alterations caused by nicotine in other physiological systems. These effects might also contribute to the dependence properties of nicotine through influences on arousal.

[References: 92] PT - Journal Article PT - Research Support, Non-U.S. Gov't PT - Research Support, U.S. Gov't, P.H.S.

PT - Review

<8>

UI - 9160116

ST - MEDLINE

AU - Colquhoun LM

AU - Patrick JW

FA - Colquhoun, L M

FA - Patrick, J W

IN - Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030, USA.

TI - Pharmacology of neuronal nicotinic acetylcholine receptor subtypes.

[Review] [150 refs] SO - Advances in Pharmacology. 39:191-220, 1997.

AB - The search for the physiological function of nicotinic receptors on neurons in the brain began with their discovery. It was initially assumed that, as in ganglia and at the neuromuscular junction, nicotinic receptors would gate fast synaptic transmission in the brain. The best functional evidence now, however, points to a role in modifying the release of other transmitters. This does not preclude a postsynaptic role in transmission for nicotinic receptors in the brain, but attempts to locate such a synapse have not been successful. If fast nicotinic synapses are present in the brain, they are probably low in number and may be masked by other more prevalent synapses (such as glutamatergic) so identification will not be easy. The extent of diversity of nicotinic receptors is substantial. At the molecular level this is reflected in the number of different genes that encode receptor subunits and the multiple possible combinations of subunits that function in expression sys!

tems. From the cellular level there is a broad diversity of properties of native receptors in neurons. Some useful pharmacological tools allow the limited identification of subunits in native receptors. For example, block by alpha-bungarotoxin identifies alpha 7, alpha 8, or alpha 9 subunits; activation of a receptor by cytisine indicates an alpha 7 or beta 4 subunit; and neuronal bungarotoxin block identifies a beta 2 subunit. Despite the clues to identity gained by careful use of these agents, we have not been able to identify all the components of any native receptor based on pharmacological properties assessed from expression studies. When both pharmacological and biophysical properties of a receptor are taken into consideration, none of the combinations tested in oocytes mimics native receptors exactly. The reason for this discrepancy has been debated at length; it is possible that oocytes do not faithfully manufacture neuronal nicotinic receptors. For example, they ma!

y not correctly modify the protein after translation or they may allow a combination of subunits that do not occur in vivo. Another possibility is that correct combinations of subunits have not yet been tested in oocytes. Data from immunoprecipitation experiments suggest that many receptors contain three or more different subunits. Results from further experiments injecting combinations of three or more subunits into oocytes may be enlightening. The diversity of receptors may allow targeting of subtypes to specific locations. Nicotinic receptors are located presynaptically,

preterminally, and on the cell soma. The function of the nicotinic receptors located on innervating axons is presumably to modify the release of other neurotransmitters. It is an attractive hypothesis that nicotinic receptors might be involved in modifying the weight of central synapses; however, in none of the regions where this phenomenon has been described is there any evidence for axoaxonal contacts. The presynaptic receptors described so far are pharmacologically unique!

e; therefore, if there are different subtypes of nicotinic receptors modifying the release of different transmitters, they may provide a means of exogenously modifying the release of a particular transmitter with drugs. There are still many basic unanswered questions about nicotinic receptors in the brain. What are the compositions of native nicotinic receptors? What is their purpose on neurons? Although there is clearly a role presynaptically, what is the function of those located on the soma?

Neuronal nicotinic receptors are highly permeable to calcium, unlike muscle nicotinic receptors, and this may have important implications for roles in synaptic plasticity and development. Finally, why is there such diversity?

(ABSTRACT TRUNCATED) [References: 150] PT - Journal Article PT - Research Support, U.S. Gov't, P.H.S.

PT - Review

Nicotinic–glutamatergic interactions and attentional performance on an operant visual signal detection task in female rats

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Abstract

Nicotinic systems have been shown to be critically involved in cognitive function including attention. Nicotine has been shown to improve performance on attentional tasks in humans with Alzheimer's disease, schizophrenia and attention deficit hyperactivity disorder. Nicotine has mixed effects on attentional accuracy in unimpaired rats with findings of increased, reduced or unaltered accuracy under different conditions. Nicotine effects on attentional function in rats might be more clearly seen in reversing impaired performance. The current study determined nicotine effects on attentional accuracy reduced by the NMDA receptor antagonist dizocilpine (MK-801). Sprague–Dawley rats ($N=35$) were trained on a food-motivated two-lever operant task with one lever correct after a brief visual signal (0.027–1.22 lx) for hits and the other lever correct after the absence of a signal for correct rejections. First, a dose response study of dizocilpine was conducted to determine the threshold for impairment. The rats were administered acute doses of dizocilpine (0, 12.5, 25 and 50 $\mu\text{g/kg}$, sc). The 50 $\mu\text{g/kg}$ dose caused significant ($p<0.0005$) reduction in percent hit at the four highest signal intensities. Percent correct rejection was also significantly lowered by this dose ($p<0.005$). No effect was seen with 12.5 $\mu\text{g/kg}$ and only minimal effect seen with 25 $\mu\text{g/kg}$. Then, nicotine–dizocilpine interactions were investigated. The rats were administered acute doses of dizocilpine (0, 37.5 and 50 $\mu\text{g/kg}$, sc) and nicotine (0, 25 and 50 $\mu\text{g/kg}$, sc), alone or in combination. Percent hit was affected by nicotine and dizocilpine in a complex fashion with only the nicotine $\times$ dizocilpine $\times$ signal intensity interaction being significant ($p<0.05$). Percent correct rejection showed a more straightforward effect. Percent correct rejection was significantly reduced by 50 $\mu\text{g/kg}$ dizocilpine ($p<0.025$). The addition of 25 $\mu\text{g/kg}$ of nicotine significantly ($p<0.025$) reversed the dizocilpine-induced reduction of correct rejection. This study shows that dizocilpine reduces signal detection accuracy in a dose-dependent fashion. Nicotine can partially counteract an aspect of this reduction by reversing the dizocilpine-induced reduction of correct rejection.

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Keywords: Dizocilpine; MK-801; NMDA receptor; Nicotine; Attention; Cognition

1. Introduction

Neuronal nicotinic systems play an important role in memory and attentional functions. In humans, the most prominent effect of the cholinergic agonist nicotine on cognitive function is improved attention (Levin et al., 2001, 1998b, 1996b; Peeke and Peeke, 1984; Warburton and Mancuso, 1998; Warburton et al., 1992). Clinical studies using nicotine via other means than tobacco, such as skin patches and other nicotinic agonists, have demonstrated the efficacy in improving attentional impairments

associated with Alzheimer's disease (Lawrence and Sahakian, 1995; Newhouse et al., 1997; Sahakian et al., 1989; White and Levin, 1999), attention deficit hyperactivity disorder (Levin et al., 2001, 1996b; Wilens et al., 1999) and schizophrenia (Levin et al., 1996a).

Nicotinic compounds have been shown to improve attention and reduce distractibility in rodents (Mirza and Bright, 2001; Mirza and Stoleran, 1998; Muir et al., 1995; Rezvani et al., 2002; Stoleran et al., 2000) and nonhuman primate models (Terry et al., 2002) as well. However, nicotine-induced improvement has been more difficult to detect in rodents than in humans. Nicotinic antagonist-induced impairments in attention have been readily documented in the operant signal detection task; however, nicotinic agonist-induced improvements of attention have been more elusive

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with some studies finding nicotinic-induced improvements (McGaughy et al., 1999; Rezvani et al., 2002) and others not (Bushnell et al., 1997; Turchi et al., 1995).

It may be the case that nicotinic agonist-induced improvement is most evident in reversing impaired attentional performance. Attention may be difficult to improve in the signal detection task because with extensive training, subjects may already be performing at their best possible levels. Drug-induced attentional impairment may be a useful model for examining nicotine effects because not only do they provide compromised performance against which nicotine may act, but also because the specific receptor actions of those drugs can lend insight into the mechanisms of action of nicotinic treatment. Recently, Terry et al. (2002) found that the nicotinic agonist SIB-1553A significantly improved performance of rats on a five-choice attentional task in rats, but only when accuracy was reduced either with a distracting stimulus or by challenge with the NMDA-sensitive glutamate receptor antagonist dizocilpine (MK-801). In the current study, we sought to determine nicotinic–NMDA interactions in another test of attentional function, the operant visual signal detection task. The operant visual signal detection is a useful technique in which both sensory function and attention can be simultaneously measured (Bushnell et al., 1997). Previously, we have found nicotine-induced improvements on this task in both low and high performing rats, demonstrating that poor performance per se is not a requirement for showing nicotine-induced attentional improvement (Rezvani et al., 2002). Specific interactions of nicotinic systems with other neurotransmitter receptor systems may be important with regard to nicotinic actions on attentional function.

MK-801 is a dissociative anesthetic that, at pharmacologically relevant doses, acts as noncompetitive of the NMDA receptor by binding to a site in the channel (Tsai and Coyle, 2002). There is convincing evidence that hypofunction of NMDA receptors plays a primary role in pathophysiology of schizophrenia (Tsai and Coyle, 2002). The aims of the present study were to determine the role of NMDA–glutamate systems in attentional function using the visual signal detection task and to find out if nicotine can counteract the disruptive effects of MK-801 on attention. First, a dose response function for MK-801 was established. Then, rats were injected with different doses of MK-801 and nicotine and their performance in the visual signal detection task was assessed. We hypothesized that acute nicotine administration will diminish the disruptive action of MK-801 in rats on attention on this task.

2. Materials and methods

2.1. Animals

Adult (70 days old at the beginning of training) female Sprague–Dawley rats ($n=35$) (Taconic Farms, German-

town, NY, USA) were used. Animals were housed in groups of three in a vivarium with 12L:12D light schedule (light on at 7:00 p.m.). Room temperature was maintained at 21 ± 1 °C and relative humidity controlled at $50\% \pm 10\%$. All training and testing sessions were performed during the dark phase of the circadian cycle, between 9:00 a.m. and 4:00 p.m. Rats were fed once daily after testing such that their body weights were kept at 80–85% of free feeding values. Rats had ad libitum access to water in their home cage. The treatment and care of the animals was carried out under an approved protocol of the Animal Care and Use Committee of Duke University in an approved facility. These rats had previously been tested for nicotine and mecamylamine response on the signal detection task (Rezvani et al., 2002).

2.2. Experimental protocol

There were two sets of experiments in the current study to characterize the effects of the NMDA receptor antagonist dizocilpine (MK-801) on attentional performance in an operant signal detection task and the interaction of dizocilpine with nicotine. In Exp. 1 the dizocilpine dose effect function was determined. In Exp. 2 nicotine interactions with an effective and a threshold dose of dizocilpine were determined.

2.2.1. Exp. 1: Dizocilpine dose effect function

Animals were injected subcutaneously with saline or one of the three doses of dizocilpine (12.5, 25 and 50 μ /kg) and 10 min later were transferred in operant boxes for testing. The interval between different doses of dizocilpine was 2–4 days. All rats received all treatments following a counter-balanced design.

2.2.2. Exp. 2: Nicotine interactions with dizocilpine

To determine nicotine interactions with dizocilpine, we studied nicotine dose effect functions with two dizocilpine doses, one high clearly suprathreshold for causing impairment (50 μ /kg) and one lower near the threshold dose for causing impairment (37.5 μ /kg). The high dizocilpine dose from Exp. 1 (0 or 50 μ /kg) and nicotine (0, 25 or 50 μ /kg) were administered alone or together. To study the interaction between a threshold dose of dizocilpine and nicotine, the rats were injected with 0 or 37.5 μ /kg dizocilpine and nicotine (0, 12.5, 25 and 50 μ /kg), alone or together. The 12.5 μ /kg nicotine dose was only run with the lower dizocilpine and did not have any interactive effect. Because it was not run also with the higher dizocilpine dose, it was not included in the overall analysis of the dizocilpine–nicotine interactions. The rats began testing 10 min after drug injection. All rats received all treatments following a counterbalanced design. The interval between different treatments was 2–4 days.

2.3. Drug preparation and dosing

Both nicotine ditartrate and dizocilpine HCl (MK-801) (Sigma, St. Louis, MO, USA) were prepared in 0.09% saline solution. Both drugs were injected subcutaneously 10 min before testing in a volume of 2 ml/kg body weight in a counterbalanced order within each experiment. There was at least a 2 day-interval between experiments. All doses of nicotine and dizocilpine were calculated as the weight of the salt.

2.4. Visual signal detection task

The operant chambers $29 \times 25 \times 29$ cm (HWD) were equipped with a signal light, a house light, two retractable levers, 13 cm apart, 2.5 cm above the floor of the chamber inserted 2.5 cm horizontally into the chamber, a food cup in the center of the front panel of the chamber, 2.2 cm above the floor (Coulbourn Instruments, Lehigh Valley, PA, USA), and a white noise amplifier mounted above the signal lever generating background white noise of about 65 dB (Med Associates, Georgia, VT, USA). During training the signal or cue light was located above the designated lever and as the rats advanced in learning the task the signal light was moved to above the food cup at the center of the front panel, 28 cm above the floor of the chamber. A signal consisted of 500-ms increase in the brightness of the signal light, to levels of 1–7 (0.027, 0.073, 0.148, 0.269, 0.466, 0.762 and 1.22 lx) above a background illumination of 1.2 lx. Signals were generated using Med Associates software running a Pentium computer processor using the Windows operating system. The same software controlled all aspects of the behavioral testing.

Rats were trained to perform a visual signal detection task (Bushnell, 1998; Bushnell et al., 1997). The task, illustrated in Fig. 1, was conducted in daily 300-trial sessions, divided into three 100-trial blocks, each block approximately 20 min in duration. Two trial types, “signal”

and “blank”, were presented in equal number in each session, in groups of four (two signal and two blank, in random order) at each signal intensity. Each signal trial included a pre-signal interval, the signal (cue light) and a post-signal interval. The pre-signal intervals were selected randomly from 12 different values ranging from 0.3 to 24.4 s. Following the signal, a post-signal interval of 2, 3 or 4 s (selected randomly) occurred. These temporal parameters yield a trial presentation rate of five trials per minute. Blank trials were presented identically, except the signal light was not present. For half the rats, the left lever was defined as the signal lever and the right lever as the blank lever; the opposite assignment was made for the remaining rats.

A trial began with both levers retracted from the chamber; then both levers were inserted into the chamber simultaneously at the end of the post-signal interval. The levers were both retracted when one was pressed or if 5 s passed without a press. If no press occurred, a response failure was recorded and the trial was not repeated. Every correct response (a press on the signal lever in a signal trial or a press on the blank lever in a blank trial) was followed by the illumination of the food cup and delivery of one 20-mg food pellet. After each incorrect response (i.e. a press on the signal lever in a blank trial or a press on the blank lever in a signal trial), or response failure, the rat received a 2-s period of total darkness (time out) (Bushnell et al., 1997).

2.5. Behavioral measures and statistical analysis

Following dependent variables were recorded for each signal intensity across the three blocks of trials in each session: hits (signal-level presses on signal trials), correct rejections (blank-lever presses on blank trials), misses (blank-lever presses on signal trials) and false alarm (signal-lever presses on blank trials).

The analysis of percent hit also included signal light intensity as a within-subjects factor. The test session was divided into three blocks of 100 trials and measures of response accuracy were analyzed across blocks as a repeated measure. The threshold for significance was $p < 0.05$. The Huynh–Feldt correction was used to correct for non-sphericity of variance across the repeated measures. The Superanova/Statview computer program (SAS, Cary, NC) was used for the statistical analysis. Planned comparisons were made to test for non-additivity of nicotine and dizocilpine effects. Significant interactions were followed up by tests of the simple main effects.

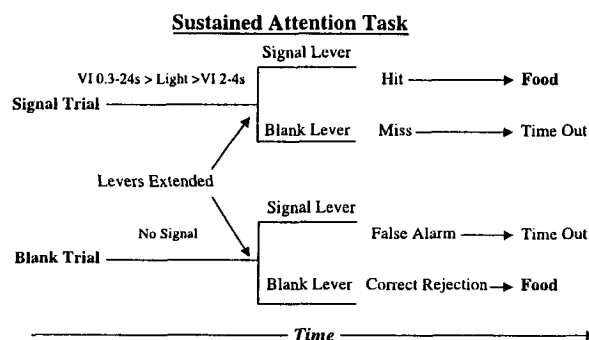


Fig. 1. Signal detection task sequence of trials. This task comprised two types of trials, signal and blank, which differed only in that a signal was presented in each signal trial and omitted in blank trials. In each trial, the rat pressed either of two retractable levers to report that a signal had or had not occurred in that trial. Four possible outcomes result: hit, miss, false alarm and correct rejection. Hits and correct rejections were followed by delivery of food; misses and false alarms by a 2-s “time out” period without food. VI stands for variable intervals for pre- and post-signal during the signal trial.

3. Results

3.1. Exp. 1: Dizocilpine dose effect function

Compared with control saline, dizocilpine at the high dose of 50 $\mu\text{g/kg}$ caused a significant impairment in percent hit response except at lowest intensity in which it caused a

significant increase in percent hit. There was a significant dizocilpine $\times$ session time block interaction ($F(6,168)=4.13, p<0.005$). As shown in Fig. 2, follow-up tests of the simple main effects demonstrated that there were significant reductions in percent hit caused by the 50 $\mu\text{g/kg}$ dizocilpine dose relative to control injections during the middle ($p<0.001$) and late ($p<0.0005$) time blocks of the session.

There was also a significant dizocilpine $\times$ signal intensity interaction ($F(18,504)=5.39, p<0.0001$). As shown in Fig. 3, the 50 $\mu\text{g/kg}$ dizocilpine dose caused significant ($p<0.0005$) reductions in percent hit relative to control injections for levels 4–7 of signal intensities. At level 3 of signal intensity, the 25 $\mu\text{g/kg}$ dizocilpine dose caused a significant ($p<0.05$) reduction in percent hit relative to control. With the lowest signal intensity, the 50 $\mu\text{g/kg}$ dizocilpine dose caused a significant increase in percent hit ($p<0.01$). The three-way interaction of dizocilpine $\times$ session time block $\times$ signal intensity was also significant ($F(36,1008)=1.50, p<0.05$). As shown in Fig. 4, at the levels 4–7 of signal intensities, the 50 $\mu\text{g/kg}$ dizocilpine dose caused significant ($p<0.005$) reduction in hit percent relative to control vehicle during the middle and late parts of the session. No effect was detected at these signal intensities during the initial part of the session. At the two lowest signal intensities during the middle portion of the session, significant dizocilpine-induced increases in hit percent were seen. The 50 $\mu\text{g/kg}$ dizocilpine dose significantly ($p<0.05$) increased percent hit at the lowest signal intensity during the middle portion of the test. The 12.5 $\mu\text{g/kg}$ dizocilpine dose significantly ($p<0.05$) increased percent hit at the level 2 of signal intensity during the middle portion of the test. Interestingly, at the level 3 of signal intensity, the 50 $\mu\text{g/kg}$ dizocilpine dose significantly ($p<0.05$) increased percent hit in the first session block and significantly ($p<0.05$) reduced percent hit in the last session block. This alteration in the valence of the dizocilpine effect relative to control was mainly due to the great change in performance from the

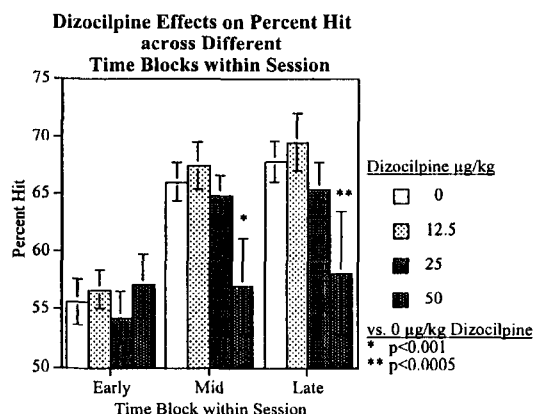


Fig. 2. Effects of different doses of dizocilpine on percent hit (mean $\pm$ S.E.M.) during the early, mid and late parts of the test session. $N=35$.

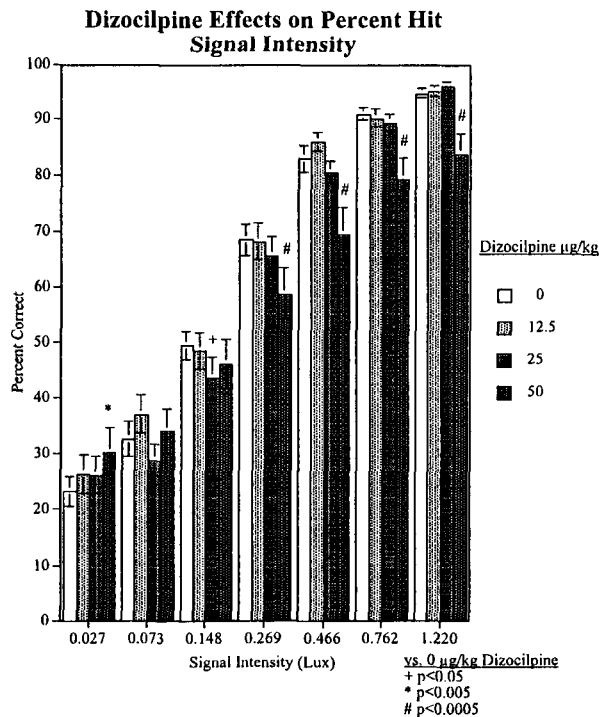


Fig. 3. Effects of different doses of dizocilpine on percent hit (mean $\pm$ S.E.M.) over the range of different signal intensities. $N=35$.

first to the third session block. In the first session block with control injections the rats had 35.7 ± 4.6 (mean $\pm$ S.E.M.) percent hit while with 50 $\mu\text{g/kg}$ dizocilpine they had 45.0 ± 4.4 percent hit. In contrast, in the third session block the rats had 61.1 ± 3.5 percent hit, while with 50 $\mu\text{g/kg}$ dizocilpine they had 50.3 ± 6.3 percent hit. In all cases, significant dizocilpine effects were consistently in the direction of random responding (50%).

Percent correct rejection was significantly lowered by the high dose of dizocilpine (Fig. 5). The main effect of dizocilpine treatment was significant ($F(3,84)=8.88, p<0.005$). Comparisons of the different dizocilpine doses vs. control showed that the 50 $\mu\text{g/kg}$ dose significantly ($p<0.005$) lowered percent correct rejection from $85.6 \pm 2.0\%$ after control treatment to $75.3 \pm 3.8\%$ after 50 $\mu\text{g/kg}$ dizocilpine. There was also a significant dizocilpine $\times$ session time block interaction ($F(6,168)=5.50, p<0.005$). As shown in Fig. 5, there were significant reductions in percent correct rejection with 50 $\mu\text{g/kg}$ dizocilpine relative to control in each session block, but the effect was smaller in the first session block ($p<0.05$) than in the second and third blocks ($p<0.0005$). Unlike with percent hit control performance did not appear to change over blocks within the session.

Response latency was significantly ($F(3,96)=4.48, p<0.001$) increased by dizocilpine (Fig. 6). Across the dose range tested, there was a significant increase in latency ($p<0.025$) with the highest dose of 50 $\mu\text{g/kg}$ dizocilpine

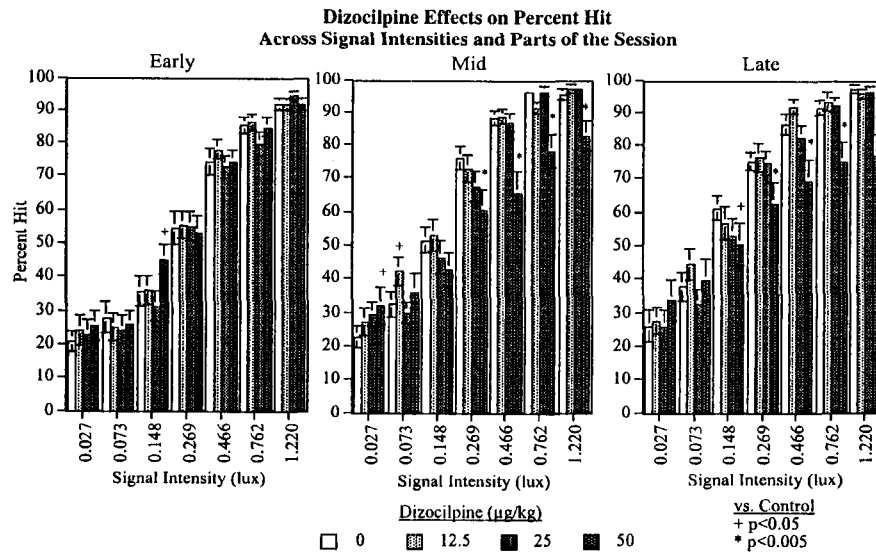


Fig. 4. Effects of different doses of dizocilpine on percent hit (mean $\pm$ S.E.M.) during the early, mid and late parts of the test session and the range of different signal intensities. $N=35$.

compared with control. Neither of the lower doses significantly affected response latency.

Dizocilpine caused a significant increase in the number of nonresponse trials ($F(3,99)=33.15$, $p<0.0001$). As with latency, it was only the highest dizocilpine dose (50 $\mu\text{g/kg}$) that caused a significant increase in nonresponse trials ($p<0.0001$). With saline the rats averaged 1.0 ± 0.6 nonresponse trials/session, with 12.5 $\mu\text{g/kg}$ dizocilpine they averaged 1.4 ± 0.5 , with 25 $\mu\text{g/kg}$ 3.4 ± 1.7 , and with 50 $\mu\text{g/kg}$ 30.3 ± 4.4 .

3.2. Exp. 2: Nicotine interactions with dizocilpine

Percent hit was significantly affected by nicotine and dizocilpine in a complex fashion. The three-way interaction of nicotine $\times$ dizocilpine $\times$ signal intensity was significant

($F(24,480)=1.83$, $p<0.05$). Fig. 7 shows the differential effects of nicotine and dizocilpine across the signal intensity range. Tests of the simple main effects at the individual signal intensities showed that without dizocilpine, 50 $\mu\text{g/kg}$ nicotine significantly ($p<0.025$) increased percent hit relative to control at the lowest signal intensity. No differential effect of nicotine across time blocks was detected. At the level 7 of signal intensity ($p<0.025$) and the level 6 of signal intensity, 50 $\mu\text{g/kg}$ nicotine significantly ($p<0.005$) decreased percent hit relative to control. With 37.5 or 50 $\mu\text{g/kg}$ dizocilpine co-administration, significant nicotine-induced percent hit increase at the low signal intensity and percent hit decrease at high intensities were no longer seen. Rather, there were occasions of significant nicotine-induced reductions in percent hit in the lower range of signal intensities. With 37.5 $\mu\text{g/kg}$ dizocilpine, 50 $\mu\text{g/kg}$ nicotine significantly ($p<0.05$) reduced percent hit relative to con-

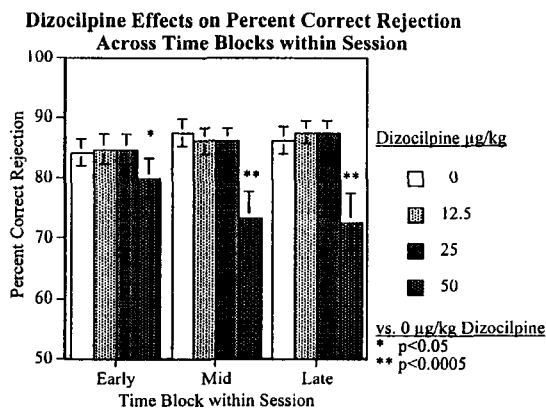


Fig. 5. Effects of different doses of dizocilpine on percent correct rejection (mean $\pm$ S.E.M.) during the early, mid and late parts of the test session. $N=35$.

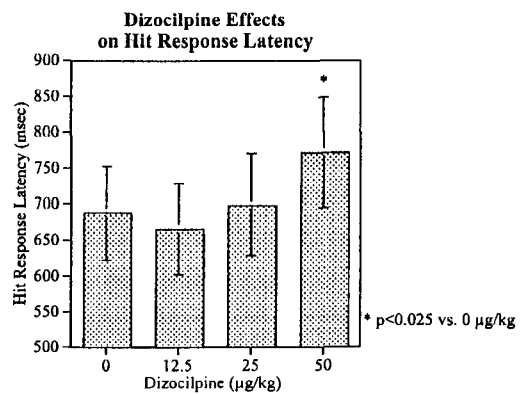


Fig. 6. Effects of different doses of dizocilpine on hit response latency (mean $\pm$ S.E.M.). $N=35$.

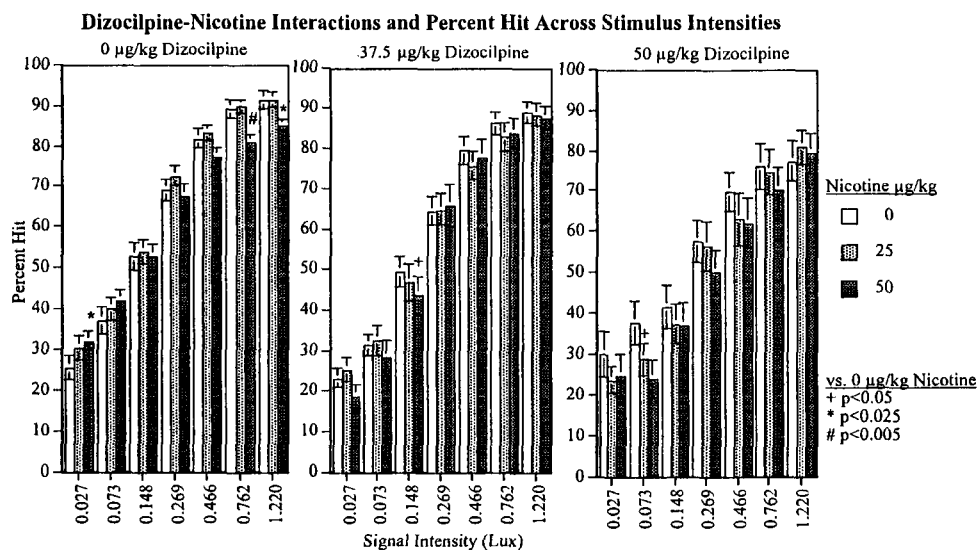


Fig. 7. Dizocilpine interactions with nicotine, percent hit (mean $\pm$ S.E.M.) across the range of signal intensities. $N=35$.

trol at the level 3 of signal intensity. With 50 $\mu\text{g/kg}$ dizocilpine, 25 $\mu\text{g/kg}$ nicotine significantly ($p<0.05$) reduced percent hit relative to control at the level 2 of signal intensity.

Percent correct rejection was affected by nicotine and dizocilpine in a much more straightforward fashion (Fig. 8). Percent correct rejection was reduced by 50 $\mu\text{g/kg}$ dizocilpine ($p<0.025$) compared to control injections. The addition of 25 $\mu\text{g/kg}$ of nicotine to 50 $\mu\text{g/kg}$ dizocilpine significantly ($p<0.025$) blocked this attenuating effect of dizocilpine on correct rejection.

Response latency was significantly lengthened by dizocilpine ($F(2,60)=5.28$, $p<0.01$) from an average of 637 ± 55 ms without dizocilpine to an average of 804 ± 84 ms with 50 $\mu\text{g/kg}$ dizocilpine for a significant slowing of response with the higher dose ($p<0.01$). The lower dose of dizocilpine did not cause a significant slowing of response.

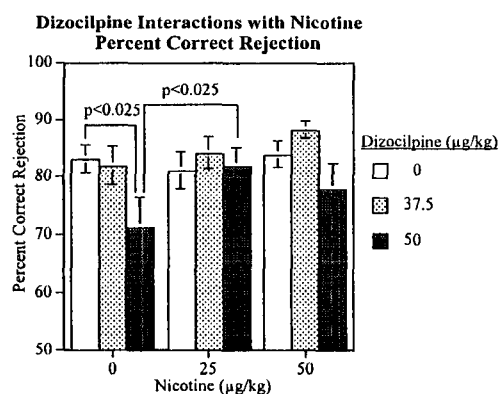


Fig. 8. Dizocilpine interactions with nicotine, percent correct rejection (mean $\pm$ S.E.M.). $N=35$.

There was no significant effect of nicotine or nicotine $\times$ dizocilpine interaction.

The incidence of nonresponse trials was significantly increased by dizocilpine ($F(2,60)=27.49$, $p<0.0001$). With saline the rats averaged 1.3 ± 0.4 response omissions and with 50 $\mu\text{g/kg}$ of dizocilpine they averaged 18.0 ± 3.4 for a significant increase with the higher dose ($p<0.0001$). The lower dose of dizocilpine did not cause a significant effect. Nicotine did not have an effect of its own or significantly influence the dizocilpine effect.

4. Discussion

This study demonstrated that NMDA blockade with dizocilpine effectively impaired sustained attention as measured by choice accuracy on this visual signal detection operant task. The dizocilpine-induced deficit was seen both in terms of percent hit and percent correct rejection. Nicotine co-treatment was effective in reversing the dizocilpine-induced correct rejection deficit. This effect was selective to correct rejection as no consistent effect was seen with reversal of dizocilpine-induced deficits in percent hit. Nicotinic-induced glutamate release (McGehee et al., 1995) may be involved in nicotine's reversal of dizocilpine-induced attentional impairment.

Dizocilpine was shown to cause a significant impairment in attentional function by reducing percent hit responses. Only the high dose of 50 $\mu\text{g/kg}$ was effective. The threshold for effect on this measure was between 25 and 50 $\mu\text{g/kg}$. When the rats were administered 50 $\mu\text{g/kg}$ of dizocilpine, they did not show the improvement in percent hit over the course of the session that was seen with control and lower doses of dizocilpine (12.5 and 25 $\mu\text{g/kg}$) (Fig. 2). This improvement in percent hit over the course of the

session has previously been seen in this task (Rezvani et al., 2002). However, in the study of Bushnell et al. (1997), the performance of rats in this task was maintained over session blocks. It is possible that rats in the current study had not attained full asymptotic performance levels. The 50 $\mu\text{g/kg}$ dizocilpine dose had the most pronounced impairment in percent hit when the stimulus was bright and clearly differentiable (Fig. 3). This suggests that the dizocilpine effect was not due to visual dysfunction. In that case, one would expect the most pronounced effect with the lower intensity stimuli, which are more difficult to detect. Interestingly, there was a significant increase in percent hit caused by 50 $\mu\text{g/kg}$ of dizocilpine at the lowest intensity. At this barely detectable intensity, the rats responded far more often when there was no stimulus than when there was. The effect of 50 $\mu\text{g/kg}$ may have been one of loss of stimulus control with performance retreating closer to chance (50%) at both the high and low stimulus intensities. The 50 $\mu\text{g/kg}$ dizocilpine dose caused a slight, though significant, increase in hit response latency. It does not appear that the magnitude of this effect (84 ms) would in itself cause the observed choice accuracy impairments. The dizocilpine impairment of percent correct rejection also emerged over the course of the session (Fig. 5), consistent with the effect on percent hit. The fact that the effect of dizocilpine is more pronounced in mid and late blocks of the session may suggest its pharmacokinetic profile in its action.

Nicotine did have significant interactions with dizocilpine. Nicotine (25 $\mu\text{g/kg}$) significantly reversed the dizocilpine (50 $\mu\text{g/kg}$)-induced percent correct rejection deficit (Fig. 8). The nicotine interactions with dizocilpine with regard to percent hit performance were more complicated with a significant ($p < 0.05$) three-way interaction of nicotine $\times$ dizocilpine $\times$ signal intensity (Fig. 7). Nicotine attenuation of dizocilpine-induced percent hit impairment was not seen. The nicotine-induced reversal of dizocilpine-induced impairments on this task appears to be selective to percent correct rejection. The neurobehavioral mechanisms for this selective effect are not entirely clear. This dose range of nicotine has previously been shown on this task to modestly improve correct rejection performance but have no apparent effect on percent hit (Rezvani et al., 2002). Bushnell et al. (1997) have demonstrated that nicotine at a higher dose (0.083 mg/kg) increased percent hit significantly. One difference between hit and correct rejection in this task is that the signal for hit is often near the threshold for sensory discrimination, as evidenced by the less than chance (50%) performance for the lower end to the signal intensity range, whereas correct rejection trials are always the same with no stimulus. The hit measure results from a combination of sensory and attentional function, whereas the correct rejection measure does not have the same component of sensory function. The correct rejection measure may be a clearer measure of attentional performance without variable demand on sensory function.

Nicotine may enhance cognition by direct effects on attention and by interacting with the presynaptic nicotinic acetylcholine receptors to facilitate the release of acetylcholine, dopamine, serotonin, γ -aminobutyric acid, norepinephrine and glutamate (Wonnacott, 1997). The NMDA–glutamate receptor system has been demonstrated to be involved in cognitive function (Wozniak et al., 1990) and there is neuropharmacological evidence that NMDA–glutamatergic and nicotinic systems in the brain are functionally related. Levin et al. (1998a) have shown that nicotine can attenuate the memory impairment caused by the NMDA receptor antagonist dizocilpine (MK-801) in rats. Tizabi et al. (1998) have demonstrated that nicotine and mecamylamine dose-dependently blocked dizocilpine-induced popping in mice. It has been shown that dizocilpine can impair attention. Dai and Carey (1994) found a dose-dependent decrease in animal's response to a stimulus object, suggesting impairment of attention by dizocilpine.

NMDA receptor blockade has been proposed as a model of schizophrenia, inasmuch as this treatment is psychotomimetic. Nicotine's attenuation of dizocilpine-induced attentional impairment in the current study and the recent study of Terry et al. (2002) as well as the nicotine's attenuation of dizocilpine-induced memory impairment (Levin et al., 1998a) may be relevant to reported nicotine-induced cognitive improvement in schizophrenia (Levin et al., 1996a) and for the development of novel nicotinic treatments for schizophrenia-related cognitive impairment.

Acknowledgements

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NICOTINIC ANTAGONIST ADMINISTRATION INTO THE VENTRAL HIPPOCAMPUS AND SPATIAL WORKING MEMORY IN RATS

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Abstract—Nicotinic acetylcholine receptors are important for maintaining optimal memory performance. In order to more fully characterize the involvement of nicotinic systems in memory, the contributions of nicotinic acetylcholine receptor subtypes were investigated. This study targeted the $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors in the ventral hippocampus, an area known to be important for spatial working memory. Antagonists of $\alpha 7$ and $\alpha 4\beta 2$ receptors were locally infused into the ventral hippocampus of rats and the effects on memory were examined with the radial-arm maze. The subtype-specific competitive antagonists infused into separate groups of rats were methyllycaconitine citrate (an $\alpha 7$ antagonist) and dihydro- β -erythroidine hydrobromide (an $\alpha 4\beta 2$ antagonist). Their effects on radial-arm maze performance were contrasted with the non-specific competitive antagonist, p-tubocurarine chloride. Significant deficits in radial-arm maze choice accuracy performance were found at 78.7 $\mu\text{g}/\text{side}$ for methyllycaconitine and at 106.9 $\mu\text{g}/\text{side}$ for dihydro- β -erythroidine. Increased response latency was also seen at these doses. Tubocurarine induced seizures at doses previously reported to have no effect. Wet dog shakes were seen in most rats at 0.1 $\mu\text{g}/\text{side}$ with tubocurarine, 26.3 $\mu\text{g}/\text{side}$ with methyllycaconitine and 106.9 $\mu\text{g}/\text{side}$ with dihydro- β -erythroidine.

This study suggests that both $\alpha 7$ and $\alpha 4\beta 2$ nicotinic acetylcholine receptor subtypes are involved in working memory formation and that the hippocampus is a critical site for nicotinic cholinergic involvement in memory function, though the high doses of antagonists needed to produce the memory impairment may have had less than completely specific effects. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: hippocampus, nicotinic, methyllycaconitine, dihydro- β -erythroidine, memory, radial-arm maze.

Understanding the role of nicotinic cholinergic systems in cognitive function is an important step in the development of therapeutic strategies for Alzheimer's disease. Substantial reductions in nicotinic receptor density are found in the hippocampus and cortex regions of the brains of Alzheimer's disease patients.^{49,50} Treatments using selective stimulation of nicotinic receptors are being designed to counteract the cognitive deficits associated with this disease.^{41,46} Nicotine, a prototypic agonist of acetylcholine receptors, has already had some success in improving the working memory of Alzheimer's disease patients.⁴² The memory-enhancing effects of nicotine have been documented in both experimental animals and humans.³¹ Further evidence for the importance of nicotinic acetylcholine receptors (nAChRs) in the CNS has been gained through studies with nicotinic antagonists. Many studies have used mecamylamine, a centrally active nicotinic

antagonist, to show the effects of nAChR blockade on cognitive function. Potent antagonists for specific nicotinic receptor subtypes are well documented using *in vitro* methods, but little is known about the effects of these drugs on memory and behavior.

Acute nicotine administration has been found in many studies in our laboratory and others to cause a consistent improvement in working memory performance in rats^{17,31,32} and monkeys.²⁸ In addition, other nicotinic agonists such as lobeline,¹⁹ dimethylethanolamine,³⁴ ABT-418¹⁸ and GTS-21⁵⁵ have also been found to be effective in improving learning and memory performance. The nicotinic antagonist mecamylamine has been shown to significantly impair radial-arm maze (RAM) performance of rats when administered systemically.³¹ Nicotine-induced improvements in working memory can be attenuated by mecamylamine. A variety of other effects of nicotine have been shown to be attenuated by mecamylamine as well (e.g., Ref. 11). Mecamylamine has been found to block nicotine-induced effects in brain slices that have not been blocked by other nAChR antagonists.^{21,53} A number of factors complicate the interpretation of the effects of mecamylamine with respect to the involvement of

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Abbreviations: DHBE, dihydro- β -erythroidine; dTC, p-tubocurarine; ETR, entry to repeat; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; RAM, radial-arm maze.

specific nAChR subtypes in working memory. Mecamylamine is a non-competitive nAChR channel blocker which also shows some affinity for *N*-methyl-D-aspartate ion channels. Most of the other commonly employed nicotinic antagonists act through a competitive mechanism with binding sites that are distinct from the non-competitive site.²² It is hoped that the use of specific competitive antagonists in this study will allow behavioral responses to be directly attributed to nAChR subtypes.

Neuronal nAChR subunits are currently classified into the α -type ($\alpha 2$ – $\alpha 8$) and the β -type ($\beta 2$ – $\beta 4$). A limited number of $\alpha\beta$ or α -only subunit combinations have been found to form functional pentameric ion channels.³⁸ The most common and widely distributed combination of subunits is $\alpha 4\beta 2$.⁵¹ Nicotine binds with the highest potency to this subtype.¹² Mecamylamine has a higher potency for the $\alpha 3\beta 2$ receptor, which is less widely distributed and found only in moderate concentrations in the CA3 region of the hippocampus.^{13,17} High concentrations of the homomeric $\alpha 7$ nAChR are found within the CA3 and dentate granule cells of the hippocampus.¹³ The $\alpha 7$ receptor has been characterized in rat hippocampal neurons as a fast inactivating Ca^{2+} channel with a low affinity for the endogenous substrate acetylcholine.^{2,5,10} This subtype may be the ideal candidate for the desensitization effects of nicotine. The $\alpha 7$ receptors may be particularly important for the effects of nicotinic drugs on memory. Meyer and co-workers^{20,27,39} have found that the nicotinic agonists GTS-21 and DMAC, which improve memory performance, are also relatively selective for $\alpha 7$ nicotinic receptors.

Hippocampal nAChRs appear to be critically involved in the sensory gating function of the hippocampus.³⁶ A regulatory role for nAChRs is supported by the existence of presynaptic nicotinic receptors on hippocampal neurons. The $\alpha 4\beta 2$ receptor has been implicated as an autoreceptor that modulates acetylcholine release.⁵² Synaptosomal preparations have also been used to demonstrate the existence of nicotinic receptors on hippocampal neurons using transmitters other than acetylcholine.⁵⁴ The subtle effects of nicotinic compounds on memory and learning are consistent with a modulatory function of nAChRs in the hippocampus.

Local infusion of mecamylamine directly into the ventral hippocampus of rats has been shown to impair working memory. Ohno *et al.*⁴³ found that 10- and 18- μg doses of mecamylamine could significantly increase the number of errors rats made on their working memory task. RAM studies, a long-standing measure of working memory (for review see Ref. 30), also suggest that local infusion of mecamylamine into the ventral hippocampus can impair working memory performance.²⁹ An almost significant effect on the arm choice accuracy (measured by the entries to a repeat entry) was detected with 3.3 μg of mecamylamine. The arm choice latency (seconds per

entry) of the rats increased in a linear fashion with increasing mecamylamine dose. Interestingly, nicotine had no effect when infused directly into the ventral hippocampus. The lack of response with nicotine (and, to some extent, mecamylamine) could be due to the discrete distribution of nAChRs in the hippocampus. In addition, rapid desensitization due to a local high concentration of agonist/antagonist may have occurred. Both competitive and non-competitive antagonist binding to nAChRs is thought to stabilize and therefore prolong the lifetime of the desensitized receptor state.^{23,48}

The action of D-tubocurarine (dTC) on peripheral, muscle-type nAChRs has been known for many years. Recently, dTC has been shown to antagonize a number of different neuronal subunit combinations, including $\alpha 7$ and $\alpha 4\beta 2$ receptors.^{9,24} Intrahippocampal injections of dTC cause limbic seizures and convulsions at doses between 5 and 10 μg .¹⁶ This behavior was accompanied by selective damage to hippocampal pyramidal cells. According to Dasheiff,¹⁶ low doses (1–3 μg) do not induce seizure activity, although some cellular damage was seen in the dentate granule cells. Doses lower than 1 μg did not induce cellular damage or epileptiform activity. Interestingly, co-administration of nicotine did not prevent dTC-induced seizures. Nicotine was successful in significantly reducing the neuronal damage caused by dTC. In another study, dTC was shown to block auditory gating in the rat hippocampus.³⁶ The aim of using sub-threshold doses of dTC (<1 μg) in our study was to elicit working memory deficits without seizure or pre-seizure activity [i.e. "wet dog shakes", catatonia (staring), excessive grooming]. This would allow comparison of the general competitive blockade of nicotinic receptors with subtype-specific receptor antagonism.

Over the last six years, two nicotinic antagonists have emerged from *in vitro* experiments as specific probes for the $\alpha 7$ and $\alpha 4\beta 2$ subtypes.^{26,48} Both of these antagonists are neuromuscular blockers with some affinity for peripheral nicotinic receptors. As such, they exhibit curare-like effects when administered systemically, limiting their use in animal studies. However, local administration into the brain through surgically implanted cannulae overcomes these peripheral effects and allows only the central actions to be observed.

Methyllycaconitine (MLA), a seed toxin from *Delphinium browni*, is now widely accepted as a selective probe for $\alpha 7$ receptors.^{1,3,4,6,26,48} Intracerebroventricular (i.c.v.) injections of up to 30 nmol did not disrupt the nicotine cue in rats trained to respond to nicotine injections.⁸ Mecamylamine did block the nicotine cue, suggesting that the $\alpha 7$ subunit is not involved in nicotine-induced responses and providing further support for a functional diversity of nAChR subtypes.

Dihydro- β -erythroidine (DHBE), a hydrogenated curare alkaloid, is a potent agonist of the $\alpha 4\beta 2$

neuronal nicotinic receptor.³⁵ Intrathecal injections into mice were successful in attenuating some, but not all, of the behavioral effects elicited by nicotine.¹⁵ DHBE was equipotent with mecamylamine in its blocking of the effects of nicotine in mice, except for antinociception, where mecamylamine was clearly more potent. Recently, a 300 nmol (or 106.9 µg) i.c.v. infusion of DHBE was shown to cause a significant deficit in RAM and Morris water maze choice accuracy.¹⁴ DHBE was also able to disrupt the enhancement of horizontal motor activity elicited by i.p. injection of nicotine. The suppressive effect of nicotine on rearing behavior was not blocked. The ability of DHBE to block only the stimulatory effects of nicotine may again be due to differences in nicotinic receptor subtype function.

The current study was conducted to determine the involvement of hippocampal nAChR subtypes in working memory function. The extent of the dissociation of function between nAChR subtypes and how these differences may relate to working memory is unclear at present, although there is evidence for the involvement of $\alpha 4\beta 2$ and $\alpha 3\beta 2$ receptors. The involvement of the hippocampal $\alpha 7$ receptor has not been characterized to date.

EXPERIMENTAL PROCEDURES

General design

The role of specific subtypes of nAChRs in the hippocampus on spatial working memory of rats was evaluated through RAM testing. The rats were trained on an eight-arm radial maze to asymptotic levels of choice accuracy (entries to repeat). Local infusion cannulae were then implanted bilaterally into the ventral hippocampus using stereotaxic techniques. After a short period of post-surgery training, the effects of a nicotinic antagonist were assessed. The drugs were given at doses to establish dose-response curves, with additional dose points being added after the initial testing to incorporate a range of effects. Once the dosing phase was complete, cannulae placement was checked by histological slicing of the rat brains.

Subjects

Adult female Sprague-Dawley rats (Zivic-Miller, Allison Park, PA, U.S.A.) that weighed between 230–406 g were used. The rats were approximately four to eight months of age. Female Sprague-Dawley rats were used in the current study because they have been shown in our previous study to be sensitive to the amnesic effects of the nicotinic antagonist mecamylamine infused into the ventral hippocampus.²⁹ During the RAM training phase, the rats were housed in groups of two to three, and then one per cage after cannulation. The body weight of the rats was maintained at 80–85% of their free feeding levels by a restricted diet. The rats had *ad libitum* access to water and were subjected to a 12-h light/12-h dark cycle. Testing occurred during the dark phase of the cycle. These studies were conducted with the approval of the Animal Use and Care Committee of Duke University.

Radial-arm maze and training

The RAM was constructed of wood and painted black. The central platform was 35 cm in diameter, and each of the eight arms was 10 × 80 cm² with a food cup at the distal end.

The maze was raised 30 cm from the floor. The testing room was soundproof and contained many extra-maze visual cues.

The rats were handled for at least two consecutive days prior to being placed on the maze. Shaping of the rats consisted of placing them in an opaque plastic cylinder in the center of the maze with eight half-pieces of Kellogg's Fruit Loops, and then allowing a maximum of 5 min for them to eat the fruit loops. Training began after two days of shaping. The procedure for running the rats on the maze, described below, was used throughout both the training and drug-test phases of the study. The rats were trained four to five days/week.

Before each testing session on the maze, each arm of the maze was baited with a half-piece of a Kellogg's Fruit Loop (a sugar-coated cereal). At the beginning of each session, the rat was placed in the opaque cylinder for 10 s to allow for orientation. The cylinder was then removed and timing begun. The rat was allowed to move freely about the maze. The rat was said to enter an arm if all four paws crossed the line at the entrance to the arm. Arm choice was recorded. The arms were not re-baited. The session continued until all eight fruit pieces had been eaten or 5 min had elapsed. The choice accuracy measure was the number of entries until an arm was re-entered (entries to repeat, ETRs). The response duration measure was the total session duration divided by the number of arms entered (seconds per entry or choice latency). After 20–22 training sessions, the rats were able to score consistently high ETRs and were cannulated.

Cannulation and drug administration

Twenty-eight rats were cannulated in the ventral hippocampus. An i.p. injection of a ketamine (70 mg/kg) and xylazine (8 mg/kg) mixture was administered as an anesthetic prior to the surgery. Stainless steel 22-gauge guide cannulae were implanted bilaterally into the ventral hippocampus of the rat whilst held in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, U.S.A.). The rat was secured in the instrument using ear and bite bars that held the head at an angle elevated 5 mm above the intra-aural line. An electric blanket was used to maintain the rat's body temperature at 98.6°F. An incision was made such that the bregma could be clearly seen and marked. The zero coordinates for the cannula placement were taken from the bregma. The coordinates for the ventral hippocampus were taken from the atlas of Pellegrino *et al.*⁴⁵ The guide cannulae were lowered through holes drilled in the skull to the following coordinates from bregma: AP +3.2, ML ±5.5, DV -8. The guide cannula tips were aimed 1 mm dorsal to the intended infusion site to minimize damage to the target structures. Dental cement and four screws tied together with wire were used to secure the cannulae. Dummy cannulae were placed in the indwelling cannulae to prevent infection or blockage.

The rats were given at least one full day of rest after surgery, after which two post-surgery training sessions were performed to establish that the surgery had not affected the ability of the rat to run the maze. At least two saline-only infusions were then given on consecutive days in order to introduce the rats to the infusion procedure.

Both drug and saline-only infusions were given in a volume of 0.4 µl per side over 3 min through 28-gauge infusion cannulae. The infusion cannulae were connected via polyethylene tubing to 10-µl Hamilton syringes held in a gear-driven Harvard Instrument infusion pump. The rats were run on the maze 10 min after the infusion.

The infusion of each drug was performed in a repeated measure counterbalanced design. The drugs were dissolved in 0.9% bacteriostatic sterile saline. Stock solutions of each dose were made prior to the dosing phase of the study and stored for up to one month at -20°C. The doses of dTC chloride were aliquoted such that there was one aliquot for each dosing session. Stock solutions of DHBE and MLA

were defrosted before and re-frozen after each session. The dTC was given in two phases, with a lowering of the dose range after the finding of seizures at the initial higher dose range. The MLA was given in dime phases, with an increasing of the dose range in the second and third phases. Appropriate vehicle control infusions were given in a counterbalanced order in each test phase. The doses and initial number of rats (*n*) in each drug group at the start of each set of sessions are shown below:

dTC sessions 1-4:	0, 0.025, 0.05, 0.1 µg/side (<i>n</i> =8)
dTC sessions 5-8:	0, 0.0125, 0.2, 0.4 µg/side (<i>n</i> =6)
MLA sessions 1-4:	0, 2.63, 8.75, 26.3 µg/side (<i>n</i> =10)
MLA sessions 5 and 6:	0, 52.6 µg/side (<i>n</i> =9)
MLA sessions 7 and 8:	0, 78.7 µg/side (<i>n</i> =9)
DHBE sessions 1-4:	0, 10.7, 35.6, 106.9 µg/side (<i>n</i> =10)

MLA and DHBE were obtained from Research Biochemicals International (Natick, MA, U.S.A.). dTC was purchased from Sigma Chemical Company (St Louis, MO, U.S.A.).

Histology

After the drug dosing phase was complete, the rats were anesthetized with sodium pentobarbital (50 mg/kg) and infused with 1 µl of dilute Chicago Sky Blue dye. The rats were then exsanguinated and perfused with saline followed by 10% paraformaldehyde in order to preserve the brains. The excised brains were stored in formaldehyde until slicing using a cryostat was performed. The locations of the infusion sites were then confirmed and recorded.

Statistical analysis

The choice accuracy and response latency measures were assessed by a within-subjects design analysis of variance (ANOVA). Planned comparisons were made between the saline-only and drug doses used. A *P*-value of <0.05 (two-tailed) was considered significant. A *P*-value of 0.05-0.10 (two-tailed) was considered to be nearly significant and strongly suggestive of an effect.

RESULTS

Histology

The placement of the cannulae was verified histologically. The placements for the three experiments are shown in Fig. 1a-c. Only those rats with cannula placements in the ventral hippocampus are shown and only those with accurate placements were used for statistical analysis of the behavioral data.

D-Tubocurarine infusions

A number of adverse events occurred during dosing with dTC. Three rats exhibited mild wet dog shakes (frequency 1-2 per minute) during the time on the maze when dosed with 0.1 µg/side. One rat had a full seizure when dosed with 0.4 µg/side. The rat was foaming at the mouth for a short time. The seizure lasted 10 min and the rat appeared normal after 30 min of dosing. A repeat dosing was not performed and therefore the data for this rat were not included in the analysis for sessions 5-8. Some rats seemed to have a higher activity of face washing and head scratching when dosed with dTC. However, a full record of this behavior was not kept because, unlike wet dog shakes, it is difficult to distinguish from

normal behavior. No significant effect on mean choice accuracy could be detected at any dose of dTC in rats that did not have seizures. Similarly, dTC did not significantly affect choice latency in these rats. Only tentative conclusions can be drawn from such a small number of rats. Because of the seizures induced, this drug was not investigated further.

Methyllycaconitine infusions

No drug-related seizures were seen with MLA, except for a single rat at the highest dose of 78.7 µg/side that had limbic seizures at 3 and 18 min after dosing. The maze data for this rat were filled in with the mean of the rest of the rats for this dose. Four of the seven rats dosed with the 52.6 µg/side dose had wet dog shakes at a frequency of four to five per minute for 10 min post-dose. At the 78.7 µg/side dose, two rats had wet dog shakes for 1-2 min after dosing.

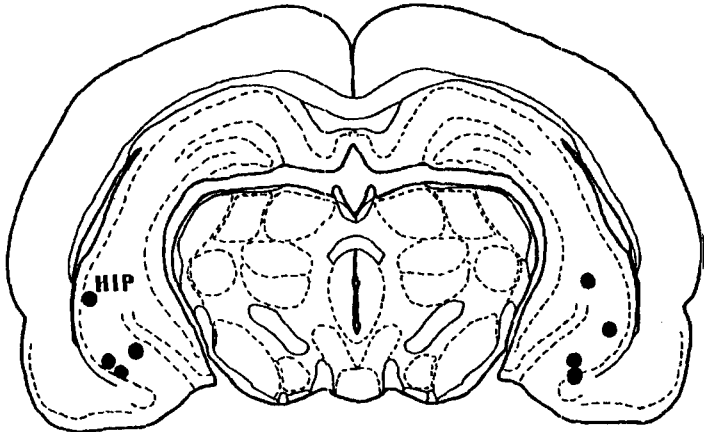
An MLA-induced memory deficit (Fig. 2) was seen as a significant linear dose-related MLA-induced choice accuracy deficit ($F_{1,30}=5.26$, $P<0.05$). At the highest dose of MLA, the mean ETR $\pm$ S.E.M. was 5.50 ± 0.56 compared with the control mean of 7.00 ± 0.26 , which is a significant negative effect ($F_{1,6}=13.56$, $P<0.025$). MLA also slowed performance (Fig. 3). There was a significant linear dose-related slowing of performance ($F_{1,30}=12.34$, $P<0.005$). No individual dose of MLA had a significant effect on mean choice latency. The correlation of response accuracy and response latency at the highest dose was not significant ($P=0.27$).

Dihydro- β -erythroidine infusions

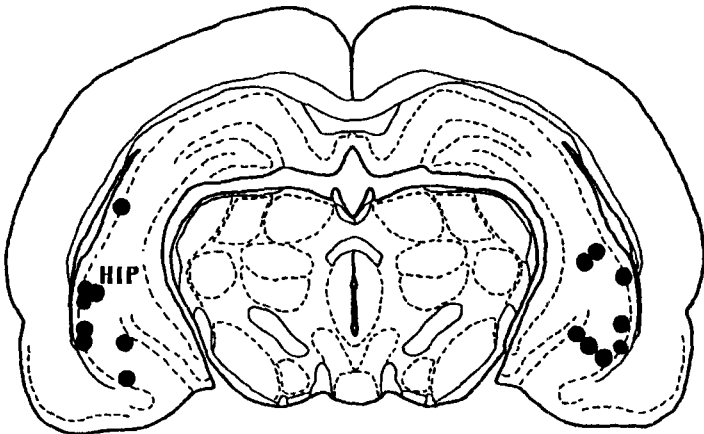
Wet dog shakes were seen in seven of eight rats with correct cannulae placement at the highest dose of DHBE. The length of time observed and number of wet dog shakes varied from the occasional shake during the time on the maze to 12 shakes per minute. The onset of the shakes was generally 5 min after dosing. In the rats with the higher numbers of wet dog shakes, there was considerable slowing of locomotor activity for a short period (2 min) while on the maze. After this time they returned to normal. The rats with only one or two shakes showed no other adverse effects. One rat that was dosed with 10.7 µg/side had wet dog shakes at a frequency of seven to eight per minute when first placed on the maze. Another rat (data not shown) had multiple limbic seizures when infused with DHBE for 30 min post-dose before returning to normal.

DHBE induced a significant memory impairment (Fig. 4). DHBE caused a significant linear dose-related decrease in the mean choice accuracy of the rats ($F_{1,21}=7.03$, $P<0.025$). The highest dose of DHBE caused a significant decrease in mean choice accuracy ($F_{1,21}=5.54$, $P<0.05$). The 106.9 µg/side dose gave a mean ETR of 4.88 ± 0.81 compared with 6.88 ± 0.40 for the control dose.

(a) dTC



(b) MLA



(c) DHBE

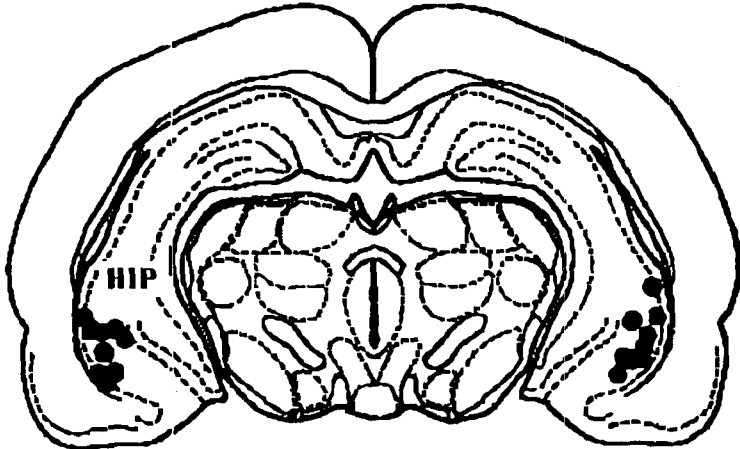


Fig. 1. Cannulae placements within the ventral hippocampus (HIP) for dTC- (a), MLA- (b) and DHBE-dosed (c) rats.

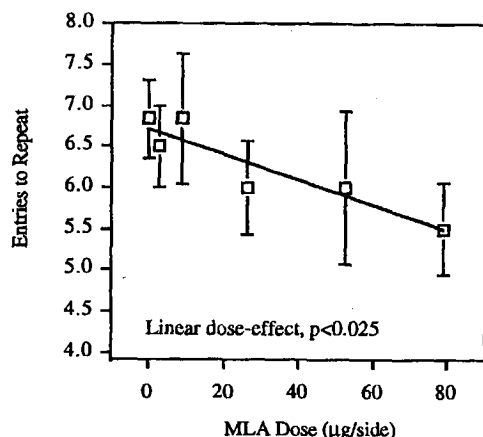


Fig. 2. The effect of the nicotinic antagonist MLA in the ventral hippocampus on ETRs (mean ± S.E.M.). Drug doses are in µg/side, $n=9$ or 10.

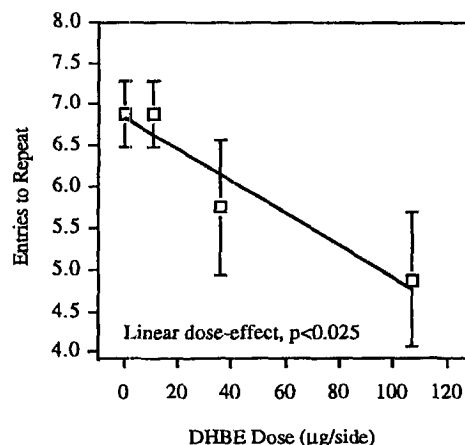


Fig. 4. The effect of the nicotinic antagonist DHBE in the ventral hippocampus on ETRs (mean ± S.E.M.). Drug doses are in µg/side, $n=10$.

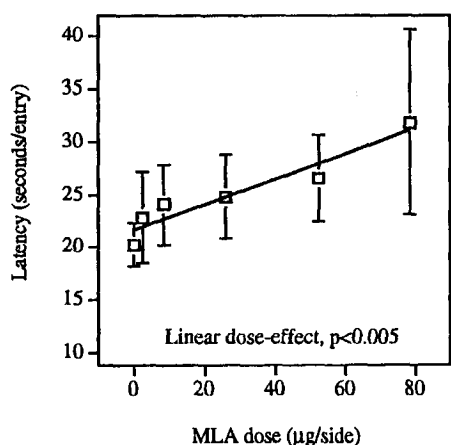


Fig. 3. The effect of the nicotinic antagonist MLA in the ventral hippocampus on seconds per entry (mean ± S.E.M.). Drug doses are in µg/side, $n=9$ or 10.

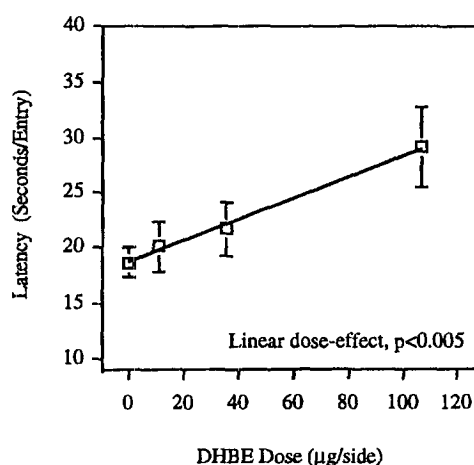


Fig. 5. The effect of the nicotinic antagonist DHBE in the ventral hippocampus on seconds per entry (mean ± S.E.M.). Drug doses are in µg/side, $n=10$.

Response latency (Fig. 5) was significantly increased by DHBE in a linear dose-related manner ($F_{1,21}=11.00$, $P<0.005$). The highest dose of DHBE significantly increased the seconds per entry from 18.6 ± 1.3 s per entry for the control to 29.1 ± 3.6 s per entry ($F_{1,21}=11.06$, $P<0.005$). The correlation of response accuracy and response latency at the highest dose was not significant ($P=0.24$).

DISCUSSION

Both MLA and DHBE caused spatial working memory deficits in this study. These effects were accompanied by a slowing of response, but the two effects were not found to be correlated. dTC was not found to impair choice accuracy in the RAM, but investigation of this drug was limited by its convulsant activity. All three drugs also had convulsant and preconvulsant actions. These data point to roles for

both $\alpha 7$ and $\alpha 4\beta 2$ nicotinic hippocampal receptors in working memory function, though the high doses of the antagonists needed to produce the impairment may have limited their specificity.

Nicotine has been shown in a variety of studies to improve working memory function.³¹ The nicotinic receptor subtypes and the anatomical location of the nicotinic receptors responsible for this effect are not known. The current study, as well as previous studies from this laboratory and others,^{29,43} point to the hippocampus as a critical substrate for nicotinic involvement in memory function. The fact that both DHBE and MLA impaired memory performance in this study suggests either a lack of nicotinic receptor subtype specificity of nicotinic receptor involvement in memory or some cross-reactivity of these drugs across different receptor subtypes at the doses used. The involvement of both $\alpha 4\beta 2$ and $\alpha 7$

nicotinic subtypes with working memory in the hippocampus suggests a complex neural mechanism for nicotine effects on memory. More than one particular neurotransmitter system are likely to be involved.

The magnitude of effect was fairly large. Perfect performance with the ETR measure on the eight-arm maze is 8. Random chance performance for this measure is 3.25 ETRs. Thus, the total response space is 4.75 units. The difference detected of approximately 2 units represents 42% of the total response space for the ETR measure.

It is possible that different locations within the hippocampus play different roles in working memory function. The precise location of the cannula placements within the hippocampus and the effects of MLA and DHBE on memory performance were not seen in the current study to be related. A more definitive assessment would compare the memory effects of nicotinic antagonist infusion aimed at different areas within the ventral hippocampus. MLA and DHBE effects in other parts of the brain are likewise unexplored. As we have seen with mecamylamine,^{29,33} there are likely to be multiple areas important for nicotinic effects on memory, while some areas with nicotinic innervation seem to be relatively uninvolved with memory function.

Slowing of response with higher doses of MLA and DHBE was not likely by itself to have accounted for the choice accuracy impairments seen at these doses. Because the RAM is a choice task, just the same amount of motor activity is needed to make a correct as an incorrect choice. Using a variety of drugs, we and others have seen that speeding or slowing of response is relatively unrelated to choice accuracy.³⁰ The lack of correlation between choice accuracy and choice latency at the highest doses of MLA and DHBE supports the independence of these effects.

The effects of dTC, MLA and DHBE in causing preconvulsant and convulsant activity raises important issues. Wet dog shakes seen in the current study have been shown to be reliable indicators of limbic seizures or generalized convulsions at higher doses.⁴⁷ The $\alpha 7$ receptors have recently been implicated in presynaptic enhancement of excitatory synaptic transmission³⁷ and may therefore prove to be a link between nAChRs and seizure/pre-seizure activity. The susceptibility of mice to nicotine-induced seizures may involve $\alpha 7$ receptors. Strains of mice with higher densities of α -bungarotoxin binding (another $\alpha 7$ selective antagonist) are more susceptible to the

convulsant effects of nicotine.⁴⁰ However, these results should be taken as only suggestive, since the relationship seen was correlative. The rapid inactivating properties of the $\alpha 7$ receptor may be such that nicotine is able to elicit desensitization and therefore inhibition of these receptors at high doses. Nicotine may have its convulsant actions mediated via desensitization of $\alpha 7$ receptors. Although MLA has a high specificity for the $\alpha 7$ receptor and DHBE for the $\alpha 4\beta 2$ receptor, it may be that the higher concentrations used in this study were sufficient to elicit more general nicotinic binding.^{7,44} Common binding to the $\alpha 3\beta 2$ receptor may also play a role in the amnesic effects of these drugs. Blockade of $\alpha 3\beta 2$ receptors in the frontal cortex has been found to have amnesic effects.²⁵ Binding to $\alpha 4\beta 2$ receptors probably still has a role to play in wet dog shakes, as is suggested by the results for DHBE infusion. The wider, more general distribution of the $\alpha 4\beta 2$ nAChR may account for wet dog shakes being seen mostly at the highest dose of DHBE.

It is unclear whether working memory impairment is part of this pathway or, conversely, an unrelated event that occurs at similar dose ranges. With MLA, wet dog shakes are observed at the same dose or lower levels as memory impairment. DHBE working memory deficits can apparently be elicited without the appearance of wet dog shakes by dosing outside of the hippocampus.¹⁴ The hippocampus may be a critical center in the development of wet dog shakes. However, the occurrence of non-cognitive effects such as increased latencies and wet dog shakes at the highest doses of MLA and DHBE suggests that the choice accuracy deficits may be part of a broader neurobehavioral syndrome. Whether memory deficits can be documented in isolation from other behavioral effects remains to be determined.

CONCLUSIONS

The nicotinic antagonists used in this study had similar effects on memory performance when infused into the ventral hippocampus. Both $\alpha 4\beta 2$ and $\alpha 7$ receptor subtypes may be important for the spatial working memory in rats, although the high doses of each drug necessary to produce the memory impairment may also have produced less specific nicotinic receptor subtype antagonism.

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Nicotinic Receptor Subtypes and Cognitive Function

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ABSTRACT: Nicotinic receptor systems are involved in a wide variety of behavioral functions including cognitive function. Nicotinic medications may provide beneficial treatment for cognitive dysfunction such as Alzheimer's disease, schizophrenia, and attention deficit hyperactivity disorder (ADHD). Nicotine has been shown to improve attentional performance in all of these disorders. Better efficacy with fewer side effects might be achieved with novel nicotinic ligands selective for particular nicotinic subtypes. To develop these novel selective nicotinic ligands it is important to use animal models to determine the critical neurobehavioral bases for nicotinic involvement in cognitive function. Nicotine-induced cognitive improvement in rats is most consistently seen in working memory tasks. We have found that both acute and chronic nicotine administration significantly improves working memory performance of rats in the radial-arm maze. The pharmacologic and anatomic mechanisms for this effect have been examined in our laboratory in a series of local drug infusion studies. Both $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors in the

ventral hippocampus and basolateral amygdala are involved in working memory function. Working memory impairments were caused by local infusion of either $\alpha 4\beta 2$ or $\alpha 7$ antagonists. Ventral hippocampal $\alpha 4\beta 2$ blockade-induced working memory deficits are reversed by chronic systemic nicotine treatment, while ventral hippocampal $\alpha 7$ blockade-induced working memory deficits were not found to be reversed by the same nicotine regimen. Interestingly, $\alpha 4\beta 2$ and $\alpha 7$ induced deficits were not found to be additive in either the ventral hippocampus or the basolateral amygdala. In fact, in the amygdala, $\alpha 7$ antagonist cotreatment actually reversed the working memory impairment caused by $\alpha 4\beta 2$ antagonist administration. These studies of the neural nicotinic mechanisms underlying cognitive function are key for opening avenues for development of safe and effective nicotinic treatments for cognitive dysfunction.

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Keywords: nicotine; nicotinic; $\alpha 4\beta 2$ receptors; $\alpha 7$ receptors; hippocampus; amygdala; working memory; radial-arm maze

INTRODUCTION

Nicotine has been shown to improve cognitive function including memory and attention in humans and experimental animals (Newhouse et al., 1997; Levin and Simon, 1998; Rusted et al., 2000). Nicotinic re-

ceptor deficits have been shown to be key in the cognitive impairments of Alzheimer's disease (Nordberg and Winblad, 1986; Shimohama et al., 1986; Whitehouse et al., 1988; Giacobini et al., 1989; London et al., 1989; Kellar and Wonnacott, 1990; Rinne et al., 1991; Perry et al., 1995) and schizophrenia (Durany et al., 2000; Leonard et al., 2000). Nicotine delivered through means other than smoking such as transdermal patches may prove to be a safe and effective treatment for cognitive impairment seen in Alzheimer's disease, schizophrenia and attention deficit hyperactivity disorder (ADHD) and other types of cognitive dysfunction (Wilson et al., 1995; Levin et al., 1996b; Levin et al., 1996d; Newhouse et al., 1997;

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White and Levin, 1999). Novel nicotinic agonists that are selective for particular nicotinic receptor subtypes may provide more specific therapeutic benefits for treating cognitive dysfunction with fewer adverse side effects. Different types of cognitive dysfunctions seem to involve different types of nicotinic receptor subtypes. For example, $\alpha 4$ -containing receptors seem to be particularly decreased in Alzheimer's disease (Martin-Ruiz et al., 1999), while $\alpha 4\beta 2$ and $\alpha 7$ receptors both seem to be decreased in schizophrenia (Durany et al., 2000; Leonard et al., 2000). The key to the development of safe and effective novel nicotinic agonists for different types of cognitive dysfunction is better understanding of the differential role nicotinic receptor subtypes play in cognitive function. Animal models of cognitive function and dysfunction are essential in the development of novel nicotinic medications. Not only do animal models provide a test for the functional effects of new nicotinic ligands; they can also help determine the critical neurobehavioral mechanisms underlying nicotinic-induced cognitive improvement.

PHARMACOLOGICAL AND GENETIC APPROACHES TO RECEPTOR SUBTYPE ANALYSIS

Great advances have been made in recent years using molecular approaches to the study of receptor function. As described in other articles in this issue, very specific manipulations can be made using genetic methods to eliminate one type of nicotinic receptor subunit. However, data from genetic knockouts of individual nicotinic receptors can be difficult to interpret. The absence of these receptors from the zygote stage onward can cause a variety of cascading effects. Nicotinic receptors play important roles in the control of neurodevelopment (Levin and Slotkin, 1998; Broide and Leslie, 1999). Eliminating select populations of nicotinic receptors can have effects on neurodevelopment that last a lifetime. It would be difficult to differentiate the persisting effects of these neurodevelopmental alterations from the effects of lacking a particular nicotinic receptor subtype in adulthood. Among the effects on neurodevelopment may be compensatory changes to "wire around" the missing receptor system, such that function is relatively preserved. These developmental problems can be sidestepped with the use of inducible knockouts. Inducible knockout techniques can also make use of local infusion of the inducing agent so that regional selectivity can be used to determine the anatomic definition of the nicotinic receptor subtype involve-

ment in cognitive function. Thus, the onset and subsequent adaptation to decreased receptor action can be studied much in the same way it can be studied with local infusion pharmacologic methods. There may be the drawback of being unable to de-induce the conditional knockout to study the reexpression of the nicotinic receptor system after compensation has taken place. This is relatively straightforward with pharmacologic methods with the cessation of chronic local infusion. However, there remains the distinct advantage of the selectivity of the knockout methodology in contrast to the invariable multimodal actions of drug probes. Any drug has a variety of actions. Greater specificity can be achieved with lower doses of drug administration, but even at low doses multiple actions cannot be completely avoided.

Pharmacologic and molecular approaches have complementary advantages. Combined approaches may be useful (Ohno et al., 2001). When both kinds of studies are conducted the field can use the converging evidence to more accurately determine the mechanisms by which nicotinic receptors influence cognitive function.

SYSTEMIC NICOTINE EFFECTS ON MEMORY

We have used the pharmacologic approach to help determine the involvement of nicotinic systems in cognitive function as well as testing nicotinic treatment in models of cognitive dysfunction. In rat (for reviews see Brioni et al., 1997; Levin and Simon, 1998) and monkey (for review see Buccafusco et al., 1996) models of nicotine-induced cognitive improvement is more clearly seen in terms of effects on memory performance than attention, although attentional performance has been documented to be improved by nicotine in some studies (Mirza and Stolerma, 1998; McGaughy et al., 1999; Stolerma et al., 2000). These animal models are vital for the determination of the mechanisms of nicotinic-induced cognitive improvement.

Importantly, for possible clinical use we have found that the efficacy of nicotine improvement of memory does not diminish with chronic administration. Rats were pretrained on the eight-arm radial maze with a food reinforcement at the end of each arm. They learned to avoid entering an arm more than once because each arm was only baited once. After they reached asymptotic levels of performance (they improved to their best level and maintained that level), nicotinic drug treatments were started. The choice accuracy used in the eight-arm maze where all

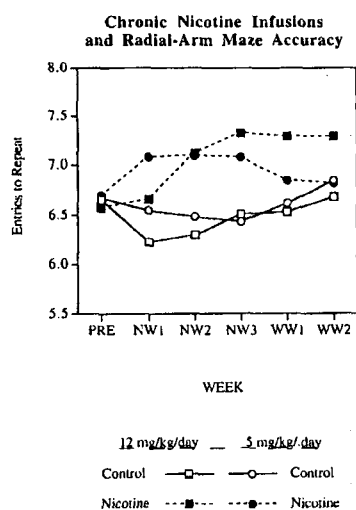


Figure 1 Chronic nicotine (5 or 12 mg/kg/day) effects on rats' eight-arm radial maze choice accuracy as measured by entries to repeat (the number of correct entries before the first repeated entry) (Levin et al., 1990; Levin and Rose, 1990; Levin et al., 1993a, 1993b; Levin and Rose, Levin et al., 1995; Levin et al., 1996c; Levin and Torry, 1996). PRE = pretreatment baseline performance; NW1, NW2 and NW3 = Weeks 1 through 3 of chronic nicotine treatment; WW1 and WW2 = Weeks 1 and 2 after withdrawal from chronic nicotine treatment. The choice accuracy measure is entries to repeat (the number of correct entries before an error occurs). Both chronic nicotine continuous infusion doses caused improvement during the period of administration. Only the 12 mg/kg/day dose caused persisting improvement after withdrawal.

the arms are baited with a reinforcer is entries to repeat which is the number of correct entries the rat makes before it repeats a choice. We have found in 20 years of research that entries to repeat is a sensitive and reliable measure of choice accuracy in the eight-arm radial maze when all arms are baited. As shown in Figure 1, we have found that chronic infusion of either 5 or 12 mg/kg/day of nicotine significantly improves memory performance on the eight-arm maze over 3–4 weeks of treatment (Levin et al., 1990; Levin and Rose, 1990; Levin et al., 1993a, 1993b; Levin and Rose, Levin et al., 1995; Levin et al., 1996c; Levin and Torry, 1996). Chronic coadministration of mecamylamine with chronic nicotine infusion blocked the nicotine-induced memory improvement (Levin et al., 1993a). Further testing was conducted with other sets of rats using a 16-arm maze to differentiate working memory (session-specific changing memory) from reference memory (invariant across session memory). Twelve of the arms were baited at the beginning of each session to test working

memory, while four were never baited to test reference memory. Those four unbaited arms were kept constant for each rat. Chronic nicotine treatment specifically improved working memory performance in the 16-arm radial maze, but had no discernible effect on reference memory (Levin et al., 1996a). This selective improvement of working memory is similar to the effect seen with acute nicotine injection (Levin et al., 1997).

More than one nicotinic receptor subtype seems to be involved in cognitive function. Nicotinic receptor subtype selective drugs are being developed to provide cognitive improvement with fewer side effects. Both $\alpha 4 \beta 2$ and $\alpha 7$ nicotinic agonists are effective. Anabaseine-related analogs that are selective $\alpha 7$ agonists significantly improved working memory in younger and aged rats (Meyer et al., 1994; Arendash et al., 1995; Meyer et al., 1997). Systemic administration of another type of $\alpha 7$ agonist ARR 17779 significantly improved learning on the radial-arm maze and reversed the working memory impairment caused by fimbria-fornix sections (Levin et al., 1999a). ABT-418, an $\alpha 4 \beta 2$ agonist, improved memory in monkeys (Buccafusco et al., 1995) and reversed the memory impairment caused by septal lesions (Decker et al., 1994). Another $\alpha 4 \beta 2$ agonist RJR 2403 significantly improved working memory (Levin and Christopher, 2002), an effect that persisted for at least 6 h after a single subcutaneous injection. Both $\alpha 7$ and $\alpha 4 \beta 2$ nicotinic agonists have been found to improve memory function. However, this does not mean that other nicotinic receptor subtypes may not be involved as well. Filling in that blank part of the map will depend on the development of more differentially selective nicotinic agonists and antagonists so that more complete information concerning the involvement of a broader diversity of nicotinic receptor subtypes on memory can be determined.

HIPPOCAMPAL INVOLVEMENT IN NICOTINIC ACTIONS ON MEMORY

For local infusion studies, antagonists offer the advantage of having a unidirectional effect of blocking nicotinic receptors. In contrast, agonists often have a bidirectional effect of stimulation and desensitization. This can be especially problematic for interpretation of local infusion studies in which there is a spatial concentration gradient to consider. Close to the local infusion site, high doses of agonist may have a more pronounced desensitizing effect than further from the infusion site where net stimulation of nicotinic receptors may be more preserved with lower concentrations

of the agonist. These concentric spheres of nicotinic receptor desensitization and activation could make interpretation of local infusion of nicotinic receptor agonists quite confusing. Local nicotinic receptor antagonist infusion has in our series of studies demonstrated the importance of nicotinic receptors in the limbic system and the midbrain for spatial working memory function.

The hippocampus was the initial region we investigated. A plethora of studies had demonstrated the importance of the hippocampus for working memory function. In particular, cholinergic innervation of the hippocampus by neurons in the medial septal area was shown to be critical for optimal memory performance. Muscarinic ACh receptors in the hippocampus were the focus of much of the early work in this area. Certainly, hippocampal muscarinic receptors are important. Local hippocampal scopolamine infusion has been shown in our studies and others to significantly impair working memory performance (Kim and Levin, 1996). Septohippocampal cholinergic innervation uses not only muscarinic but also nicotinic receptors. Autoradiographic studies have demonstrated the presence of $\alpha 7$ (Whiteaker et al., 1999; Fabian-Fine et al., 2001) and $\alpha 4$ (Didier et al., 1995) containing nicotinic receptors in the hippocampus. We have found the hippocampus to be important for chronic nicotine effects on memory function. Small hippocampal ibotenic acid lesions, which did not significantly impair working memory performance, blocked the chronic nicotine-induced memory improvement (Levin et al., 1999b). The critical site of action for chronic nicotine in the hippocampus appears to be postsynaptic to the septohippocampal projection. Chronic nicotine-induced memory improvement was not attenuated by knife-cut lesions of this fiber bundle (Levin et al., 1993b).

Ohno and coworkers initially demonstrated that local infusion of the nonspecific nicotinic receptor antagonist mecamylamine into the hippocampus caused working memory but not reference memory impairment in rats in a three spatial choice maze task (Ohno et al., 1993). We subsequently replicated their finding. As shown in Figure 2, the same dose range of mecamylamine significantly impaired choice accuracy in the eight-arm radial maze after infusion into the ventral hippocampus (Kim and Levin, 1996). This effect was not specific to the hippocampus. The same dose range of mecamylamine in the midbrain dopamine nuclei (ventral tegmental area and substantia nigra) also caused significant working memory impairments (Fig. 2) (Levin et al., 1994). Interestingly, the specificity of this dose range of mecamylamine was seen in its locus of action with no discernable

Local Infusions of Mecamylamine and Radial-Arm Maze Accuracy

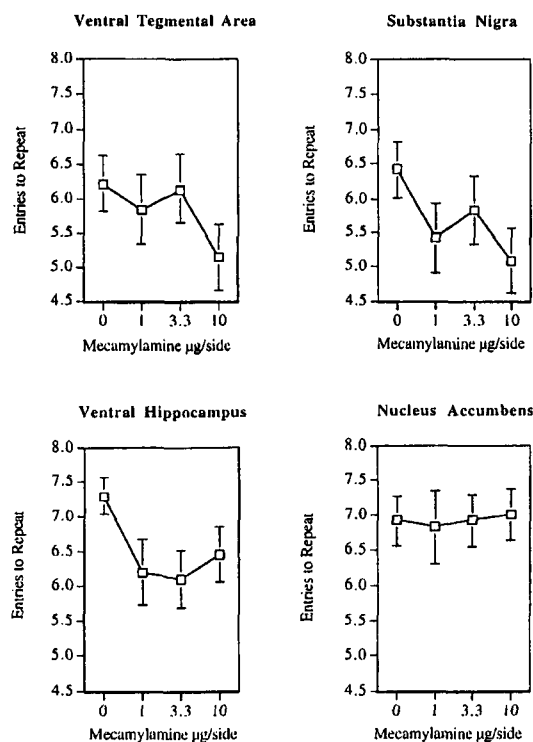


Figure 2 Acute infusion of the nonspecific nicotinic antagonist mecamylamine into the ventral tegmental area, substantia nigra, ventral hippocampus and nucleus accumbens on eight-arm radial maze choice accuracy (entries to repeat, mean $\pm$ S.E.M.) (Levin et al., 1994; Kim and Levin, 1996). Impairments seen with mecamylamine infusions into the ventral tegmental area, substantia nigra and ventral hippocampus, but not with the same doses infused into the nucleus accumbens.

effect after infusion into the nucleus accumbens (Fig. 2) (Kim and Levin, 1996).

Subsequent studies with more specific nicotinic receptor antagonists in the ventral hippocampus showed the involvement of both $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors with working memory function. As shown in Figure 3, significant dose-effect induced radial-arm maze working memory deficits were seen after acute ventral hippocampal infusion of either the $\alpha 7$ antagonist MLA or the $\alpha 4\beta 2$ antagonist DH β E (Felix and Levin, 1997). One caveat of this experiment is that at the high end of the dose range tested, these drugs may have had lessened specificity.

To determine if lower, more specific doses of MLA and DH β E would affect memory performance, their

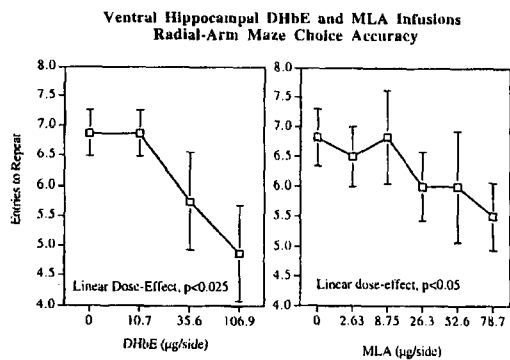


Figure 3 Acute nicotinic antagonist infusion the $\alpha 4\beta 2$ nicotinic antagonist DHβE and the $\alpha 7$ nicotinic antagonist MLA into the ventral hippocampus and eight-arm radial maze choice accuracy (mean $\pm$ S.E.M.) (Felix and Levin, 1997). Both antagonists caused working memory impairments in the eight-arm maze, albeit at high doses.

effects were tested in animals trained on the more difficult 16-arm radial maze (Levin et al., 2002). As shown in Figure 4, both MLA and DHβE significantly impaired working memory at lower doses in the more difficult 16-arm maze. Reference memory was only very slightly affected (data not shown). Interestingly, the two nicotinic receptor antagonists did not have additive effects when administered together. This was not due to a ceiling in the number of errors possible on this maze. We have seen substantially more errors in response to other drug manipulations such as NMDA receptor blockade with dizocilpine (Levin et al.,

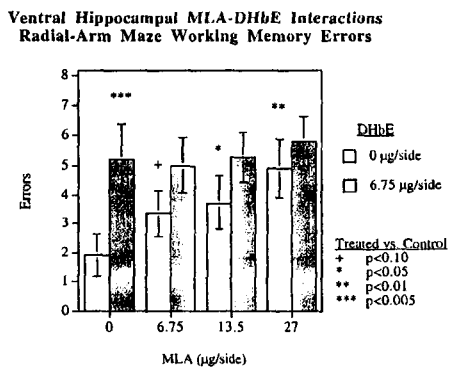


Figure 4 Low-dose acute nicotinic antagonist infusion of the $\alpha 4\beta 2$ nicotinic antagonist DHβE and the $\alpha 7$ nicotinic antagonist MLA into the ventral hippocampus and 16-arm radial maze choice accuracy (mean $\pm$ S.E.M.) (Levin et al., 2002). Both antagonists caused working memory impairments in the 16-arm maze at lower doses than in the eight-arm maze. The impairments caused by DHβE and MLA were not additive.

Chronic Nicotine and Acute Hippocampal DHβE and MLA Effects on Radial-Arm Maze Choice Accuracy

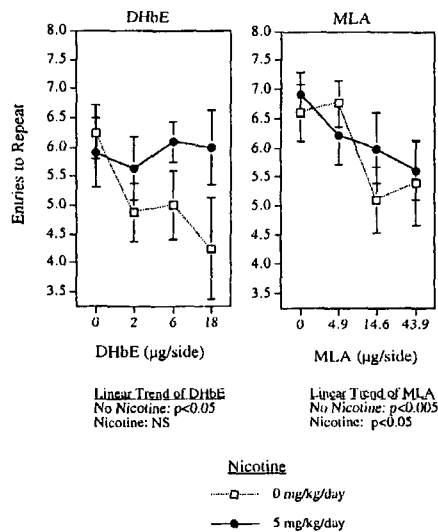


Figure 5 Chronic systemic nicotine infusion (5 mg/kg/day) and low-dose acute nicotinic antagonist infusion the $\alpha 4\beta 2$ nicotinic antagonist DHβE and the $\alpha 7$ nicotinic antagonist MLA into the ventral hippocampus and eight-arm radial maze choice accuracy (mean $\pm$ S.E.M.) (Bancroft and Levin, 2000; Bettany and Levin, 2001). Both DHβE and MLA caused working memory impairments. Chronic systemic nicotine infusion reversed the DHβE-induced impairment but not the MLA-induced impairment.

1998). The nonadditivity of simultaneous $\alpha 7$ and $\alpha 4\beta 2$ blockade in the ventral hippocampus may be due to some mechanistic interaction that limits their total common effect, such as convergence on hippocampal GABA interneurons (Alkondon and Albuquerque, 2001).

Both acute DHβE and MLA infusion into the ventral hippocampus caused significant working memory impairment but they respond to systemic nicotine in different ways. Figure 5 shows two experiments in which equimolar doses of these antagonists were infused into the ventral hippocampus (Bancroft and Levin, 2000; Bettany and Levin, 2001). They had similar effects impairing working memory accuracy in the eight-arm radial maze. However, chronic systemic nicotine infusion (5 mg/kg/day) had a differential effect on the impairments caused by these two drugs. The DHβE-induced memory deficit was eliminated by chronic systemic nicotine while the MLA-induced deficit was not.

Both $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptor subtypes in the hippocampus are important for working memory function. As shown in Figure 3, memory in the radial-

arm maze was impaired by local hippocampal infusion of either DH β E, the α 4 β 2 antagonist, or MLA, the α 7 antagonist (Felix and Levin, 1997; Levin et al., 2002), as well as the nicotinic receptor channel blocker mecamylamine (Kim and Levin, 1996). Chronic systemic nicotine was found to effectively overcome the working memory deficit caused by hippocampal infusion of the α 4 β 2 antagonist DH β E (Bancroft and Levin, 2000). However, the same dose of chronic systemic nicotine that reversed DH β E-induced working memory impairments was ineffective in attenuating intrahippocampal infusions of equimolar doses of the α 7 antagonist MLA (Bettany and Levin, 2001). These data suggest that although ventral hippocampal α 7 and α 4 β 2 nicotinic receptors are important for memory function, α 7 receptors may be more essential for the expression of the actions of systemic nicotine on working memory function.

TOPICS FOR FURTHER RESEARCH

Certainly, the hippocampus is not the only brain area important for nicotinic involvement in memory function. As discussed above, our earlier results showed that infusions of the nonspecific nicotinic antagonist mecamylamine into the ventral tegmental area and substantia nigra significantly impaired working memory performance, whereas the same doses infused into the nucleus accumbens did not significantly affect memory performance. The amygdala appears to be important for nicotinic actions on spatial memory. Amygdalar nicotinic antagonist infusion effects on memory have also been examined. In an initial study (Addy et al., 2002), we found that as in the ventral hippocampus, local infusion of MLA or DH β E into the basolateral amygdala caused significant impairments in working memory as measured by 16-arm maze choice accuracy. Also, like with ventral hippocampal infusion, the combination of MLA and DH β E did not cause an additive effect. In fact, with basolateral amygdalar infusion coadministration of DH β E with MLA actually significantly reduced the working memory error rate back down to control levels (Fig. 6). The basolateral amygdala has been known to be important for memory of fear conditioning (Maren, 1999), but its role in spatial appetitive memory has not been well characterized. Other brain areas such as the frontal cortex and thalamic nuclei are also likely to be important in nicotinic involvement in memory function.

A wide diversity of nicotinic receptor subtypes have been identified. Research has focused on the α 4 β 2 and α 7 nicotinic receptors to date principally

Basolateral Amygdalar Infusion of MLA and DH β E Effects on Working Memory in the 16-Arm Radial Maze

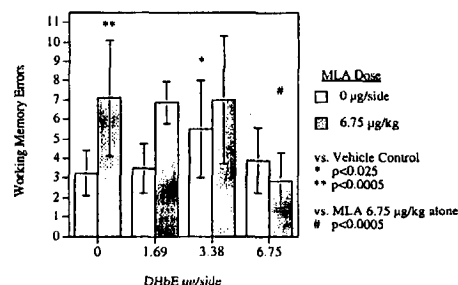


Figure 6 Low-dose acute nicotinic antagonist infusion the α 4 β 2 nicotinic antagonist DH β E and the α 7 nicotinic antagonist MLA into the basolateral amygdala and 16-arm radial maze choice accuracy (mean $\pm$ sem) (Addy et al., 2002). Both antagonists caused working memory impairments in the 16-arm maze. The impairments caused by DH β E and MLA were not additive. In fact, the 6.75 μ g/side dose of DH β E reversed the impairment caused by 6.75 μ g/side of MLA.

because of the availability of relatively selective ligands for these receptor subtypes. As other nicotinic receptor subtype selective agonists and antagonists become available, the other nicotinic receptor subtypes can be more easily studied.

CONCLUSIONS

Nicotinic systems are vital in the neural basis of cognitive function. Nicotinic-based therapeutic treatments may be useful for the treatment of a variety of cognitive disorders including Alzheimer's disease, schizophrenia, and attention deficit hyperactivity disorder (ADHD). Whole animal models are critical for determining the neurobehavioral mechanisms of the cognitive effects of nicotinic treatments, including critical nicotinic receptor subtypes and their anatomic localization and interactions with other neural systems. Mechanistic whole animal studies can help improve the understanding of the neurobiology of memory and aid in the development of therapeutic agents for memory dysfunction.

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HIPPOCAMPAL $\alpha 7$ AND $\alpha 4\beta 2$ NICOTINIC RECEPTORS
AND WORKING MEMORY

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Abstract—Nicotine and other nicotinic receptor agonists have been found in a variety of studies to improve memory, while nicotinic receptor blockade can impair memory. The critical neural mechanisms for nicotinic involvement with memory are still under investigation. Initial evidence supports the involvement of the ventral hippocampus. Lesions in this area block nicotine-induced memory improvement and mecamylamine-induced impairment. Local ventral hippocampal application of the nicotinic channel blocker mecamylamine impairs memory in the 8-arm radial maze. Both $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors seem to be involved. Ventral hippocampal infusions of high doses of the $\alpha 4\beta 2$ nicotinic antagonist dihydro- β -erythrodine (DH β E) and the $\alpha 7$ nicotinic antagonist methyllycaconitine (MLA) impair memory performance on the 8-arm radial maze. However, high doses of these drugs may limit specificity and they cause pre-convulsant effects, which in themselves may affect memory. The current study used the more challenging 16-arm radial maze to determine the effects of lower doses of these drugs on memory and to differentiate effects on working and reference memory.

Adult female Sprague-Dawley rats were trained on a working and reference memory task in the 16-arm radial maze and then were implanted with bilateral chronic guide cannulae directed to the ventral hippocampus. After recovery from surgery, the rats received acute intrahippocampal infusions of dose combinations of DH β E and MLA. In the first study, DH β E (0 and 6.75 μ g/side) and MLA (0, 6.75, 13.5 and 27 μ g/side) were administered in a counter-balanced order. In the second study, lower doses of DH β E (0, 1.6375, 3.275 and 6.75 μ g/side) were administered alone or with MLA (0 and 6.75 μ g/side) in a counter-balanced order. In the first study, DH β E caused a significant increase in both working and reference memory errors. MLA at a dose of 27 μ g/side caused a significant increase in working memory errors, but this dose had no significant effect on reference memory errors. Interestingly, no additive effects were seen with combined administration of DH β E and MLA in this study, and at the doses used, no effects were seen on response latency. In the second study, lower doses of DH β E did not cause a significant deficit in working memory performance. Co-administration of MLA with these subthreshold doses did precipitate a memory impairment. The current results confirm the specificity of the memory deficits caused by these drugs.

These results support the involvement of $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors in the ventral hippocampus as being critical for memory function. © 2002 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: cholinergic, methyllycaconitine, dihydro- β -erythrodine, $\alpha 7$, $\alpha 4\beta 2$, radial-arm maze.

Nicotinic acetylcholine receptors have been found in a variety of studies to be important for working memory function in humans and experimental animal models (Brioni et al., 1997; Levin and Simon, 1998b; Newhouse et al., 1997). Nicotine and nicotinic receptor agonists have been found to improve memory performance, while nicotinic receptor antagonists, such as mecamylamine, have been found to impair memory function. Different nicotinic receptor subtypes have been found in many different parts of the brain. The relative involvement of particular nicotinic receptor subtypes in specific parts of the brain with memory function is still being characterized. The present experiment was conducted to determine the involvement of ventral hippocampal $\alpha 7$

and $\alpha 4\beta 2$ nicotinic receptors with the neural basis of working and reference memory.

Nicotinic involvement in memory function has important clinical implications. Memory impairments in Alzheimer's disease have been associated with dramatic reductions in nicotinic receptor concentration in the hippocampus and neocortex (Whitehouse et al., 1995; Whitehouse et al., 1988). Nicotinic treatment for Alzheimer's disease and other disorders of cognitive function have shown initial promise (Arneric et al., 1995; Newhouse et al., 1997). In Alzheimer's disease, nicotine treatment by intravenous, subcutaneous or transdermal routes has been found to improve attention learning, memory and attention (Jones et al., 1992; Newhouse et al., 1988; Newhouse et al., 1986; Sahakian et al., 1989; Sahakian et al., 1991; White et al., 1999; Wilson et al., 1995). With schizophrenic patients, we have found that transdermal nicotine significantly improves attentional performance and attenuates the memory deficit caused by the antipsychotic drug haloperidol (Levin et al., 1996a). Attention deficit/hyperactivity disorder symptoms in adults have also been shown in our

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Abbreviations: MLA, methyllycaconitine; DH β E, dihydro- β -erythrodine.

laboratory to be reduced with application of nicotine skin patches (Conners et al., 1996; Levin et al., 1996b). Although further research is necessary, these preliminary studies provide the proof of principle that nicotinic treatments hold promise for treatment of cognitive deficits.

For the development of nicotinic-based therapeutic treatments and for a more accurate understanding of the basic neurobiology of memory function, it is important to determine the critical neural substrate of nicotinic actions on cognitive function. There is initial evidence that the hippocampus is important for nicotinic effects on memory function. Lesions of the ventral hippocampus block the amnesic effects of the nicotinic receptor antagonist mecamylamine (Chambers et al., 1996) and the memory improvement caused by nicotine (Levin et al., 1999b). Local infusions of the nicotinic receptor antagonist mecamylamine into the ventral hippocampus have been found to significantly impair memory performance in rats (Kim and Levin, 1996). High concentrations of $\alpha 7$ nicotinic receptors are found in CA3 and dentate granule cells of the hippocampus. The $\alpha 7$ nicotinic receptor has been characterized as a fast inactivating non-selective cation channel with high permeability to calcium and with low affinity for acetylcholine (Alkondon et al., 1993; Barrantes et al., 1995; Castro and Albuquerque, 1993; Court and Clementi, 1995). The $\alpha 7$ receptors are found on hippocampal interneurons and seem to regulate neurite outgrowth and survival. These $\alpha 7$ nicotinic receptors may be critical targets for $\alpha 7$ nicotinic receptor agonists that improve memory performance (de Fiebre et al., 1995; Hunter et al., 1994; Levin et al., 1999a; Meyer et al., 1994). The nicotinic receptors in the hippocampus have been shown to be critically involved in the sensory gating function of the hippocampus as well as some suggestion of a regulatory role by their presence presynaptically on hippocampal neurons (Luntz-Leyman et al., 1992; Wilkie et al., 1993; Wonnacott et al., 1989).

In the current study, we used two nicotinic receptor antagonists, methyllycaconitine (MLA), a seed toxin from *Delphinium brownii*, and dihydro- β -erythrodine (DH β E), a hydrogenated curare alkaloid. MLA has been characterized in binding and electrophysiological studies to be a preferential antagonist at $\alpha 7$ nicotinic receptors, while DH β E has been characterized as a preferential antagonist at $\alpha 4\beta 2$ nicotinic receptors (Aiyar et al., 1979; Alkondon et al., 1995; Alkondon et al., 1992; Briggs et al., 1995; Hardick et al., 1995; Luetje et al., 1990; Ward et al., 1990). We have previously found that both MLA and DH β E, when locally infused into the ventral hippocampus, cause significant impairments in choice accuracy on the 8-arm radial maze (Felix and Levin, 1997). However, in that study, the MLA and DH β E doses needed to produce choice accuracy impairments in the 8-arm radial maze were high enough to cause proconvulsant behavior, which might in itself cause memory impairment. Thus, the specificity of the effects of these antagonists directly affecting memory was left in question. Like any drug, MLA and DH β E are not completely specific to a single receptor. At high concentrations, they can affect both $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors.

Greater discrimination between nicotinic receptor subtypes can be gained by using lower doses. The current studies used the more challenging 16-arm maze to determine whether these nicotinic receptor antagonists would impair memory performance at lower doses, which have fewer side effects. Also, the behavioral specificity of MLA and DH β E effects was tested. The 16-arm maze has a sufficient number of arms that some can be devoted to assessing reference memory while leaving enough arms to measure working memory in a challenging fashion. Finally, the combination of $\alpha 7$ and $\alpha 4\beta 2$ receptor antagonists were assessed to determine whether there was a mutually potentiating interaction of these nicotinic receptor subtypes with regard to memory function.

EXPERIMENTAL PROCEDURES

Subjects

Young adult female Sprague-Dawley rats (Taconic Farms, Germantown, NY, USA) weighing between 200 and 325 g were used in this study (study 1, $n=11$; study 2, $n=8$). The rats were housed in groups of three to four during initial training and singly after cannulation. The rat colony room had a reverse 12:12 light:dark cycle. All testing was conducted in the dark phase. The rats had *ad libitum* access to drinking water but were kept on a restricted feeding schedule to maintain body weights of approximately 85% of free feeding levels, adjusted for growth. Daily feeding took place after testing. All efforts were made to minimize both the suffering and number of animals used, and that all experiments conformed to the Duke University and federal guidelines on the ethical use of animals.

Radial-arm maze

The 16-arm radial maze was used to determine spatial working and reference memory. Rats were trained to asymptotic levels of accuracy before local infusion, cannulae were then implanted bilaterally into the ventral hippocampus and the drugs were administered.

The 16-arm radial maze was constructed of wood and painted black. The center platform was 50 cm in diameter, the 16 arms were arranged radially from the platform and measured 10 $\times$ 60 cm. There were food cups placed at the distal end of each arm. The maze was raised 30 cm off the floor. There were abundant visual cues within the maze test room.

The rats were handled for at least two consecutive days prior to being placed on the maze. The rats were shaped by placing them in an opaque plastic cylinder on the center of the maze with 12 pieces of bait, half pieces of sweetened cereal, Kellogg's Froot Loops® (Kellogg, Battle Creek, MI, USA). They were then allowed a maximum of 5 min or until they ate all the Froot Loops®. Training began after 2 days of shaping. The procedure for running the rats was standard during both the training and drug testing phases. The rats were trained 4–5 days a week.

Before each testing session on the maze, 12 of the 16 arms were baited with Froot Loops®. For any particular rat, the same arms were always baited with no more than two adjacent arms left unbaited. Each rat had a different baiting pattern. At the beginning of the session, the rat was placed in the opaque cylinder in the center of the maze for 10 s to allow for orientation, then the cylinder was removed and timing begun. The rat was allowed to move freely about the maze and entry to an arm was recorded if all four paws of the rat crossed the entrance of the arm. The session continued until all the baited arms were entered or 10 min had elapsed. Reference and working memory errors could be differentially scored, with reference memory

errors being any entries into the arms which were never baited, and working memory errors being re-entries into arms which were previously baited. Baited arms that were not entered by the maximum session length (600 s) were counted as working errors of omission with a maximum of three such errors per session. Errors of omission were occurred only very occasionally when a rat did not finish the maze in the time allotted (10 min). Working memory omission (those of not entering baited arms) and commission (those of re-entering previously baited arms) errors were not found to be differentially affected by the drug treatments and were summed for analysis. Latency of the rat was said to be the average time per entry, the total time divided by the number of entries made. The rats were trained for 18 sessions or until they were consistently scoring on the maze, before cannulation was performed.

Cannulation

The rats were cannulated in the ventral hippocampus. An i.p. injection of ketamine (70 mg/kg) in combination with xylazine (8 mg/kg) or edetomidine (0.3 mg/kg) was administered as an anesthetic prior to surgery. During surgery, body temperature of the rat was maintained by use of an electric heating pad. The rat was secured in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA) with ear and bite bars that securely held the head elevated at an angle 5 mm above the intra-aural line. The stainless steel 22-gauge guide cannulae were implanted bilaterally to the ventral hippocampus.

In preparation for surgery, the fur was shaved from between the eyes to behind the ears of the rat's head, an incision was made lengthwise such that the bregma could be clearly seen and marked. The zero coordinates for the cannulae positions were taken from the bregma. The coordinates for the ventral hippocampus were taken from the Pellegrino atlas (Pellegrino et al., 1979). The guide cannulae were lowered through holes drilled into the skull to the following coordinates from bregma: anteroposterior +3.2, mediolateral ± 5.3 , dorsoventral -7.0. The guide cannulae were aimed 1 mm dorsal to the intended site of infusion to minimize damage to the target structures. Four screws were fixed in between the cannulae and tied together with wire. These were used to help anchor the protective cap that was formed by cranioplastic cement. The cement, a two-part acrylic-based medium, was mixed and applied to surround and secure the cannulae and cover the incision area. Dummy cannulae were placed in the indwelling cannulae to prevent infection or blockage.

The rats were given at least one full day of rest after surgery before being run on the maze. Two postsurgery trials were run to assess the rats' performance in order to establish any surgery-related effects. The rats were given two saline infusions to familiarize them with the infusion procedure.

Drug administration

The drug sessions were run no more than three times a week to allow the rats 2 days of recovery between sessions. The infusions were given in a volume of 0.4 μ l/side at a rate of 0.126 μ l/min through 28-gauge infusion cannulae. The infusion cannulae were connected via polyethylene tubing to 10 μ l Hamilton syringes held in a gear-driven Harvard instruments infusion pump. The rats were run on the maze 10 min after infusion.

In both studies the interactions of MLA and DH β E (RBI Sigma, Natick, MA, USA) were studied. The drugs were administered in a repeated-measure counter-balanced design in both studies so that each rat received every drug combination once in different order. Therefore, the possible impact of synchronized female cyclicity would not have been confounded with drug dose. In the first study, doses of MLA (0, 6.75, 13.5 and 27 μ g/side) were administered with and without DH β E (0 and 6.75 μ g/side). Drug solutions were made to the desired concentrations from salts in artificial cerebrospinal fluid, which served as the control infusion. The infusions were given at 0.4 μ l/side and the drugs were kept frozen between sessions and were allowed to reach room temperature before infusions. In the sec-

ond study, lower doses of DH β E (0, 1.69, 3.38 and 6.75 μ g/side) were administered with and without MLA (0 and 6.75 μ g/side) in a counter-balanced order. The rats were run on the 16-arm radial maze 10 min after drug administration.

Histology

After all drug sessions were complete, the rats were killed and the cannula placement was determined using histological methods. Once testing was completed, each rat was anaesthetized with sodium pentobarbital (50 mg/kg). A solution of saline and Chicago sky-blue dye was then infused, approximately 1 μ l into each cannula, in order to mark the cannulae placement and area of drug infusion. The rats were then perfused with a 9% phosphate-buffered saline solution followed by a 4% solution of formaldehyde. After perfusion, the brain was removed and stored in 4% formaldehyde. Histological slides were then made by freezing the brain on dry ice and taking slices on a cryostat. The slides were then studied to confirm cannula placement before including data for analysis. Only the rats with cannula placement within the ventral hippocampus were included in the data analysis. Placement of cannulae is shown in Fig. 1A, B. The rostrocaudal extent of the points of infusion extended from -3.0 to -3.4 mm anteroposterior.

Statistical analysis

The working memory errors, reference memory errors and latency were assessed by a within-subjects design analysis of variance. $P=0.05$ or lower (two-tailed) was considered significant. A P -value of between 0.05 and 0.1 was considered suggestive of an effect. Planned comparisons were made for vehicle vs. each dose of MLA and DH β E alone and the combinations of MLA with DH β E vs. MLA alone.

RESULTS

Study 1

Both DH β E and MLA caused a significant increase in working memory errors (Fig. 2). With MLA, a significant increase in working memory errors was seen with the high dose of 27 μ g/side ($F(1,30)=9.13$, $P<0.01$). The low MLA dose (6.75 μ g/side) did not cause a significant working memory impairment and the intermediate MLA (13.5 μ g/side) did not cause a significant increase in working memory errors ($F(1,30)=3.35$, $P<0.08$). There was a significant linear dose-effect of MLA ($P<0.05$). The DH β E dose used (6.75 μ g/side) caused a significant increase in working memory errors ($F(1,30)=10.87$, $P<0.005$). Interestingly, despite the significant impairments caused by both of these nicotinic antagonists, they did not show any sign of having additive effects. None of the comparisons of the MLA doses in combination with DH β E showed significant increases in working error rates compared with those doses of MLA alone (Fig. 2).

As shown in Fig. 3, there were also significant impairments in reference memory caused by MLA and DH β E. Interestingly, it was the low dose of MLA (6.75 μ g/side) that caused a significant increase in reference memory errors relative to control ($F(1,30)=4.46$, $P<0.05$), while the higher MLA doses did not cause significant impairments, suggesting that the MLA effect on reference memory was not very robust. As with working

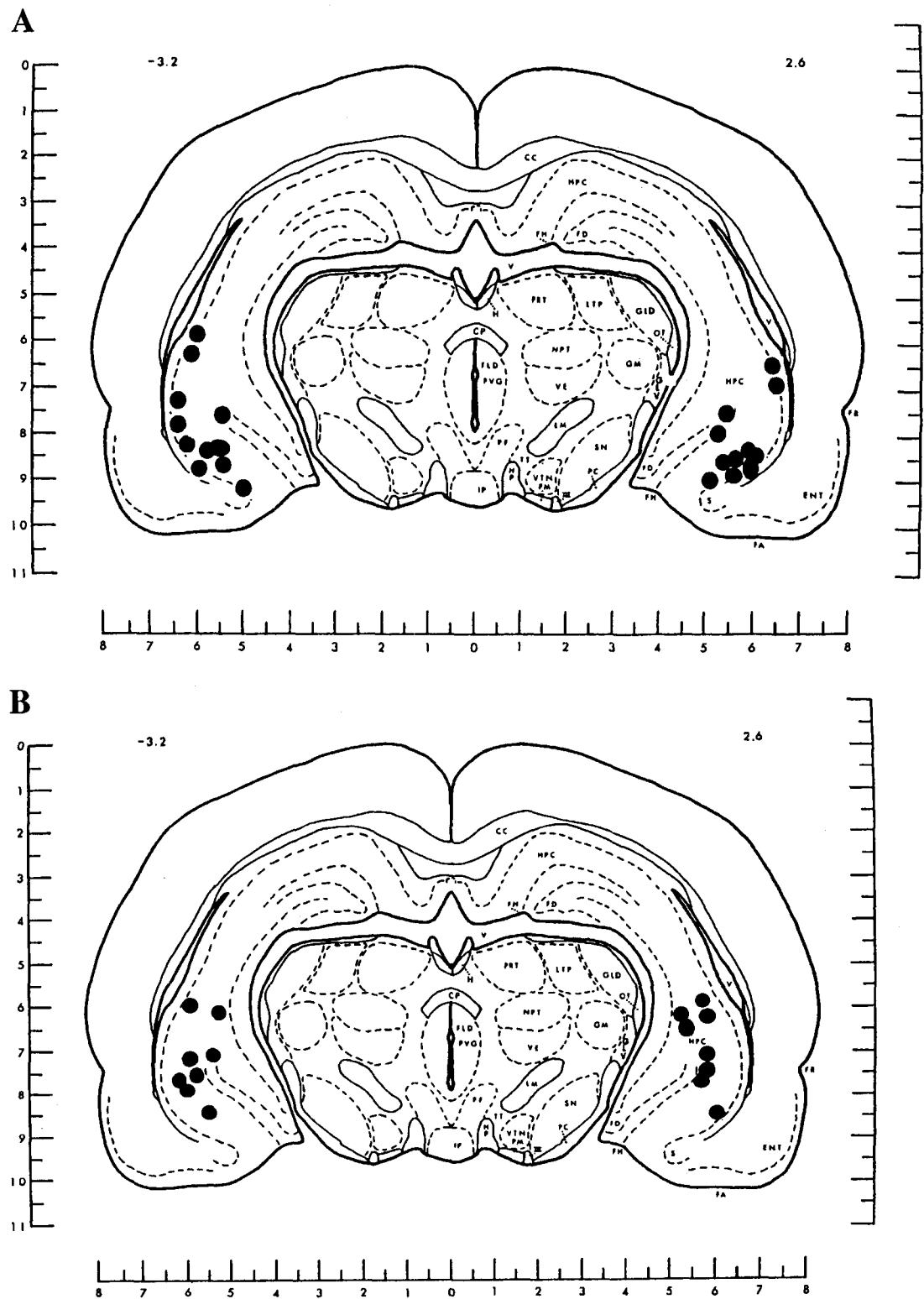


Fig. 1. Cannula locations within the ventral hippocampus (HPC). Axes are in mm in the Pellegrino rat brain atlas (Pellegrino et al., 1979). (A) Study 1; (B) study 2.

Ventral Hippocampal MLA-DH β E Interactions Radial-Arm Maze Working Memory Errors (study 1)

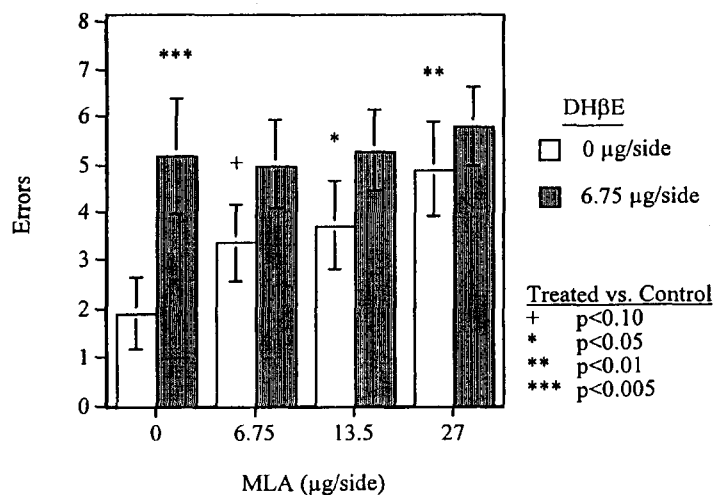


Fig. 2. Effects of hippocampal infusions of MLA and DH β E in study 1 ($n=11$) on working memory (mean $\pm$ S.E.M.) of rats in the 16-arm radial maze. Significant MLA-induced impairments were seen with the 13.5- ($P<0.05$) and 27- μ g/side ($P<0.01$) doses and with the 6.75- μ g/side DH β E dose ($P<0.005$). However, there was no indication of additive effects of the two drugs.

memory, the dose of DH β E used (6.75 μ g/side) caused a significant impairment in reference memory ($F(1,30)=4.46$, $P<0.05$), but this effect was much less pronounced than the DH β E effect on working memory. As with working memory none of the comparisons of the MLA doses in combination with DH β E showed significant increases in reference error rates compared with those doses of MLA alone (Fig. 3).

There were no significant effects of either drug on the latency of the rats in this experiment. In all of the dose conditions of MLA or DH β E or their combinations, response latency averaged between 18.8 and 21.0 s/entry.

After infusion, increased grooming behavior was witnessed in some rats. In our previous study (Felix and Levin, 1997), high doses of MLA and DH β E caused increases in wet-dog shakes. In the current study with

Ventral Hippocampal MLA-DH β E Interactions Radial-Arm Maze Reference Memory Errors (study 1)

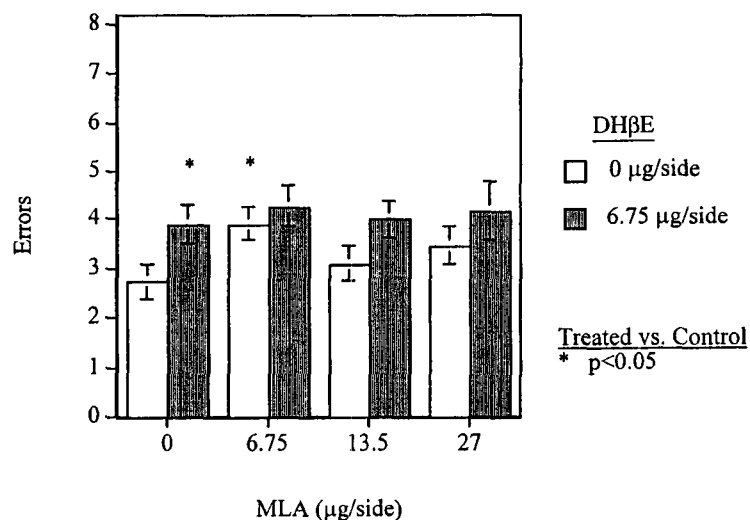


Fig. 3. Effects of hippocampal infusions of MLA and DH β E in study 1 ($n=11$) on reference memory (mean $\pm$ S.E.M.) in rats in the 16-arm radial maze. The 6.75- μ g/side doses of MLA and DH β E caused significant impairments relative to vehicle ($P<0.05$). However, unlike with working memory errors, significant effects were not seen with higher doses of MLA.

Ventral Hippocampal Low Dose DHβE-MLA Interactions
Radial-Arm Maze Working Memory Errors (study 2)

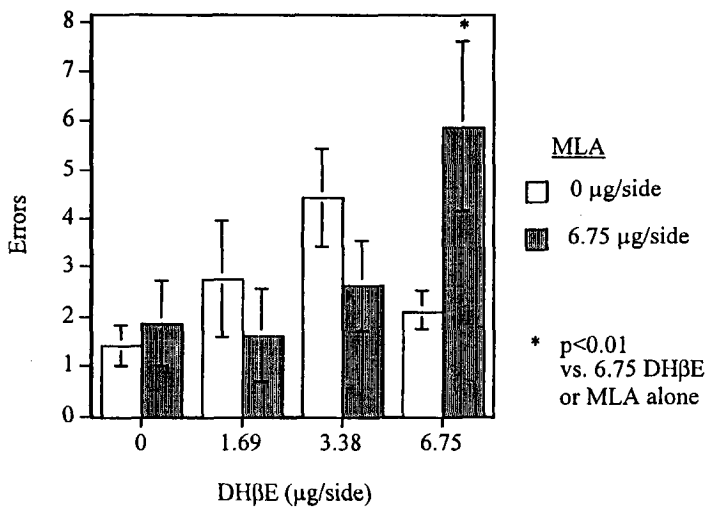


Fig. 4. Effects of hippocampal infusions of 6.75 μg/kg of MLA and DHβE on working memory in rats in the 16-arm radial maze (mean ± S.E.M.) in study 2 (n=8). The combination of 6.75 μg/side of DHβE and 6.75 μg/side of MLA significantly impaired ($P < 0.01$) working memory performance relative to either drug alone.

lower doses, wet-dog shakes were seen in only a single rat. This occurred with the highest dose of MLA (27 μg/side).

Study 2

The interaction of lower ventral hippocampal doses of DHβE with a threshold dose of MLA was assessed in study 2 to determine if the lack of additive effects seen in study 1 was due to a maximal level of impairment

reached. With working memory performance there was a significant interaction of DHβE and MLA ($F(3,21) = 3.75, P < 0.05$). As shown in Fig. 4, the combination of low doses of MLA and DHβE produced a significantly greater effect than either alone ($P < 0.01$). No significant working memory deficits were seen when the DHβE and MLA were given alone. With reference memory performance (Fig. 5), the interaction of DHβE and MLA was not quite significant. The main effect of MLA was not significant and the main effect of DHβE

Ventral Hippocampal Low Dose DHβE-MLA Interactions
Radial-Arm Maze Reference Memory Errors (study 2)

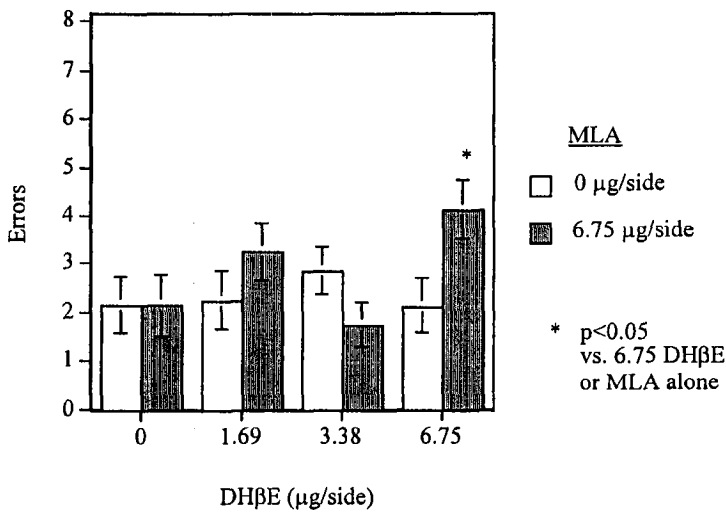


Fig. 5. Effects of hippocampal infusions of 6.75 μg/kg of MLA and DHβE on reference memory in rats in the 16-arm radial maze (mean ± S.E.M.) in study 2 (n=8). The combination of 6.75 μg/side of DHβE and 6.75 μg/side of MLA significantly impaired ($P < 0.05$) working memory performance relative to either drug alone.

was not quite significant ($P < 0.09$). There were no significant drug effects on latency.

Combined analysis of errors

Both the first and second study evaluated the impact of 6.75 $\mu\text{g}/\text{side}$ of MLA and DH β E on working and reference memory. An overall statistical analysis of the effect of this dose was conducted with study (study 1 and study 2) as a between subjects factor and MLA and DH β E treatments as within subjects factors. No rats participated in both studies. Individual treatment comparisons showed that relative to vehicle, 6.75 $\mu\text{g}/\text{side}$ of DH β E caused a significant ($P < 0.05$) working memory impairment. This was selective in as much as this dose of DH β E did not cause a significant reference memory impairment. In contrast to DH β E, 6.75 $\mu\text{g}/\text{side}$ of MLA did not significantly ($P = 0.31$) impair working memory performance relative to control. This dose of MLA also did not cause a reference memory impairment. The combination of 6.75 $\mu\text{g}/\text{side}$ of DH β E and MLA caused a significant working memory impairment relative to 6.75 $\mu\text{g}/\text{side}$ of MLA alone ($P < 0.01$), but did not cause a significant impairment relative to DH β E alone ($P < 0.07$). The combination dose significantly impaired reference memory performance relative to either MLA or DH β E alone ($P < 0.025$).

DISCUSSION

These results show that significant working memory deficits can be produced by either $\alpha 7$ or $\alpha 4\beta 2$ nicotinic receptor antagonist treatment in the ventral hippocampus. This replicates our earlier finding in the 8-arm radial maze (Felix and Levin, 1997). In that study, however, significant deficits caused by MLA and DH β E were not seen unless high doses were given. In the current study with the more challenging 16-arm maze, significant impairments were seen at lower doses of both MLA and DH β E. This reinforces the conclusion that antagonism of $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors are responsible for the working memory deficits observed.

Previously (Felix and Levin, 1997), we saw that higher doses of MLA and DH β E caused working memory impairments in the 8-arm radial maze. However, the doses of MLA and DH β E necessary to produce the choice accuracy deficit also caused significantly increased response latency and preconvulsant behavior. With the more challenging 16-arm maze in the current study we were able to demonstrate significant memory impairments caused by both MLA and DH β E at doses that did not cause latency and preconvulsant effects. Interestingly, there was a very low incidence of wet-dog shakes in the current even with the highest MLA dose given (27 $\mu\text{g}/\text{side}$). In our previous study (Felix and Levin, 1997), we saw this dose to produce wet-dog shakes in most rats. The difference may have derived from the repeated measures design used in these studies. In the previous study, the 27- $\mu\text{g}/\text{side}$ dose was given interspersed with much higher doses, which caused

more severe side effects. In the current study, this was the highest dose given. Sensitization of this type of preconvulsant behavior may have rendered the rats in the previous study more susceptible to wet-dog shakes at the 27- $\mu\text{g}/\text{side}$ dose.

The 16-arm radial maze permitted simultaneous assessment of working and reference memory. Both MLA and DH β E caused significant deficits in reference memory. However, these reference memory impairments were less pronounced than those seen with working memory. The effects on reference memory did not seem very robust. With MLA, the impairment did not increase with higher doses and in the overall analysis of both experiments, the 6.75- $\mu\text{g}/\text{side}$ dose of DH β E did not cause a significant effect on reference memory, even though it did significantly impair working memory performance, which was consistently dose-related (Fig. 2). The impairment produced by the 6.75- $\mu\text{g}/\text{side}$ dose of DH β E on working memory was apparent with the sample size of the first study and was also significant in the combined analysis using the larger sample size ($n = 19$) of both studies. The greater nicotinic antagonist-induced impairments in working vs. reference memory seen in the current study are consistent with our earlier research which found that systemic administration of the non-selective, non-competitive nicotinic antagonist mecamylamine produced deficits in working memory but not reference memory on the 16-arm radial maze (Levin et al., 1997).

The greater working memory load (12 locations vs. 8) in the current study compared with the earlier study (Felix and Levin, 1997) appeared to provide sufficient challenge to increase sensitivity to the memory impairments caused by low-dose MLA and DH β E hippocampal infusion. This increased sensitivity is important because it permits the demonstration of significant working memory impairment caused by either MLA and DH β E at doses which cause much less non-cognitive behavioral disruption, such as preconvulsant behavior. Only one rat in the current studies showed increased incidence of wet-dog shakes and the only other observed non-cognitive sign was an increase in grooming behavior in a few rats. The grooming was just an increase in this normally occurring component of the rats' behavioral repertoire and did not appear to interfere with maze choice accuracy. The principal effect of MLA and DH β E was to increase working memory errors. There was weaker evidence for increases in reference memory errors. This was seen only intermittently with no overall effect with MLA and a non-dose-related effect with DH β E. This is congruent with previous work with the non-selective, non-competitive nicotinic antagonist mecamylamine, which produced deficits in working memory but not reference memory on the 16-arm radial maze (Levin et al., 1997).

Even though both MLA and DH β E each caused significant working and reference memory deficits, at the higher doses (study 1), there was no evidence for additive effects in this study. The addition of the doses of MLA and DH β E did not produce either greater impairment in memory or greater frequency of other adverse effects as

do simple increases in doses of either of these drugs alone. This lack of additivity was not due to a ceiling effect on further increases in number of errors. There is no theoretical ceiling for the number of errors obtainable other than the number of arms that can be chosen within the maximum time allotted for the session (10 min). Another practical ceiling would be the number of errors expected if the rats were choosing arms randomly. Our Monte Carlo simulation of choice performance for 10 000 sessions in various-sized radial mazes has shown that the random chance rate of errors with 12 baited arms is 25.23. With other amnesic treatments such as the *N*-methyl-D-aspartate antagonist dizocilpine, substantially higher error rates result that do approach the random chance rates (Levin et al., 1998a). It may be the case that the doses used produced the maximum possible effect achievable with nicotinic blockade. However, our previous work has shown that further degradation in performance accuracy is possible with higher doses of both MLA and DH β E (Felix and Levin, 1997). In contrast, with the lower doses used in study 2, there was a greater memory impairment seen with combined MLA and DH β E exposure. In study 2 the combination of 6.75 μ g/side of MLA and DH β E caused greater working and reference memory impairment than either drug alone.

There are some interesting differences between studies 1 and 2. The 6.75- μ g/side dose of DH β E caused a significant working and reference memory impairment in study 1 but not in study 2. Combined analysis of the data from the two studies detected a significant working memory impairment with the 6.75- μ g/side dose of DH β E. The overall effect of 6.75- μ g/side dose of DH β E in both studies on reference memory did not reach significance. The difference between the studies with regard to working memory performance may be due to the fact that this is a near-threshold dose of DH β E. Clearly higher doses of DH β E, as tested in our previous study (Felix and Levin, 1997), caused robust memory impairments and lower doses, tested in study 2 of the current study, caused little if any effect. Since the 6.75- μ g/side dose is a just suprathreshold dose, it is not surprising that individual experiments may sometimes detect the effect and sometimes miss it. There may also be an impact of the repeated testing of doses in each study. In study 1, the 6.75- μ g/side dose of DH β E was

given repeatedly either alone or together with an equal or higher doses of MLA. In contrast, in study 2, the 6.75- μ g/side dose of DH β E was given only once alone and once together with an equal dose of MLA. The other treatments administered were lower doses of DH β E. There may be some carryover effect of the repeated 6.75- μ g/side dose of DH β E with MLA, which potentiated the effect when it was given alone. Both studies had a counter-balance design such that each rat received the dose combinations in a different order. This was done to make the dose unconfounded with order. The doses were given with at least a 2-day interval to attenuate carryover effects. Nonetheless, it is possible that carryover effects may have potentiated the effects DH β E.

The amnesic sites of action of MLA and DH β E within the hippocampus are currently unknown. Alkondon et al. (1999) have demonstrated the presence of functional α 7 and α 4 β 2 nicotinic receptors on hippocampal CA1 interneurons. They found that both MLA and DH β E increase the frequency and amplitude of GABAergic postsynaptic currents in CA1 interneurons. The ventral hippocampus is a vital region for the expression of the memory improvement caused by nicotine. Ibotenic acid lesions of this area block chronic nicotine-induced memory improvements (Levin et al., 1999b). Lesions in this area also block the amnesic effects of systemic administration of the nicotinic antagonist mecamylamine (Chambers et al., 1996). The current results demonstrate that low doses of either the α 7 antagonist MLA or the α 4 β 2 antagonist DH β E significantly impaired working memory function with little effect on non-cognitive behavior. The effects on working memory function were much more robust than suggestions of effects on reference memory. As in the earlier study with higher doses (Felix and Levin, 1997), the effects of these two drugs had similar effects in impairing working memory function, but did not have additive effects. Determining the relative roles of α 7 and α 4 β 2 nicotinic receptors in the hippocampus for memory function will be important for the development of novel nicotinic agonists for memory impairments such as seen in Alzheimer's disease.

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Research report

Nicotinic mechanisms of memory: effects of acute local DH β E and MLA infusions in the basolateral amygdala

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Abstract

Nicotine has been shown to improve working memory. The neural mechanisms underlying this effect are still being determined. The ventral hippocampus is critical for nicotinic effects on memory. Local ventral hippocampal infusions of either the nicotinic $\alpha 7$ nicotinic receptor antagonist methyllycaconitine (MLA) or the $\alpha 4\beta 2$ nicotinic receptor antagonist dihydro- β -erythroidine (DH β E) caused working memory impairments, but no additive effects were seen. Other areas, such as the amygdala, also likely play important roles in nicotinic effects on memory. Amygdalar lesions cause memory impairment and there is a dense concentration of nicotinic receptors in the basolateral amygdala. The current study used local basolateral amygdalar infusions of the nicotinic antagonists MLA and DH β E to determine the involvement of $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors in spatial working and reference memory. Rats ($n=8$) were trained in the 16-arm radial maze and were implanted with bilateral infusion cannulae into the basolateral amygdala. Acute infusions of MLA (6.75 μ g/side, $P<0.0005$) or DH β E (3.38 μ g/side, $P<0.025$) caused significant working memory impairments. When given together MLA and DH β E did not produce an additive effect. In fact, the 6.75 μ g/kg dose of DH β E produced a significant ($P<0.0005$) attenuation of the MLA-induced working memory impairment. Significant effects were not seen with reference memory or response latency. Nicotinic systems in the basolateral amygdala, as in the ventral hippocampus, are important for spatial working memory. In both the basolateral amygdala and the ventral hippocampus, MLA and DH β E individually caused working memory impairments. The lowest effective dose of DH β E was lower in the basolateral amygdala than in the ventral hippocampus. In both the basolateral amygdala and the ventral hippocampus, combined MLA and DH β E treatment did not produce additive working memory deficits. Unlike in the ventral hippocampus, the addition of DH β E to MLA in the basolateral amygdala significantly reduced the MLA-induced working memory deficit. © 2002 Elsevier Science B.V. All rights reserved.

Theme: Neural basis of behavior*Topic:* Learning and memory: pharmacology*Keywords:* Nicotinic; Antagonist; MLA; DH β E; Amygdala; Memory; Radial-arm maze**1. Introduction**

In animal models, nicotine effects on memory performance have been extensively demonstrated. Systemic administration of nicotine or other nicotinic agonists have been shown to improve working memory performance. This is clearly seen in the radial-arm maze where acute and chronic nicotine administration induced working memory

improvements [12]. In contrast, systemic administration of nicotinic antagonists, like mecamylamine, have been shown to produce working memory deficits (for review, see Ref. [15]). Further, co-administration of mecamylamine with chronic or acute nicotine blocks the nicotine-induced improvements [14]. In contrast to nicotinic effects on working memory, nicotine administration did not lead to a significant change in reference memory performance [12].

Given these nicotinic memory effects, it is clear that the nicotinic receptor system is important for memory function. Currently the receptor subtypes and anatomic locations of the nicotinic receptors critical for working memory are being explored. Previous studies have shown that

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nicotinic receptors in the ventral hippocampus are involved in working memory function [11,22]. In particular, we have shown that both the $\alpha 4\beta 2$ and $\alpha 7$ nicotinic acetylcholine receptor subtypes are involved. In the hippocampus, antagonism of either the $\alpha 4\beta 2$ or the $\alpha 7$ nicotinic receptors led to an impairment in working memory performance [5,13]. In addition to hippocampal work we have also begun to explore the memory involvement of the amygdala in recent studies.

The amygdala, like the hippocampus, has been shown to play an important role in memory function [19]. Classically, the amygdala has been shown to be important for aversively-motivated memory. Much of this work has been carried out using paradigms such as passive (inhibitory) avoidance. Martinez et al. found that disrupting electrical stimulation in the amygdala caused amnesia for passive avoidance [17]. This involves a long-term form of memory for aversive experience similar to reference memory. Others have also shown amygdalar involvement in memory consolidation during passive avoidance performance [23,28]. In addition, amygdalar involvement in non-aversively motivated memory has been demonstrated using several memory paradigms. Basolateral amygdala lesions in rats led to working memory impairments in paradigms such as delayed non-matching-to-sample and the three-panel runway appetitive task [21,24]. Working but not reference memory was impaired by basolateral amygdala lesions [21]. Systemic treatment with indirect cholinergic agonists or direct muscarinic agonists ameliorated the memory deficit. Aversive tasks may have a different neural basis in the amygdala. Blozovski and Dumery found that infusion of the nicotinic antagonist mecamylamine into the basolateral amygdala caused passive avoidance deficits in rats before day 30 after birth but not later [3]. Riekkinen Jr. et al. found that the passive avoidance deficits caused by amygdaloid lesions was not reversible by nicotinic or muscarinic treatment [30]. However, not all studies have found impairments due to amygdalar lesions. In some paradigms, such as spatial alternation, amygdala lesions did not lead to any significant changes in performance [33]. Overall, it appears that the amygdala is involved in certain aspects of memory and not others, as evidenced by several different studies.

The basolateral amygdala may be important in determining the reinforcing value of behavioral responses. Lesions of the basolateral amygdala impaired memory for the location of differential rewards on the radial-arm maze [10]. This role of the basolateral amygdala is supported by the finding that neurons in the basolateral amygdala differentially respond to reward magnitude on the radial-arm maze [29].

The current study was conducted to determine the memory involvement of the basolateral amygdala in memory performance on the eight-arm radial maze, a non-aversive appetitively motivated task. In addition to the amygdalar involvement mentioned earlier, the amygdala

has been shown to be involved in memory consolidation for reward tasks and for appetitively-motivated memory [20,25]. In previous tests, it was shown that excitotoxic amygdalar lesions caused significant impairments in radial-arm maze choice accuracy [4]. Furthermore, lesions of the basolateral amygdala in rats inhibit exploration on the eight-arm radial maze [6]. In the current study, we aimed to explore the specific receptors in the amygdala that may play a role in memory function.

Our motivation to explore the role of the $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors in the amygdala stems from our previous work with these receptors in the hippocampus and from other work exploring the possible mechanisms of amygdalar involvement in memory. Several studies have suggested the role of cholinergic activity as a mechanism by which the amygdala modulates memory [20,21,30]. This has been shown in both the muscarinic cholinergic and nicotinic cholinergic systems [20,21]. Since we have previously explored the role of the nicotinic cholinergic system in the hippocampus, we aimed to explore this system in the amygdala as well. The amygdala is also responsive to nicotine exposure. In fact, nicotinic stimulation increased Fos activity in the amygdala [18,31,34]. The basolateral amygdala receives cholinergic innervation from acetylcholine-containing neurons in the basal forebrain [32] and this innervation may be relevant to amygdalar roles in memory function [2,9,16,27,30].

To test the involvement of amygdalar nicotinic receptor systems in working memory, this study was carried out with local infusions of specific nicotinic cholinergic receptor antagonists. Methyllycaconitine (MLA) and dihydro- β -erythroidine (DH β E) are nicotine antagonists that have been used to study nicotinic receptors and their involvement in working memory. MLA is an $\alpha 7$ antagonist while DH β E is an $\alpha 4\beta 2$ antagonist [5]. In a previous study, when administered into the hippocampus, both drugs induced working memory deficits in rats on the radial arm maze [5]. Thus these receptors seem to be important with respect to working memory in the hippocampus. The current study continued to explore the effects of these nicotine antagonists on working memory in the amygdala.

2. Methods

2.1. Subjects

Adult female Sprague–Dawley rats ($n=8$) were used in this study. Rats that did not have cannula placements within the basolateral amygdala were eliminated from the study. Rats were housed three to a cage on a 12 h light/12 h dark cycle and were tested during the dark phase. Rats were first placed on ad libitum feeding and then were fed at 85% of their free-feeding levels. Following cannulation surgery, rats were housed singly.

2.2. Radial arm maze

Rats were tested on a 16-arm radial maze housed in a test room with a variety of visual cues. The entire maze was 30 cm above the ground, with a central platform 50 cm in diameter. The dimensions of the arms were 10×60 cm and each arm contained a food cup at the end farthest from the central platform. Clear Plexiglas barriers were placed between each of the 16 arms to prevent cross arm jumping.

During training, 12 of the 16 arms were baited with a half piece of Kellogg's Froot Loops®. During each training session, the same four arms were left unbaited for each individual rat. During a training session, rats were first placed on the central platform inside of an opaque plastic bucket for 10 s. Then the bucket was removed and the rats were allowed to explore the maze. An entry was recorded when all four of the animal's paws crossed the threshold of an arm. The rat was allowed a maximum of 600 s to complete the maze by entering every baited arm.

Working memory errors were recorded as the number of times the rat re-entered any of the arms that were baited at the beginning of the session. Working memory data was only recorded when no more than three baited arms were left not entered. Reference memory errors referred to the number times the rat entered the arms that were never baited. Latency was recorded as the total time to run the maze divided by the total number of entries.

2.3. Cannulation

Rats were anesthetized with an intra-peritoneal cocktail injection of 0.75 mg/kg of ketamine and 0.3 mg/kg of medetamine and were secured in the stereotaxic instrument (David Kopf Instruments, Tazunga, CA, USA). Cannulae (22 gauge, Plastics One, Roanoke, VA, USA) were inserted bilaterally at the following coordinates from bregma: AP -1.4, ML ±5.5, DV -7.5 and the bite bar was set at +5.0 mm [26]. Four screws were also drilled into the skull and a wire was placed around them to help anchor the cannulae at the completion of surgery. Cranioplastic powder and liquid was used to create a plastic cap to hold the cannulae in place. Dummy cannulae were then placed into the guide cannulae to prevent blockage or infection. After surgery, rats were revived with an intra-peritoneal injection of 0.3 mg/kg of antisedan.

2.4. Drug administration

Following surgery, the rats were allowed to recover for 1 week. Then the rats were tested on the radial arm maze task with no drug administration. Following this post surgery trial, the rats were then tested on the maze with drug administration. Infusions were carried out using a gear driven Harvard Instruments infusion pump. The drug was delivered bilaterally through two 10- μ l Hamilton

syringes to 28 gauge cannulae via a polyethylene tube. The drug was administered in a volume of 0.4 μ l per side at a rate of 0.126 μ l for 3 min and 10 s. The rats were then run on the maze 10 min after drug administration and this was repeated for all drug testing.

During the first two trials on the radial-arm maze, artificial cerebrospinal fluid (aCSF) was administered as a control. For DH β E testing, the animals received dosages of 0, 1.69, 3.38, and 6.75 μ g/side. During MLA treatment, the rats received either 0 or 6.75 μ g/side. In these drug solutions, DH β E and MLA were dissolved in aCSF. On trials with dual drug administration, the rats received 6.75 μ g/side MLA with 1.69 μ g/side DH β E, 6.75 μ g/side MLA with 3.38 μ g/side DH β E, and 6.75 μ g/side with 6.75 μ g/side DH β E. In dual drug administration, the drugs were administered as a cocktail. Each of the eight rats was given the eight experimental drug treatments (including control) in a repeated measures counterbalanced design. There was at least 1 day between each drug administration trial on the radial-arm maze. All drug solutions were refrigerated for storage when not in use.

2.5. Histology

After testing was completed, the rats were anesthetized with pentobarbital and infused with 0.5 μ l of Chicago sky blue dye in each cannula. The rats were then perfused with saline followed by formaldehyde. Following perfusion, the brains were removed, placed in formaldehyde, and then frozen. The brains were then sliced on a cryostat and placed onto slides. The cannula placement was then checked under a microscope with the Chicago blue dye serving as a visual marker for localization.

2.6. Data analysis

The choice accuracy (working and reference memory errors) and response latency measures were assessed by repeated measures analysis of variance, with factors of MLA and DH β E dose. Significance was assumed at α P <0.05 for the planned comparisons of the MLA and DH β E doses alone vs. vehicle and the DH β E doses alone vs. the DH β E doses plus MLA. Alpha levels between 0.05 and 0.10 were considered suggestive of an effect.

3. Results

3.1. Histology

All of the rats included in the data set (n =8) had bilateral cannula placements with infusion flow into the basolateral amygdala. Rats that did not have appropriate cannulae placements for flow into the basolateral amygdala were eliminated from the study. The tips of the cannulae were located at -1.4 AP, between 5.0 and 6.0 ML, and

between 8.0 and 9.0 DV (Fig. 1) measured from the cortical surface. The infused dye from the tips was consistently observed in the basolateral amygdala on both sides in all animals, but some overflow into the adjacent lateral amygdala was also observed in some animals.

3.2. Working memory

MLA infusion into the basolateral amygdala caused a significant [$F(1,21)=19.38$, $P<0.0005$] working memory impairment on the 16-arm radial maze (Fig. 2). The 6.75 $\mu\text{g}/\text{side}$ MLA dose increased working memory errors to 7.12 ± 2.98 (mean $\pm$ S.E.M.) vs. 3.25 ± 1.16 with vehicle infusion. DH β E also significantly [$F(1,21)=6.53$, $P<0.025$] impaired working memory performance at the 3.38 $\mu\text{g}/\text{side}$ dose (5.50 ± 2.51). Interestingly, despite the working memory deficit induced by DH β E alone, a dose of 6.75 $\mu\text{g}/\text{side}$ DH β E together with 6.75 $\mu\text{g}/\text{side}$ MLA significantly [$F(1,21)=23.31$, $P<0.0005$] attenuated the MLA-induced working memory impairment (Fig. 2). The addition of 6.75 $\mu\text{g}/\text{side}$ of DH β E reduced the MLA-induced working memory deficit from 7.12 ± 2.98 with 6.75 $\mu\text{g}/\text{side}$ of MLA alone to 2.88 ± 1.43 with 6.75 $\mu\text{g}/\text{side}$ of MLA plus 6.75 $\mu\text{g}/\text{side}$ of DH β E.

3.3. Reference memory

With reference memory, similar mean differences were

seen as with working memory errors (Fig. 3). However, the effects were more modest and lacked significance. The difference between reference memory errors after vehicle administration (3.88 ± 0.40) and 6.25 $\mu\text{g}/\text{side}$ of MLA alone (5.62 ± 1.08) was nearly significant ($P<0.07$). The addition of 6.25 $\mu\text{g}/\text{side}$ of DH β E to 6.25 $\mu\text{g}/\text{side}$ of MLA caused a nearly significant ($P<0.07$) reduction in reference memory errors to 3.88 ± 0.35 .

3.4. Latency

Response latency on the 16-arm radial maze was not significantly affected by these doses of either DH β E or MLA alone or in combination infused into the basolateral amygdala (data not shown).

4. Discussion

Acute basolateral amygdalar infusion of either DH β E or MLA individually induced working memory deficits in the radial-arm maze. Both $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors in the basolateral amygdala appear to be important for appetitively-motivated spatial working memory function. The effect was relatively specific to working memory. Reference memory performance was not significantly affected by DH β E. There was only a marginally significant MLA reference memory deficit and a marginally signifi-

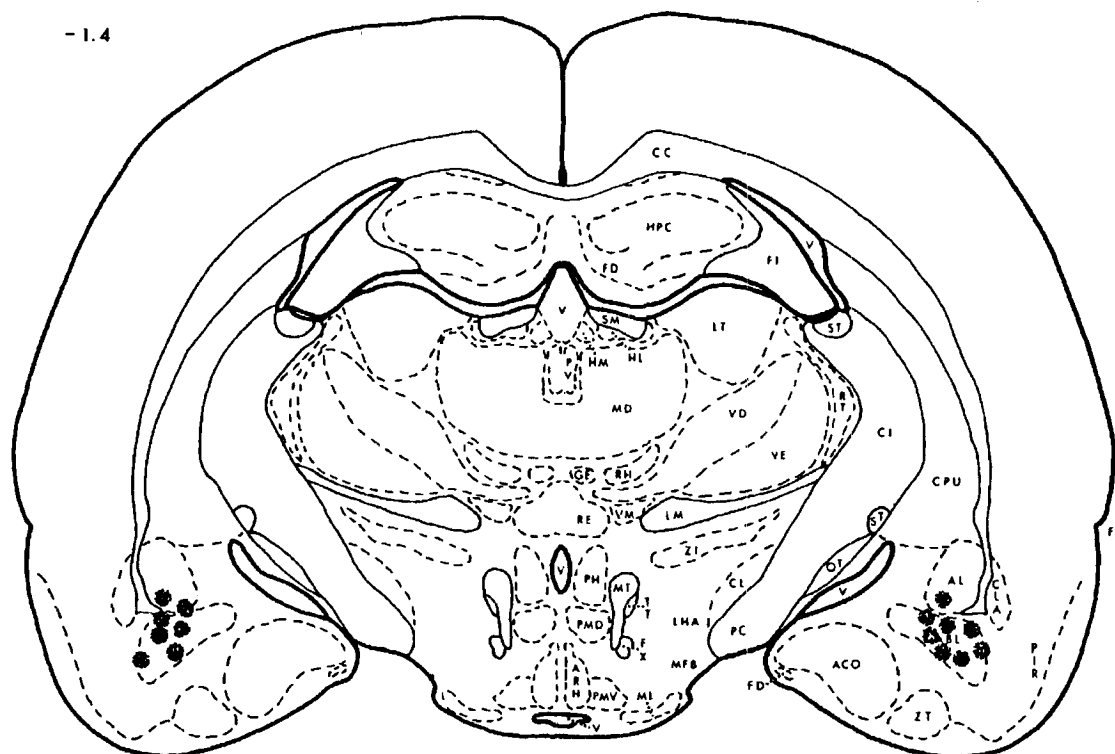


Fig. 1. Coronal diagram of the bilateral cannula placements [26] to the basolateral amygdala in this study ($n=8$).

Basolateral Amygdalar Infusion of MLA and DHbE Effects on Working Memory in the 16-Arm Radial Maze

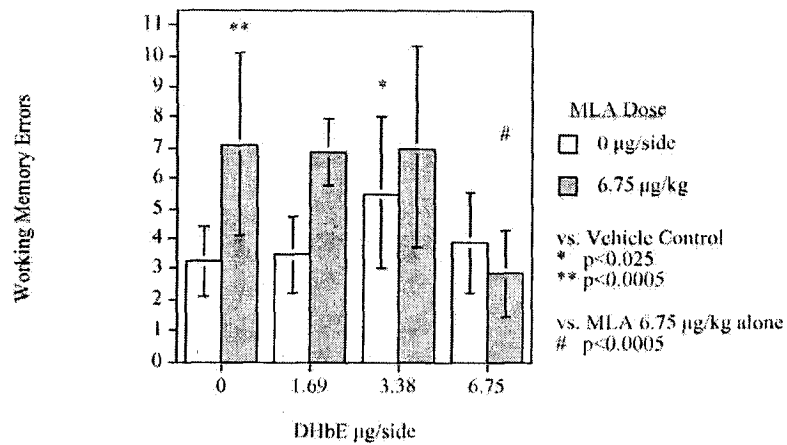


Fig. 2. Acute bilateral basolateral amygdalar DHbE and MLA infusion effects on working memory performance on the 16-arm radial maze (mean ± S.E.M.) ($n=8$). The 3.38 µg/side dose of DHbE ($P<0.025$) and the 6.75 µg/side dose of MLA each alone caused significant impairments relative to vehicle control. The two treatments did not have additive effects. Rather, DHbE (6.75 µg/side) significantly ($P<0.0005$) attenuated the impairment caused by MLA treatment.

cant reversal of that deficit with DHbE. While a larger study may have detected a significant effect of DHbE or MLA on reference memory, it is clear that these possible effects are much more subtle than the effects of the same drug doses on working memory. In a similar finding Ohno et al. found that working but not reference memory was impaired by basolateral amygdala lesions [21]. Neither DHbE nor MLA affected response latency. This specificity

does not support explanations of the choice accuracy data involving drug-induced motivation or sensorimotor changes. DHbE and MLA did not have additive effects with regard to working memory. In fact, despite the fact that both antagonists individually impaired working memory performance, DHbE significantly reduced the MLA-induced working memory deficit.

In interpreting these results, it is important to take into

Basolateral Amygdalar Infusion of MLA and DHbE Effects on Reference Memory in the 16-Arm Radial Maze

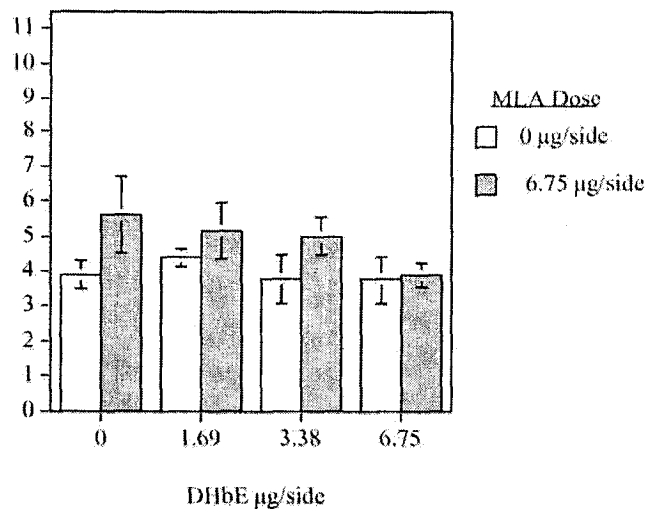


Fig. 3. Acute bilateral basolateral amygdalar DHbE and MLA infusion and reference memory performance on the 16-arm radial maze (mean ± S.E.M.) ($n=8$). No significant ($P<0.05$) effects were seen.

account the possibility that the infused drugs may have spread to other areas of the brain. All cannulae placements were located in the basolateral amygdala and were verified by infusion of 0.5 μ l of Chicago sky blue dye. The tips of the cannulae were located for bilateral infusion flow into the basolateral amygdala in all animals, but the dye was also observed in the adjacent lateral amygdala of some animals. Tissue damage from the infusions was not seen to be a problem. Even subtle tissue damage with repeated drug infusion would not have been confounded with the observed drug effects because the drugs were administered in a repeated measures counterbalanced design so that order of drug infusion was not confounded with dose. Thus, the data from this study supports the involvement of both $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors of the basolateral amygdala in working memory and suggest that their relationship is complex.

The data from the current study is important in light of our earlier work in the hippocampus. Previous studies in our laboratory have shown that ventral hippocampal infusions of DH β E and MLA also cause spatial working memory deficits on the radial-arm maze [1,5,13]. In a similar fashion much more modest effects were seen with reference memory [13]. Compared with the ventral hippocampus [13], a lower dose of DH β E administered in the current study to the basolateral amygdala was effective in producing a working memory deficit. DH β E and MLA given together had non-additive effects in both the ventral hippocampus study [13] and in the current basolateral amygdala study. The principal difference between DH β E–MLA interactions in the ventral hippocampus and basolateral amygdala is that DH β E co-administration attenuated the MLA-induced working memory impairment with infusion into the basolateral amygdala but not the ventral hippocampus. The data illustrates the differences in nicotinic function between the amygdala and hippocampus. While this differentiation does not directly illustrate interaction between these two structures, hippocampal–amygdalar connections may be important to consider in looking at the similarities and differences of nicotinic antagonist effects in the two areas.

The DH β E-induced attenuation of the working memory impairment caused by MLA in the basolateral amygdala also provides important information concerning possible mechanisms for $\alpha 4\beta 2$ and $\alpha 7$ interactions regarding working memory. This attenuation provides evidence that the deficits caused by DH β E and MLA are not merely due to cross activity of the two ligands to a common receptor. One mechanism that could explain this result would be that these receptor subtypes act on different neural types within the basolateral amygdala. Blocking either one may disrupt the balance in neural activity but the proper ratio of doses of both antagonists may restore the balance. Nicotinic systems are known to stimulate the efflux of a variety of neurotransmitters including ones with opposite actions on downstream neural stimulation, such as the inhibitory transmitter GABA and the excitatory transmitter glutamate

[35]. The amygdala itself includes a variety of transmitter systems including GABAergic, glutamergic, noradrenergic, dopaminergic, cholinergic, and opioid peptidergic systems [19]. Thus, studies of the interactive effects of $\alpha 4\beta 2$ and $\alpha 7$ antagonists with other receptor systems in the basolateral amygdala may provide important information concerning the complex nature of nicotinic effects on working memory.

While we explored cholinergic effects on memory through reversible nicotinic receptor blockade, others have explored the memory effects removing cholinergic input through lesion studies. Mallet et al. demonstrated that removal of cholinergic input to the amygdala leads to working memory impairment in the Y-maze [16]. In another study, Ohno et al. showed that basolateral amygdaloid lesions led to working memory impairment in the three-panel runway task [21]. It is interesting to note that in these two studies, as in our current study, working memory was impaired while there was no significant change in reference memory performance [16,21]. Given the similarities in working and reference memory effects between the three studies it is possible that the working memory impairments observed in the Mallet and Ohno studies may also resulted from a decrease in amygdalar nicotinic cholinergic activity.

In addition to looking at the amygdalar nicotinic cholinergic system, it is also important to consider the muscarinic cholinergic system. We have clearly demonstrated that direct antagonism of amygdalar cholinergic receptors leads to working memory performance impairments. In a previous study by McIntyre et al., muscarinic antagonism via scopolamine did not lead to a significant change in working or reference memory performance on the radial-arm maze [20]. Thus, it appears that there is a difference in nicotinic and muscarinic effects on memory performance on the radial-arm maze. While we have not focused on the differences in nicotinic and muscarinic effects, further study of their involvement through future studies will allow for a better understanding of the mechanism of amygdalar involvement in memory.

Overall, these data point to the involvement of basolateral amygdalar $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors in working memory. Like the ventral hippocampus, the basolateral amygdala appears to be an important site for nicotinic involvement in memory. Although both $\alpha 4\beta 2$ and $\alpha 7$ receptors in the basolateral amygdala appear to be critically involved in working memory, the impairments caused by local infusions of either DH β E or MLA suggest that their effects appear to be mediated via different downstream mechanisms because DH β E actually attenuates MLA-induced working memory deficits. A full understanding of the $\alpha 4\beta 2$ and $\alpha 7$ nicotinic interactions in the basolateral amygdala in memory processes may involve their interactions with amygdalar monoaminergic, NMDA and GABA systems as well as related limbic areas known to be important for memory function [7,8]. Through future studies, we hope to gain a better understanding on the

neural mechanisms involved in nicotine effects on memory.

Acknowledgements

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Patente de Invención ☒ Patente de Modelo de Utilidad ☐

Vía Nacional ☐

Vía PCT (fase nacional) ☒ Cap. I ☐ Cap. II ☒

No. Sol. Internacional PCT/FR03/00004
Fecha de Presentación Internal. 03-01-2003

Como resultado del examen definitivo, realizado con fundamento en el artículo 48 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

<u>Descripción:</u>	Folios: 92 a 116	(como se radicó inicialmente)
	Folios 163	(modificación).
	Folios 180 a 204	(modificación)

<u>Reivindicaciones:</u>	Folios 117 a 118	(como se radicó inicialmente)
	Folios 164 a 166	(modificación).
	Folios 205 a 206	(modificación).

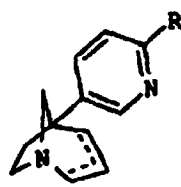
Rta. del Solicitante: Folios: 171 a 178

Prioridad: Folio 1
Fecha: 07-01-2002, país número: FR02/00109

2. Objeto de la invención

- Título de la solicitud: "DERIVADOS DE 5-(PIRIDIN-3-IL)-1-AZABICICLO [3.2.1] OCTANO, SU PREPERACIÓN Y SU APLICACIÓN EN TERAPÉUTICA".

El objeto de la presente solicitud de patente de invención consiste en: Derivados de la fórmula (I)



Reivindicaciones 1- 3: Un compuesto de la fórmula (I)

Reivindicación 3: compuestos derivados del [3.2.1] octano.

Reivindicaciones 4 a 5: Un medicamento que contiene los compuestos.

El problema planteado en esta solicitud es la necesidad de compuestos útiles para el tratamiento o la prevención de los desórdenes relacionados con una disfunción de los receptores nicotínicos, en especial al nivel del sistema nervioso central.

Para solucionar este problema técnico la solicitud en estudio proporciona una composición que contiene un derivado de 5-(piridin-3-il)-1-azabicyclo[3.2.1]octano.

Clasificación IPC: A61K31/437; A61K31/439; A61K31/444

3. De la solicitud de Patente

a. Reivindicaciones (artículo 30 D 486)

la reivindicación 1 establece que R puede ser fenilo y que este puede estar sustituido por uno o mas grupos elegidos entre un listado contenido en la misma reivindicación, en dicho listado no es claro cuales son los sustituyentes del grupo fenilo y cuales son los demás significados de R. La reivindicación 2 aclara que el anillo fenilo puede estar sustituido solo por halógeno, alquilo (C₁-C₆), alcoxi (C₁-C₆), nitro, trifluorometilo, acetilo o metilendioxi. En consecuencia el solicitante debe establecer en forma clara el fin del listado de los sustituyentes del grupo fenilo en la reivindicación 1 introduciendo una expresión luego de la palabra metilendioxi como: "R también puede representar".

La reivindicación 3 presenta un grupo de compuestos que no están relacionados con los presentados en la reivindicación 1, puesto que la reivindicación 1 establece que R puede ser un grupo fenilo sustituido por uno o más grupos. Sin embargo ninguno de los compuestos de la reivindicación 3 presenta sustitución en el anillo fenilo. En consecuencia el solicitante debe establecer cuáles son los compuestos que realmente resuelven en problema planteado en la solicitud bien sea aquellos en los que el grupo fenil esta sustituido o aquellos en los que este grupo no presenta sustitución.

En consecuencia las reivindicaciones mencionadas no cumplen con el requisito claridad o sustento por la descripción de acuerdo al art. 30 de la Decisión 486.y/o art.22 Tratado PCT.

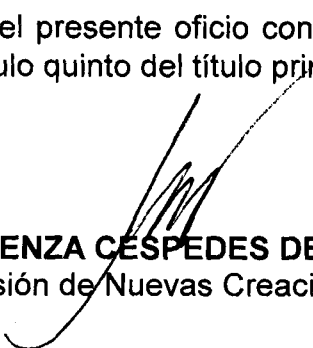
4. Respuesta a los Argumentos del Solicitante

El solicitante presenta un capítulo reivindicatorio con 5 reivindicaciones contenido en los folios 205 a 206. Esta oficina establece que este documento no implica una ampliación de la materia contenida en la solicitud original y en consecuencia es aceptado para el estudio de patentabilidad.

El solicitante presenta una tabla anexa en donde aparecen los valores de Cl_{50} frente a los receptores de nicotina que tienen subunidades $\alpha_4 \beta_2$ y α_7 con el fin de subsanar la objeción interpuesta sobre cuales son los compuestos que presentaron dicha actividad. Esta oficina acepta esta información y considera que la misma no amplía la solicitud tal y como fue presentada originalmente y se da por subsanada la objeción interpuesta.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha 08 MAYO 2009

CONCEPTO TÉCNICO No. 986	EXAMINADOR TÉCNICO Código No. 202073
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