

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 13-278000- -1	FECHA: 2014-04-23 10:18:35
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 14
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
ALICIA LLOREDA RICAURTE

Asunto: Radicación: 13-278000- -1
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 4761
 Folios: 14

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/SE2009/051493				
Fecha de Presentación Internacional	22/12/2009				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado 13-278000-00000-0000	26/noviembre/2013
Reivindicaciones:	Radicado 13-278000-00000-0000	26/noviembre/2013
Prioridad:	US 61/140,673	24/marzo/2008

2. Análisis de la Prioridad

Se acepta la reivindicación de la prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el

presente caso se estudiarán las reivindicaciones 1 a 4 del capítulo reivindicatorio radicado bajo N° 13-278000-00000-0000).

Título de la solicitud: “Compuestos etanamínicos y su uso para tratar la depresión.”

El problema planteado en esta solicitud consiste en proporcionar compuestos etanamínicos, sus sales y enantiómeros para trastornos depresivos.

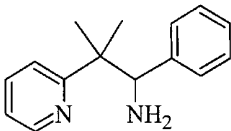
Para resolver este problema técnico la solicitud en estudio proporciona un compuesto que se selecciona entre: 1-(3 5 -fluorofenil)-2-metil-2-(piridin-2-il)propan-1-amina; (R)-1-(3-fluorofenil)-2-metil-2-(piridin-2-il)propan-1-amina; (S)-1-(3-fluorofenil)-2-metil-2-(piridin-2-il)propan-1-amina, 2-metil-1-fenil-2-(piridin-2-il)propan-1-amina; (R)-2-metil-1-fenil-2-(piridin-2-il)propan-1-amina; o (S)-2-metil-1-fenil-2-(piridin-2-il)propan-1-amina; o una sal farmacéuticamente aceptable de cualquiera de los compuestos anteriores.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D 213/38.

4. Presentación de Solicitud Fraccionaria (Art 36 D 486)

Se le informa a la solicitante que la solicitud divisional es aceptada a trámite porque porque no implica ampliación de la materia contenida en la solicitud inicial, sin embargo cabe aclarar que existe solapamiento de la materia reclamada en la reivindicación independiente 1, con materia que ya fue objeto de estudio de fondo y decisión mediante la Resolución No. 60353 del 17 de octubre de 2013 en la solicitud originaria, tal y como se evidencia en la siguiente tabla.

Tabla 1

Solicitud Original Radicado 11-081545-00006-0000	Solicitud Original Radicado 11-081545-00010-0000	Solicitud divisional Radicado 13-278000-00000-0000
Reivindicación 1 Un compuesto que es 2-metil-1-fenil-2-(piridin-2-5 il)propan-1-amina de la siguiente estructura:  o una sal farmacéuticamente aceptable del mismo	Reivindicación 1 Un compuesto que es 2-metil-1-fenil-2-(piridin-2-5il)propan-1-amina o una sal farmacéuticamente aceptable del mismo.	Reivindicación 1 Un compuesto que se selecciona entre:1-(3 5 -fluorofenil)-2-metil-2-(piridin-2-il)propan-1-amina;(R)-1-(3-fluorofenil)-2-metil-2-(piridin-2-il)propan-1-amina;(S)-1-(3-fluorofenil)-2-metil-2-(piridin-2-il)propan-1-amina; 2-metil-1-fenil-2-(piridin-2-il)propan-1-amina; (R)-2-metil-1-fenil-2-(piridin-2-il)propan-1-amina; o (S)-2-metil-1-fenil-2-(piridin-2-il)propan-1-amina;o una sal farmacéuticamente aceptable de cualquiera de los compuestos anteriores.

Por lo cual este Despacho sugiere a la solicitante excluir la materia que ya fue objeto de decisión en la solicitud original.

5. De la Solicitud de Patente

Titulo

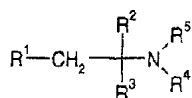
Dado que el título de una solicitud debe coincidir con el objeto revelado en la descripción, debe ser breve y descriptivo, y en este caso debe diferenciar la materia de la solicitud divisional con la de la solicitud originaria, este Despacho se permite sugerir a la solicitante como título para su solicitud divisional el siguiente: compuestos derivados de la 2-metil-1-fenil-2-(pirimidin-2-il)propan-1-amina, enantiómeros y sales de los mismos, composiciones que lo comprenden, útiles en el tratamiento de la depresión.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad más antigua: marzo 24 de 2008 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	MX PA01009448	"Uso de ciertos antagonistas de n-metil-d-aspartato de afinidad como antidepresivos"	06/agosto2002
D2	The Journal of Pharmacology and Experimental Therapeutics Vol. 297, No. 3	"Structural Modifications to an N-Methyl-D-aspartate Receptor Antagonist Result in Large Differences in Trapping Block"	13/septiembre/2007

El documento D1^[1] divulga antagonista de NMDA de baja afinidad para el tratamiento de la depresión de fórmula (I) (Pág. 4, líneas 4 a 19)



Compuesto I

El documento D2^[2] divulga 23 compuestos antagonistas de baja afinidad de NMDA estructuralmente relacionados con el diclorhidrato de la (S)-alfa-fenil-2-piridinetanamida (AR-R15896AR) (Pág. 5, tabla 1)

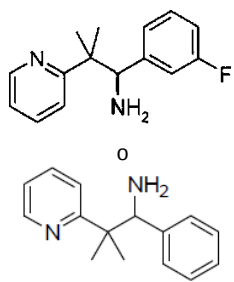
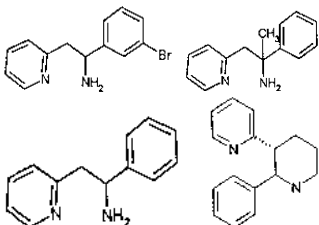
7. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art 18 D486)

La reivindicación 1 consiste en: "Un compuesto que se selecciona..." (Pág. 6 del radicado N°. 13-278000-00000-0000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

1: http://worldwide.espacenet.com/publicationDetails/originalDocument?FT=D&date=20020806&DB=EPODOC&locale=en_EP&CC=MX&NR=PA01009448A&KC=A&ND=2
2: <http://jpet.aspetjournals.org/content/297/3/906.full.pdf>

Características esenciales	D1	D2
Un compuesto seleccionado entre  o sus enantiómeros o una sal farmacéuticamente aceptable de cualquiera de los compuestos anteriores	Un compuesto de fórmula (I) $R^1-CH_2-\overset{\overset{R^2}{ }}{\underset{\underset{R^3}{ }}{C}}-N\overset{\overset{R^5}{ }}{\underset{\underset{R^4}{ }}{}}$ Compuesto I donde R¹ es piridilo; R² es fenilo o 4-fluorofenilo; R³ R⁴ y R⁵ son hidrógeno (Pág. 4, líneas 4 a 19)	Un antagonista de baja afinidad de NMDA que comprende entre otros a los siguientes compuestos:  (Pág. 5, tabla 1)

El documento D1 se considera el estado de la técnica cercano a la materia reclamada definida en las reivindicaciones 1 porque divulga compuestos derivados de la (S)-alfa-fenil-2-piridinetanamina y otros compuestos antagonistas NMDA de baja afinidad.

La diferencia entre la materia reclamada, definida en las reivindicaciones 1 y los compuestos divulgados en el documento D1 consiste en que los compuestos de la solicitud presentan como sustituyentes en la posición 2 del grupo etanamina 2 grupos metilo mientras que en el documento D1 son hidrógeno.

El efecto que logra el remplazar los dos hidrógeno en la posición 2 del grupo etanamina en los derivados de la (S)-alfa-fenil-2-piridinetanamina es obtener antagonista NMDA de baja afinidad.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo obtener obtener antagonista NMDA de baja afinidad alternativos.

Sin embargo el documento D2 da a conocer derivados de la (S)-alfa-fenil-2-piridina-etanamina donde el átomo de hidrógeno en la posición 1 de la cadena alifática es sustituido por un grupo metilo del grupo etanamina que se encuentra entre los anillos 2-piridinilo y fenilo permite obtener compuestos alternativos inhibidores de NMDA.

En consecuencia, la persona del oficio normalmente versada en la materia estaría motivada a remplazar en los compuestos divulgados en el documento D1 los átomos de hidrogeno por grupos metilo de la manera que lo sugiere el documento D2 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Puesto que las reivindicaciones dependientes 2 a 4 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

8. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	MX PA01009448	1-4	Nivel inventivo
D2	The Journal of Pharmacology and Experimental Therapeutics Vol. 297, No. 3	1-4	Nivel inventivo

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 (60) días contados a partir del día siguiente 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.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

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 No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-04-29

Elaboró: Lenin Roberto Guerrero

Revisó: Consuelo Leguizamon Leguizamon

Structural Modifications to an *N*-Methyl-D-aspartate Receptor Antagonist Result in Large Differences in Trapping Block

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ABSTRACT

Differences in the degree of trapping of initial block by *N*-methyl-D-aspartate (NMDA) receptor antagonists may affect their safety and, hence, suitability for clinical trials. In this comparative study, 23 compounds structurally related to the low-affinity, use-dependent NMDA receptor antagonist (S)- α -phenyl-2-pyridineethanamine dihydrochloride (AR-R15896AR) were examined to determine the degree of trapping block they exhibit. Compounds were tested at concentrations that produced a comparable initial 80% block of NMDA-mediated whole-cell current in rat cortical cultures. A wide range of values of trapping block was found, indicating that trapping is not an all-or-none event. Fifteen of the compounds trapped significantly more than the $54 \pm 3\%$ of initial block trapped by AR-R15896AR. The off-rates of these compounds were slower than that of AR-R15896AR. Only 2 of the 23 compounds

trapped significantly less than AR-R15896AR. AR-R15808, the piperidine analog of AR-R15896AR, appeared to trap only $8 \pm 3\%$ of its initial block, although its fast off-rate confounded accurate quantification of trapping. AR-R26952, which, like AR-R15896AR, contains a pyridine in place of a phenyl group, trapped $40 \pm 5\%$ of its initial block and exhibited kinetics comparable with AR-R15896AR. Structure-activity analysis suggested that the presence of two basic nitrogen atoms and decreased hydrophobicity led to decreased trapping. There was no correlation between trapping and lipophilicity as would be expected if closed-channel egress was due to escape through the lipid bilayer. However, there was a positive correlation between off-rate and degree of trapping. Models that can account for partial trapping are presented.

Some neuropathological states, like epilepsy, are characterized by excessive activation of receptors and cells, in terms of frequency or duration. Periods of excessive activation, prolonged ischemic depolarization, or multiple spreading depression-like periods also characterize ischemia. Some of the most useful anticonvulsants, like phenytoin, are uncompetitive, or use-dependent antagonists (Meldrum, 1993). Use-dependent compounds can only access their site of action when the receptor/channel is in an active state, resulting in a delay between receptor activation and the antagonist-induced blockade. Therefore, they are less likely to block short periods of activation, which typify normal activity. This characteristic of use-dependent compounds probably accounts for their clinical utility to block convulsant episodes without severely affecting normal function.

Antagonists of the NMDA type of excitatory amino acid receptors are neuroprotective in the *in vitro* and *in vivo* models of ischemia (Kemp et al., 1987; Olney et al., 1989). In

addition, cerebral ischemia has been associated with an elevation of extracellular concentrations of excitatory amino acids (Benveniste et al., 1984). However, the clinical use of NMDA antagonists has been hampered by the occurrence of side effects that appear to be a class effect (Small and Buchan, 1997). Hallucinations and dysphoria of the type produced by PCP appear to be due to blockade of normal activity mediated by NMDA receptor activation.

NMDA receptor antagonists that act at the PCP site produce a use-dependent block, acting only after the receptor enters the open state (Huettner and Bean, 1988). Unexpectedly, many of these use-dependent antagonists, like MK-801, also have no therapeutic value due to PCP-like neurobehavioral side effects (Leppik et al., 1988). Many of these compounds have high affinity for the receptor, whereas some lower affinity derivatives have reduced side effects. It was hypothesized that the lack of safety of high-affinity compounds was due to the fact that the concentrations needed to reach steady-state block rapidly enough to be therapeutic (via mass action) are much higher than those that already completely block receptor activity (Rogawski, 1993). For the

This work was funded by the National Research Council of Canada and AstraZeneca Canada, Inc.

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; MK-801, dizocilpine; ADCl, 5-aminocarbonyl-10,11-dihydro-5*H*-dibenzo-[a,d]cyclohepten-5,10-imine; AR-R15896AR, (S)- α -phenyl-2-pyridineethanamine dihydrochloride; MEM, Eagle's minimal essential medium; 9-AA, 9-aminoacridine.

lower affinity compounds, the concentrations that only partially modulate receptor activity are similar to those needed to achieve rapid block. In support of this hypothesis, memantine (Muller et al., 1995; Parsons et al., 1995), amantadine (Parsons et al., 1995), and ADCI (Rogawski et al., 1991, 1995), all of which have relatively low affinity (Chen et al., 1992; Parsons et al., 1993, 1995), lack serious side effects.

However, a comparison of a series of uncompetitive NMDA receptor antagonists for efficacy as antiepileptic drugs demonstrated that some lower affinity antagonists actually had worse therapeutic indices (Parsons et al., 1995), suggesting that other factors are also important. Trapping, a feature of many use-dependent NMDA receptor antagonists, may account for these neurobehavioral side effects. Antagonists such as MK-801, ADCI, memantine, amantadine, ketamine, and AR-R15896AR all exhibit trapping (Jones and Rogawski, 1992; Blanpied et al., 1997; Chen and Lipton, 1997; Mealing et al., 1997, 1999; Lanthorn et al., 2000; Sobolevsky and Yelshansky, 2000). Trapping occurs when agonist is removed while antagonist is present, and the antagonist remains bound to the receptor. During subsequent receptor activation, the trapped antagonist is already present, blocking activity immediately. Thus, a trapped antagonist functionally loses its use-dependence and produces a tonic block. With compounds like MK-801 and ADCI, trapping appears to be complete (Jones and Rogawski, 1992). However, in the case of the clinically safer compound, memantine, about one sixth of the blocked channels release, rather than trap, the blocker (Blanpied et al., 1997). We have recently reported significant differences in the degree of trapping among three low-affinity, use-dependent NMDA receptor antagonists that possess intermediate kinetics (Mealing et al., 1999). Ketamine, which induces psychotomimetic side effects (Ginski and Witkin, 1994; Krystal et al., 1994), showed 86% trapping, whereas memantine and AR-R15896AR, which have improved therapeutic safety profiles (Parsons et al., 1995; Palmer et al., 1996, 1997), trapped only 71% and 54%, respectively. This reduction in trapping, from ketamine to memantine to AR-R15896AR, is in the same order as that for reduction of severity of side effects as measured by behavioral and cognitive tests in rodents. Therefore, use-dependent NMDA receptor antagonists that exhibit less trapping may provide safer compounds for therapeutic use.

The purpose of this study was to examine a series of structurally related compounds (analogs of AR-R15896AR) to see if differences could be found in the degree of trapping block. We then looked for correlations between these differences and the physical and structural characteristics of the compounds.

Materials and Methods

Compound Selection. Starting from a library of 131 compounds, a diverse subset of clusters of compounds structurally related to the NMDA receptor antagonist, AR-R15896AR (formerly called ARL 15896AR or FPL 15896AR), were generated. The diversity metrics used for creating these clusters were two-dimensional fingerprints, molecular weight, and dipole total. These parameters were calculated from the compounds' chemical structures using SYBYL 6.7 software (Tripos, Inc., St. Louis, MO). LogP and pK_a values, computed using ACD Software (Advanced Chemistry Development, Inc., Toronto, Ontario, Canada), were also included in the cluster creation criteria. Clusters were generated using Wards clustering algorithm

(JMP software; SAS Institute, Inc., Cary, NC). Twenty-one compounds, each one selected arbitrarily from a different cluster, were tested to determine the degree of trapping they exhibited. Compounds were not selected from all clusters generated because of their unsuitability (for example, their weak antagonism). Two additional compounds, AR-R15808 and AR-R26952, were subsequently chosen to examine the impact of specific structural features (the presence of two basic nitrogen atoms) on trapping. All compounds tested were previously synthesized during the development of AR-R15896AR.

ACD_logP is the calculated LogP (octanol/water partition coefficient) for the neutral form of a molecule, accurate to ± 0.3 U or better. The calculation, made using ACD software, is based on an algorithm that uses a database containing one or more experimental LogP values for over 3600 structures with 500 different functional groups. ACD_ pK_a is the calculated acid-base ionization constant (pK_a value) for an organic structure at 25°C and zero ionic strength in an aqueous solution. The accuracy of the calculation is usually better than ± 0.2 units. This calculation is also based on ACD's database and algorithms. ACD_LogD is the calculated apparent LogP value at pH 7.4.

Chemicals and Reagents. Dulbecco's phosphate-buffered saline, HEPES, Eagle's minimal essential medium (MEM), poly-L-lysine, tetrodotoxin, and strychnine hydrochloride were purchased from Sigma Chemical (St. Louis, MO). Heat-inactivated fetal bovine serum was purchased from Life Technologies (Gaithersburg, MD), and heat-inactivated horse serum was purchased from Hyclone Laboratories, Inc. (Logan, UT). NMDA, ($\pm$)-2-amino-5-phosphonopentanoic acid, and ketamine were purchased from RBI/Sigma (Natick, MA). EGTA was purchased from Fluka Chemical Corp. (Ronkonkoma, NY). NMDA receptor antagonists were synthesized by AstraZeneca USA (Worcester, MA).

Cell Culture. Rat cortical neurons isolated from E18 fetuses were grown in primary culture as previously described (Black et al., 1995). Timed-pregnant Sprague-Dawley rats were purchased from Charles River Canada (St. Constant, PQ, Canada). After killing the mother by cervical dislocation under halothane anesthesia, the fetuses were removed from the uterus on day E18, their brains removed and placed in ice-cold phosphate-buffered saline, and the cortices isolated. The cortical neurons were dispersed by triturating with a 10-ml pipette, and the cells were centrifuged at 250g for 5 min at 4°C. The cells were gently resuspended in plating medium and viable cells, as determined by trypan blue exclusion, were counted. The cells were then plated at 10^5 cells/cm² on poly-L-lysine-coated 35 mm culture dishes (Nunc, Roskilde, Denmark) in 2 ml of plating medium at 37°C in an atmosphere of 5% CO₂/95% air. Plating medium consisted of 80% MEM, with 20 mM glucose, 10% heat-inactivated fetal bovine serum, and 10% heat-inactivated horse serum containing 2 mM L-glutamine. Since the cultures contained both neurons and glial cells, they were treated with 15 mg/ml of 5-fluoro-2'-deoxyuridine and 35 mg/ml on day 5 to minimize glial cell proliferation. On day 6, half of the medium was removed and replaced with growth medium consisting of 90% MEM and 10% horse serum. Experiments were performed on cultures after 14 to 19 days in vitro.

Whole-Cell Recording. Cortical neurons grown on 35-mm culture dishes were mounted on the stage of an inverted Zeiss microscope equipped with Hoffman modulation optics in a perfusion system flowing continuously at 1 ml/min at 22°C. The bathing solution contained 140 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 10 mM HEPES, and 3 mM glucose at pH 7.4. Bathing solutions also contained 1 μ M tetrodotoxin to block Na⁺ currents, 10 μ M glycine to saturate the glycine site on NMDA receptors, and 1 μ M strychnine to block glycine-activated Cl⁻ currents.

Patch pipettes (2–4 M Ω resistance) were constructed from 1.5 mm o.d./1.0 mm i.d. Pyrex 7740 glass (Corning, Big Flats, MN) using a Brown-Flaming P80 micropipette puller (Sutter Instrument Co., San Francisco, CA). The pipette solution contained 140 mM CsCl, 1.1 mM EGTA, 10 mM HEPES, and 2 mM Mg-ATP at pH 7.2.

Whole-cell currents were acquired using an Axopatch 1-D ampli-

fier equipped with a CV-4 headstage with a 1 G Ω feedback resistor (Axon Instruments, Foster City, CA). Voltage command and current acquisition were accomplished using a personal computer equipped with a Digidata 1200 interface and pClamp 6.0 software (Axon Instruments). Data were filtered at 1 kHz and sampled at 2 kHz.

Rapid agonist or agonist-antagonist application was accomplished using a modified DAD-12 perfusion system (ALA Scientific Instruments, Westbury, NY). The system consisted of a custom-made manifold of five quartz tubes, 100 μ m in diameter, that converged into a common 100- μ m tip with minimal dead volume. The tubes were fed from pressurized reservoirs equipped with miniature switching valves controlled by a computer, such that solution only flowed from a single reservoir at one time. The tip of the manifold was placed less than 100 μ m from the patch-clamped cell under study. Solutions were degassed prior to use, and reservoirs were pressurized at 200 to 400 mm Hg. Switching between solutions took 12 ± 1 ms ($n = 12$), as determined by junction potential measurements using a 10% Cl⁻ solution in one reservoir and normal Cl⁻ in the others. However, whole-cell current responses to agonist were slower, such that maximum agonist-induced current developed at 200 ± 25 ms. All drugs were prepared fresh daily.

Data Analysis. The block of NMDA-evoked currents was calculated according to the formula $B = [(I - I_B) / I] \times 100$, where I was determined by curve fitting the decay of the NMDA-evoked current during the NMDA application and extrapolating the fit to the end of the NMDA antagonist coapplication, and I_B was the current measured at the end of NMDA/blocker coapplication. Current decays were fit to first-order exponential curves using a Chebyshev fit method and pClamp software.

The residual block of NMDA-evoked currents was calculated according to the formula $B_R = [(I_{1st} - I_{2nd}) / I_{1st}] \times 100$, where I_{1st} was the maximal current measured 200 ± 25 ms after onset of the first

NMDA exposure and I_{2nd} was the maximal current measured 200 ± 25 ms after onset of the delayed second NMDA exposure after wash-out of blocker from the bath. The delay between onset of agonist application and current measurement was to allow development of maximal I_{1st} and to avoid possible corruption of data measurement by artifacts.

The block trapped (B_T), or the amount of block remaining at the beginning of the second NMDA application as a percent of the initial block produced at the end of the previous NMDA/antagonist coapplication, was calculated according to the formula $B_T = (B_R / B) \times 100$, where B and B_R are defined as above.

Data are expressed as means $\pm$ S.E.M. (n = number of cells). Statistical analysis was performed using Microcal Origin (Northampton, MA) software. Comparisons were made by analysis of variance, with $p < 0.01$ considered as significant.

Results

The Physiological Relevance of Trapping. In general, trapping experiments were conducted with antagonist removed between the initial agonist/antagonist coapplication and the later agonist application. However, in a therapeutic situation, antagonist would be present continuously, making it important to demonstrate that antagonist could still escape from the channel in this situation. As is shown in Fig. 1, AR-R15896AR does escape from the channel whether or not it is present in the bath between applications of NMDA. There was no significant difference in trapping whether antagonist was continually present ($61 \pm 2\%$, $n = 3$) or not ($54 \pm 3\%$, $n = 9$). This demonstrates that the partial trapping of an antagonist is a physiologically relevant phenomenon.

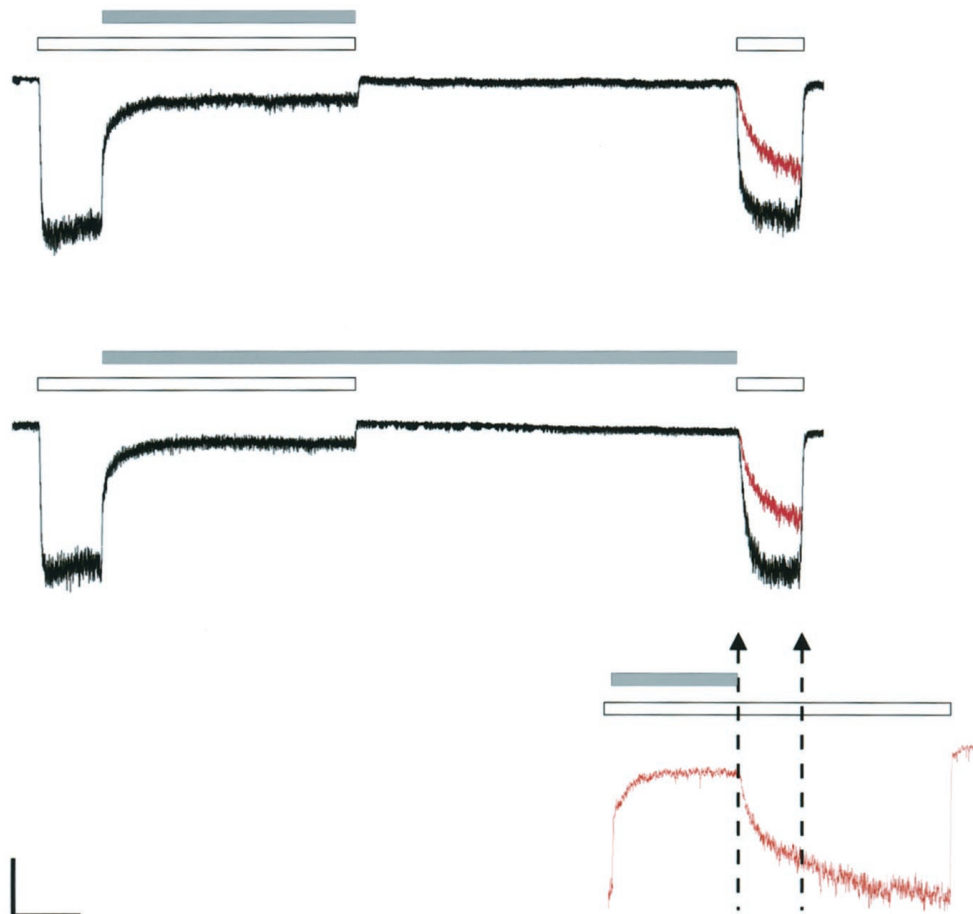


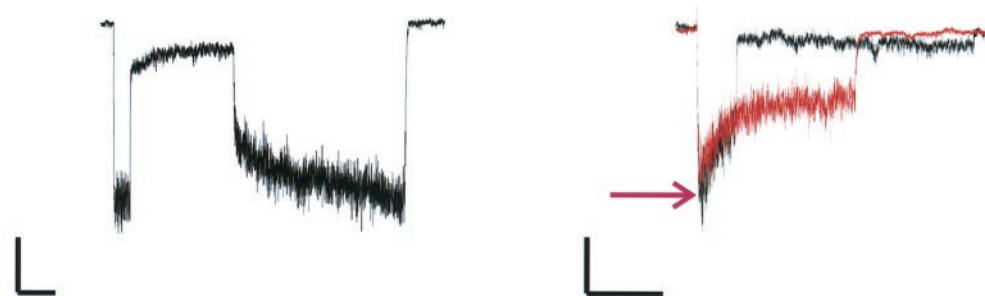
Fig. 1. Partial trapping occurs regardless of whether the antagonist is continuously present during the 30 s between agonist applications (middle trace) or not (top trace). The bottom current trace (in red) shows the onset and immediate relief of block using the same concentration of antagonist used in the top traces. The section of this trace between the dashed lines corresponds to the antagonist off-rate and has been superimposed (in red) where agonist was reapplied in the upper traces. This illustrates the difference between the similar partial trapping observed in the top two traces and their appearance if trapping were complete. Open bars indicate application of 10 μ M NMDA, and shaded bars indicate application of 50 μ M AR-R15896AR. Scale, 100 pA; 5 s.

On-/Off-Rates and Trapping of Compounds Structurally Related to AR-R15896AR. The concentration of each compound that was required to produce a block of NMDA-induced whole-cell current, not significantly different from the block of $81 \pm 1\%$ produced by $50 \mu\text{M}$ AR-R15896AR, was determined. Then on-/off-rates and degree of trapping of the initial block were measured using the appropriate concentration of antagonist. Representative current traces for selected compounds that trap significantly less than, equivalent to, or more than AR-R15896AR are shown in Fig. 2, top, middle, and bottom, respectively. On-/off-rates were measured by curve fitting the development of block, or relief from it, as neurons were exposed for 5 s to $10 \mu\text{M}$ NMDA, followed by a 30-s coapplication of NMDA + antagonist and a 50-s re-exposure to NMDA. Trapping was determined by exposing cells to $10 \mu\text{M}$ NMDA for 5 s, followed by a 30-s coapplication

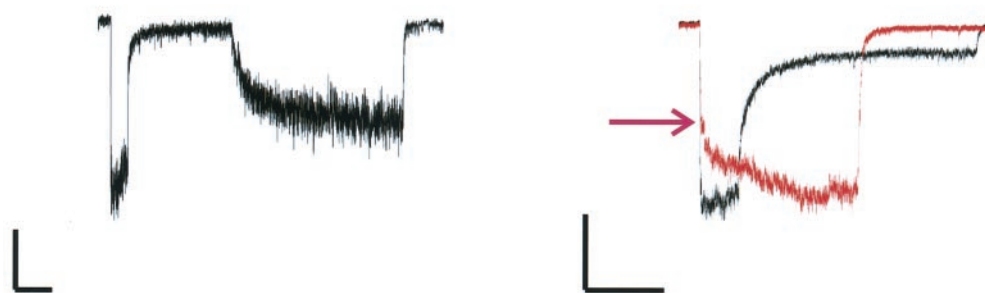
of NMDA + antagonist (in black), and then 120 s later reapplying $10 \mu\text{M}$ NMDA for 20 s (shown superimposed in red upon the initial response). The degree of trapping and the on-/off-rates for each compound at the appropriate concentration are summarized in Table 1.

Two compounds trapped significantly less than AR-R15896AR. We measured a trapping block of only $8 \pm 3\%$ with AR-R15808 (Fig. 2, top). However, this value may be an underestimation of trapping, because the off-rate of AR-R15808 exceeded the resolution of our system. A minor, slower component of its off-rate ($\tau_{\text{off}} = 6.4 \pm 1.1 \text{ s}$) was also observed in 6 of 11 cells tested. The structure of AR-R15808 resulted from changing the pyridine group of AR-R15896AR to a piperidine ring, thus increasing the basicity of this nitrogen. The second compound that trapped significantly less than AR-R15896AR was AR-R26952. Although AR-R26952

Low Trapping Compound AR-R15808



Intermediate Trapping Compound AR-R15336



High Trapping Compound AR-R15844

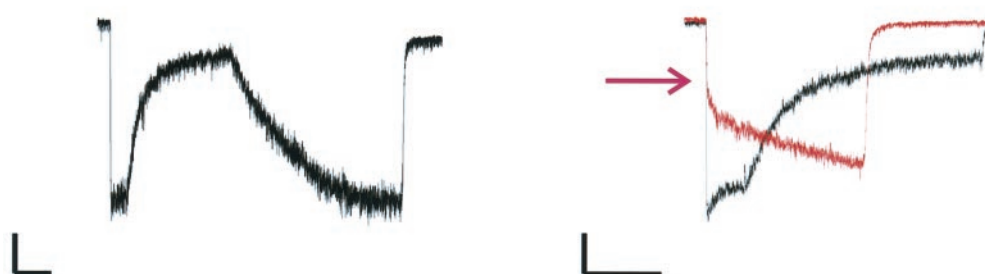


Fig. 2. The left current trace for each compound shows the response to a 5-s exposure to $10 \mu\text{M}$ NMDA, followed by a 30-s coapplication of NMDA + antagonist and a 50-s re-exposure to NMDA. The right set of two current traces shows an initial response (in black) to a 5-s exposure to $10 \mu\text{M}$ NMDA, followed by a 30-s coapplication of NMDA + antagonist. Superimposed (in red) upon this initial response is the response to a 20-s exposure to $10 \mu\text{M}$ NMDA exactly 120 s later. Arrows indicate the initial level of current upon re-application of agonist. Scale, 100 pA; 10 s.

TABLE 1
Summary of properties of compounds structurally related to AR-R15896AR

AR-R	Structure	FW (g)	[IC ₈₀] (μM) % B	% <i>B_T</i>	τ ₂ on (s)	τ ₁ on (s)	τ _{off} (s)
Low-trapping compounds ^a							
15808 See Fig. 2 (Upper)		277.2	150 84 ± 2 (22)	8 ± 3 (8) See text	0.2 ± 0.0 89 ± 1%	5.8 ± 0.6	See text
26952		311.3	150 80 ± 2 (12)	40 ± 5 (6)	0.5 ± 0.3 70 ± 4%	4.9 ± 0.5	6.9 ± 1.0
High-trapping compounds ^b							
16442		352.30	20 86 ± 2 (12)	70 ± 4 (7)	1.1 ± 0.1 48 ± 2%	5.1 ± 0.2	10.6 ± 2.2
15278		281.85	50 86 ± 2 (8)	72 ± 2 (6)	1.0 ± 0.1 52 ± 8%	7.0 ± 1.2	7.5 ± 3.6
12859 ^c		332.36	10 84 ± 1 (11)	72 ± 3 (6)	1.2 ± 0.2 49 ± 8%	8.0 ± 1.5	7.1 ± 1.8
15824 ^c		321.98	50 81 ± 2 (9)	76 ± 3 (5)	1.9 ± 0.3 49 ± 4%	13.9 ± 2.3	10.5 ± 2.1
16283		264.67	50 84 ± 1 (10)	76 ± 4 (6)	0.5 ± 0.1 51 ± 5%	2.9 ± 0.2	3.7 ± 0.6
15232		233.74	10 85 ± 2 (15)	77 ± 5 (6)	1.3 ± 0.1 61 ± 3%	5.9 ± 0.9	10 ± 0.8
15545 ^c		263.4	100 82 ± 2 (11)	78 ± 2 (5)	3.5 ± 0.3 60 ± 8%	18.8 ± 3.0	13.3 ± 1.1
15832		339.31	20 76 ± 3 (6)	78 ± 6 (3)	1.3 ± 0.2 35 ± 6%	11.5 ± 0.2	15.4 ± 1.4
18526		266.78	20 81 ± 1 (13)	80 ± 3 (8)	0.8 ± 0.1 49 ± 2%	6.4 ± 0.3	10.7 ± 1.3
15366		319.3	20 89 ± 1 (8)	81 ± 2 (6)	1.8 ± 0.1 54 ± 2%	8.1 ± 0.1	23.8 ± 0.5
15859 ^c		285.22	20 83 ± 2 (12)	82 ± 2 (6)	0.9 ± 0.1 41 ± 3%	7.2 ± 0.8	8.6 ± 2.4
15514 ^c		253.8	10 84 ± 4 (10)	84 ± 3 (5)	2.4 ± 0.3 32 ± 6%	10.9 ± 1.1	45.5 ± 0.6
15844 ^c See Fig. 2 (Lower)		259.77	20 83 ± 1 (14)	84 ± 2 (8)	NA	5.3 ± 0.2	21.7 ± 1.2
15492 ^c		395.5	200 85 ± 3 (6)	92 ± 3 (4)	2.3 ± 0.5 16 ± 6%	12.4 ± 0.2 ^e	63 ± 13
15793 ^c		310.39	20 86 ± 1 (13)	93 ± 3 (8)	1.1 ± 0.1 39 ± 2%	7.6 ± 0.6	20.8 ± 5.3
Intermediate-trapping compounds ^d							
15896		271.2	50 81 ± 1 (9)	54 ± 3 (9)	NA	2.4 ± 0.6	2.8 ± 0.9
15336 See Fig. 2 (Middle)		282.39	100 85 ± 3 (6)	57 ± 6 (5)	0.5 ± 0.2 65 ± 7%	4.9 ± 0.6	6.6 ± 1.1
15006		444.44	30 83 ± 1 (15)	60 ± 4 (10)	1.2 ± 0.1 56 ± 2%	7.2 ± 0.6	15.0 ± 0.7
15614 ^c		293.86	10 80 ± 1 (8)	60 ± 5 (5)	0.8 ± 0.1 56 ± 1%	7.8 ± 0.9	13.4 ± 0.5
15209		259.78	10 83 ± 1 (7)	61 ± 4 (5)	0.6 ± 0.1 52 ± 2%	7.5 ± 0.6	13.1 ± 6.6
15870 ^c		291.03	10 82 ± 1 (9)	62 ± 7 (5)	2.3 ± 0.2 55 ± 4%	11.0 ± 0.9	13.3 ± 3.3
15688		450.56	50 82 ± 2 (11)	68 ± 3 (6)	0.5 ± 0.1 65 ± 3%	3.8 ± 0.8	3.0 ± 1.2

FW, formula weight; B_T, block trapped; NA, not applicable

^a Significantly less trapping block than AR-R15896AR.

^b Significantly more trapping block than AR-R15896AR.

^c Fully saturated α-carbon.

^d Not significantly different trapping block than AR-R15896AR.

^e Not steady-state at 30 s.

trapped only $40 \pm 5\%$ of its initial block, its off-rate ($\tau_{\text{off}} = 6.9 \pm 1.0$ s) was well within resolution, making a quantitative determination of trapping possible. Both AR-R26952 and AR-R15896AR contain a pyridine instead of a phenyl group.

Six compounds showed intermediate trapping characteristics, not significantly different from AR-R15896AR. Onset and relief of block and trapping of one of these compounds, AR-R15336, is shown in Fig. 2, middle. Trapping in this group of compounds ranged from 54 ± 3 to $68 \pm 3\%$, whereas the τ_{off} of these compounds was between 2.8 ± 0.9 and 15.0 ± 0.7 s.

Fifteen NMDA receptor antagonists produced significantly more trapping block than AR-R15896AR, ranging from 70 ± 4 to $93 \pm 3\%$. AR-R15844, which trapped $84 \pm 2\%$ of the initial block, is shown in Fig. 2, bottom. The off-rates of these compounds were slower than that of AR-R15896AR. In fact, we observed a significant positive correlation ($p < 0.0001$; $r = 0.85$) between $\text{Log } \tau_{\text{off}}$ and the degree of trapping among all the compounds tested (Fig. 3A). The distinction between trapping and just coming off slowly could not be made for AR-R15492 ($\tau_{\text{off}} = 63 \pm 13$ s), given the time frame of the experimental protocol used. However, depolarizing the cell to $+60$ mV in the presence of the agonist could drive off AR-R15492. The on-rate for AR-R15492 was also sufficiently slow that a steady-state block was not reached after 30 s. It is noteworthy that the structures of the four highest trapping compounds have a fully saturated carbon atom in the α position. In fact, 8 of the 15 high trapping compounds have a fully saturated carbon atom in the α position, whereas only 2 of the 7 intermediate trapping compounds and none of the low trapping compounds have a fully saturated carbon atom in the α position.

Correlation between Physical Characteristics and Trapping. There was no significant correlation between lipophilic character and trapping, as estimated by ACD_LogP

(Fig. 3B). A negative correlation would be expected if partial trapping was due to lipophilic escape from the closed channel. On the contrary, a significant positive correlation was found between trapping and ACD_LogD (Fig. 3C), a parameter that takes into account the ionic character of compounds at physiological pH. This result is mirrored by a significant negative correlation between ACD_pK_a values and trapping (Fig. 3D). In addition, compounds that exhibited the least trapping contained two basic nitrogen atoms that were significantly protonated at physiological pH, thus increasing the hydrophilic nature of the molecule.

Discussion

This study demonstrates that partial trapping of block during repetitive stimulation can occur even in the continuous presence of antagonists. Therefore, the degree of trapping of an antagonist is a relevant factor to consider in the physiological situation. It is likely that trapping of channel block contributes to the side effects that have limited the clinical development of use-dependent NMDA receptor antagonists. Our studies confirm and extend the observation that trapping is not an all-or-nothing phenomenon. Among the 24 compounds tested, we observed levels of trapping that varied over a broad range, from essentially complete (93%) to at least as low as 40%. In fact, we measured trapping of only 8% for AR-R15808. However, this value may be an underestimate, since this compound's off-rate exceeded the resolution of our system. If the factors that led to these differences in degree of trapping were amenable to drug design, compounds with increased safety could be synthesized.

Another important finding of this study is that, within the initial group of 21 compounds selected, none trapped significantly less than AR-R15896AR, whereas 15 of them trapped significantly more. AR-R15896AR was chosen as a clinical

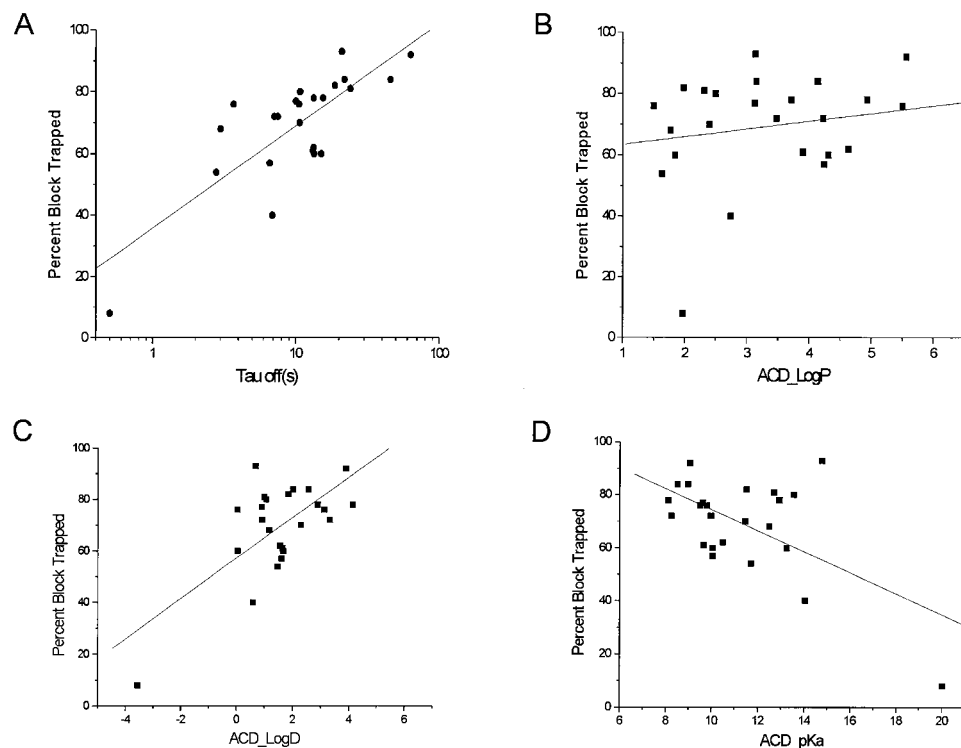


Fig. 3. A, plot of $\text{Log } \tau_{\text{off}}$ versus percent block trapped. Linear fit shows a significant positive correlation ($p < 0.0001$; $r = 0.85$). B, plot of ACD_LogP versus percent block trapped. Linear fit shows no significant correlation ($p = 0.18$; $r = 0.28$). C, plot of ACD_LogD versus percent block trapped. Linear fit shows a significant positive correlation ($p < 0.003$; $r = 0.67$). D, plot of ACD_LogpK_a versus percent block trapped. Linear fit shows a significant negative correlation ($p < 0.003$; $r = 0.59$).

candidate over other compounds in this group based strongly on its lack of PCP-like effects in rats (Palmer et al., 1999). Taken together, these observations are consistent with the hypothesis that trapping is an important factor in the PCP-like behaviors seen with many use-dependent NMDA receptor antagonists.

Specific structural features influence the degree of trapping. From examining structures of the 21 compounds in the initial group selected, the presence and nature of two basic nitrogen atoms appeared to be important. AR-R15896AR itself contains a pyridine and is less trapped than compounds containing a phenyl group in the analogous position. This suggested that a pyridine or other heterocyclic group might reduce trapping in other compounds. Two additional compounds, AR-R26952 and AR-R15808, were specifically chosen to test this hypothesis. Like AR-R15896AR, AR-R26952 contains a pyridine instead of a phenyl group. AR-R26952 trapped only 40% of its initial block, which was significantly less than that of AR-R15896AR. In the case of AR-R15808, changing the pyridine of AR-R15896AR to a piperidine ring increased the basicity of this nitrogen. This substitution resulted in a compound with the lowest apparent degree of trapping and the fastest off-rate of all compounds tested. At the other end of the spectrum, the four highest trapping compounds have a fully saturated carbon atom in the α position. In fact, 8 of the 15 high trapping compounds have a fully saturated carbon atom in the α position, whereas only two of the seven intermediate trapping and none of the low trapping compounds have a fully saturated carbon atom in the α position. Thus, changes in structural features influence the degree of trapping, suggesting that a specific binding site is responsible for differences in trapping and that partial trapping is an inherent feature of the trapping mechanism in NMDA receptors.

A simple mechanism that could account for differences in the degree of trapping is closed-channel egress via a lipophilic pathway. However, there was no significant correlation between the extent of trapping and ACD_LogP among the 24 compounds examined in this study. This observation is supported by our previous report that lipophilicity did not correlate with trapping among AR-R15896AR, memantine, and ketamine (Mealing et al., 1999). Furthermore, that study also demonstrated that trapping was stable over relatively long periods of time, which would not be predicted if lipophilic diffusion was occurring. Cumulatively, this evidence strongly argues that lipophilic diffusion through the receptor protein or lipid bilayer is not responsible for partial trapping.

Our data indicate a positive linear correlation between $\text{Log}\tau_{\text{off}}$ and degree of trapping, which is consistent with antagonist unbinding being an exponential process. Furthermore, among three NMDA receptor antagonists with intermediate kinetics that we previously used to investigate trapping, a similar trend is evident (Mealing et al., 1999). We also noted that the degree of trapping was the same across a range of concentrations of antagonist. This finding is consistent with the correlation between off-rate and degree of trapping, because off-rate is concentration-independent. Compounds with a fast off-rate could escape being trapped simply because they exit faster than the receptor closes. This could possibly be described by modifications to existing linear models of trapping block. However, a sequential or linear mechanism of trapping block would predict inward tail currents,

developing upon removal of agonist following onset of block, since the blocked receptor must return to a closed state via an open state (Lingle, 1983). In this study, tail currents were not observed with any of the compounds tested. Using similar methodology, we have previously demonstrated tail currents using the sequential channel blocker, 9-AA (Mealing et al., 1999). However, failure to observe tail currents with 23 of the compounds tested is not compelling evidence against a sequential mechanism of block. These compounds all have a much slower off-rate than 9-AA. Consequently, tail currents spanning a longer duration with smaller amplitude could have escaped detection. However, AR-R15808, whose off-rate, like that of 9-AA, was beyond our resolution, also failed to show tail currents. Therefore, although we cannot rule out a sequential mechanism of block for compounds that exhibit partial trapping, we can offer no evidence to support it.

On the other hand, cyclic models, like those of Gurney and Rang (1984) or Benveniste and Mayer (1995), explicitly allow for the possibility of the return from a closed, blocked state directly to the closed state. These models do not predict tail currents prior to channel closure. Changes in the rate constant for the escape of antagonist from the closed channel, or "closed-channel egress", could allow partial trapping to occur. However, these models give no insight into mechanisms that could mediate closed-channel egress.

A physical description of trapping channel block involves closing of the channel that is associated with trapping of the antagonist molecule. The question arises as to where the blocker is trapped. It is unlikely that these use-dependent antagonists can traverse the NMDA channel. Therefore, closing of the channel cannot prevent the antagonist from rejoining the extracellular fluid. We can visualize the channel to be a "lower barrier" through which antagonists cannot pass into the cell (Fig. 4). To account for antagonists being trapped, there must be an additional, more extracellularly located, "upper barrier", whose closure restricts the antagonist from rejoining the extracellular space. In this scheme, once the upper barrier closes, the antagonist is trapped in a "vestibule", formed by the space between these two barriers, and the receptor is in the closed, blocked state. This scheme is similar to one proposed for quaternary ammonium compounds block of potassium channels (Armstrong, 1971). Barring lipophilic diffusion, the receptor cannot return to the closed, unblocked state without going through a blocked state in which the upper barrier is open. Partial trapping could occur if the upper barrier closes more slowly than the channel. In large, structurally complex molecules like receptor channels, this is not improbable. Compounds with fast off-rates could escape trapping because they escape the vestibule faster than the upper barrier closes. Therefore, the term closed-channel egress would be more accurately defined as closed-channel/open-vestibule egress.

An interesting corollary to this model is that the antagonist should also be able to enter the vestibule during the closed-channel/open-vestibule state. A significant amount of entry would reduce apparent closed-channel egress. In our experiment to test the physiological relevance of closed-channel egress, we did not detect any significant differences suggestive of closed-channel/open-vestibule entry. However, since macroscopic on-rate is a function of concentration, it is possible that this effect would be prominent only at higher concentrations of antagonist. It is also possible that the pres-

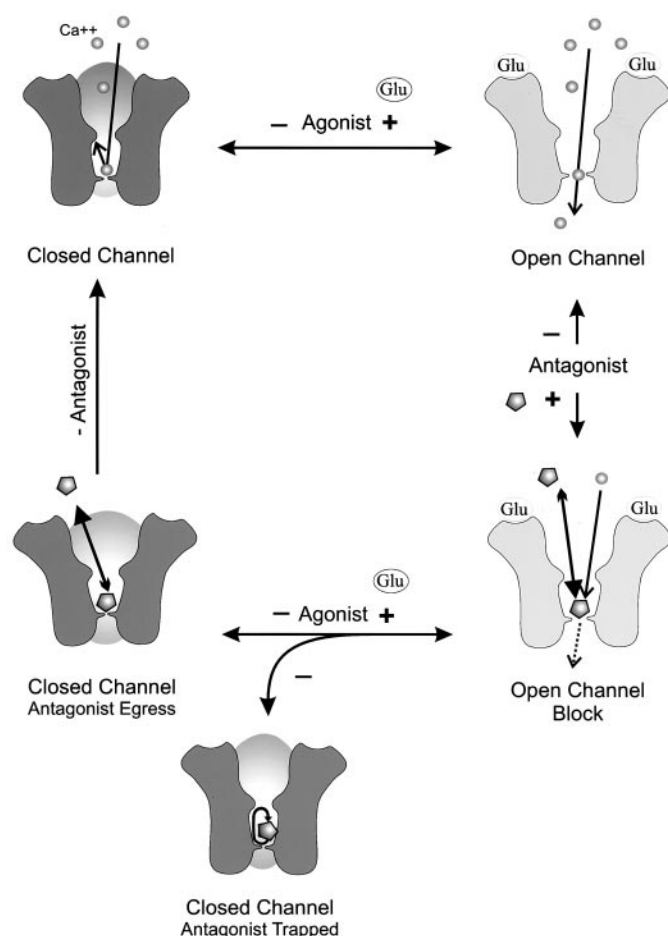


Fig. 4. Cartoon illustrating closed-channel trapping and egress. In the upper left-hand corner, a closed channel is shown whose conformation does not permit ion flow. Application of agonist causes channel opening (upper right). Concurrent application of agonist and antagonist allows antagonist into a "vestibule" to act at the channel pore, resulting in an open channel block (lower right). Removal of the agonist results in channel closure. If the "upper barrier" of the vestibule closes more slowly than the "lower barrier", the channel pore, the antagonist may escape, resulting in closed channel egress (lower left). However, if the upper barrier closes more rapidly than the antagonist can leave the channel pore, trapping results (bottom).

ence of antagonist in the vestibule retards closing, but it can then proceed rapidly following egress. In addition, physicochemical factors might favor antagonist egress over re-entry into the vestibule.

We have proposed that closed-channel egress provides a means to escape the problem that trapping presents to use-dependence. We noted that AR-R15896AR had the lowest trapping of the compounds chosen using objective selection criteria. Other post hoc comparisons (e.g., AR-R18526) suggest that compounds with high degrees of trapping produce significant amounts of PCP-like behaviors in rats at lower doses (G. Palmer, personal communication). Therefore, we would expect compounds with small degrees of trapping to have low liabilities for producing PCP-like behaviors. Unfortunately, behavioral studies using the two lowest trapping compounds, AR-R15808 and AR-R26952, could not be completed due to complications with bioavailability and specificity. Therefore, the correlation between degree of trapping and safety remains a plausible hypothesis, but presently untestable with the present inventory of compounds.

Rogawski (1993) proposed that low-affinity NMDA receptor antagonists would be safer because they prevent supra-maximal block and the delayed recovery resulting from it. However, trapping of antagonist can override the benefits of low-affinity and use-dependence. Partial trapping permits at least some degree of use-dependence to continue. We suggest a model in which partial trapping occurs because of very fast off-rates of compounds. Since fast off-rate is one determinant of low affinity, safety in use-dependent NMDA receptor antagonists can be accounted for by a modified version of Rogawski's proposal. Parsons et al. (1995) have also noted the importance of fast off-rate kinetics to compound safety, especially during strong depolarization, as would occur during excessive receptor activation.

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