

Role of Ghrelin in the Regulation of Vasopressin Release in Conscious Rats

SEIJI ISHIZAKI, TAKASHI MURASE, YOSHIHISA SUGIMURA, SATOSHI KAKIYA, HISASHI YOKOI, KAZUSHIGE TACHIKAWA, HIROSHI ARIMA, YOSHITAKA MIURA, AND YUTAKA OISO

First Department of Internal Medicine (S.I., Y.S., S.K., H.Y., K.T., H.A., Y.M., Y.O.), Nagoya University School of Medicine, Nagoya 466-8550, Japan; and Research Institute of Environmental Medicine (T.M.), Nagoya University, Nagoya 464-8601, Japan

GH secretagogue (GHS) is a small, synthetic compound that has the potential to stimulate GH release via its specific receptors (GHS-R). Ghrelin is a novel 28-amino acid peptide recently isolated from human and rat stomach, and it is thought to be the endogenous ligand for GHS-R. Ghrelin has a variety of physiological functions such as the stimulation of GH release or the increase of food intake by activating NPY neurons. In the present study, we investigated the effects of ghrelin on AVP release in conscious rats. Intracerebroventricular (icv) administration of ghrelin increased the plasma AVP concentration in a dose-dependent manner (1–1000 pmol/

rat), and its effect was observed as late as 60 min after the administration. Icv injection of ghrelin caused no significant change in plasma osmolality, plasma volume, or arterial blood pressure. Iv administration of ghrelin (10 nmol/rat) also increased the plasma AVP concentration, which was accompanied by a significant decrease in arterial blood pressure. Pretreatment with antiserum against NPY significantly reduced the plasma AVP increase induced by icv administration of ghrelin. These results suggest that ghrelin plays a stimulatory role in AVP release, which is possibly mediated by NPY neurons. (*Endocrinology* 143: 1589–1593, 2002)

GH SECRETAGOGUES (GHSs) are synthetic compounds that are potent stimulators of GH release (1, 2). GHSs have been reported to have a variety of physiological functions such as stimulating food intake in addition to GH-releasing activity (3, 4). The GHSs work via a novel G protein-coupled receptor (GPCR), the GHS receptor (GHS-R). GHS-Rs are expressed in various regions of the brain including the pituitary, hypothalamus, and hippocampus (5). Ghrelin is an endogenous ligand for GHS-R recently purified from rat and human stomach (6, 7). Ghrelin-producing cells are most abundant in the oxyntic glands of the stomach and are also located in the hypothalamus (6, 8). Central and peripheral administration of ghrelin potently stimulates GH release from the pituitary in rats and humans (6, 9–11). Ghrelin and GHSs have also been reported to affect the release of other pituitary hormones (12, 13). GHSs activate the hypothalamo-pituitary-adrenal (HPA) axis, possibly through the stimulation of AVP neurons (14). GHSs have been reported to stimulate AVP release from acute hypothalamic explants *in vitro* (15). However, the physiological role of ghrelin in the regulation of AVP release remains unknown.

Both central and peripheral injections of ghrelin reportedly stimulate food intake (13, 16–19). Ghrelin expression in the stomach is up-regulated upon fasting, insulin-induced hypoglycemia, and leptin administration in rats (20), whereas circulating ghrelin levels are decreased in human obesity (21). This evidence indicates the important role of ghrelin in the regulation of feeding behavior. This appetite-increasing effect of ghrelin is thought to be mediated by NPY

neurons because 1) GHS-Rs are localized in arcuate nucleus NPY neurons (22); 2) ghrelin induces *c-fos* expression in NPY neurons and increases NPY mRNA in the arcuate nucleus (18); and 3) antibodies and antagonists of NPY abolish ghrelin-induced feeding (18, 19). It is well known that NPY also plays important roles in the regulation of AVP release. A central injection of NPY stimulates AVP release *in vivo* (23–26). The findings that ghrelin could activate NPY neurons in the arcuate nucleus and that NPY has AVP release-stimulating activity suggest the possibility that ghrelin could stimulate AVP release through the NPY neurons.

In the present study, therefore, we examined the effects of central and systemic administration of ghrelin on AVP release in conscious rats and the possible involvement of NPY neurons in this effect.

Materials and Methods

Male Sprague Dawley rats (body weight 250–300 g; Chubu Science Materials, Nagoya, Japan) were housed in a colony room maintained at 23 C with lights on from 0900–2100 h. Rats had *ad libitum* access to standard rat chow and tap water until immediately before each experiment. Five days before the experiments, the rats were anesthetized by ip injection of sodium pentobarbital (50 mg/kg body weight), and a 21-gauge stainless steel cannula was inserted stereotactically into the right lateral ventricle for intracerebroventricular (icv) administration. The stereotaxic coordinates were 0.8 mm posterior to the bregma, 1.4 mm lateral to the midline, and 4.0 mm below the surface of the skull. The cannula was fixed with dental cement and anchored to the skull with two jeweler's screws. Appropriate placement of the icv cannula was verified by an icv injection of dye after decapitation. All icv injections were in a volume of 10 μ l infused in 1 min. Ghrelin (Peptide Institute, Osaka, Japan) was dissolved in isotonic saline and injected icv. An equal volume of vehicle was injected as the control. All experiments were performed on conscious, unrestrained rats between 0900 and 1130 h. All procedures were performed in accordance with the institutional guidelines for animal care at Nagoya University School of Medicine, which conform to NIH Guidelines for the Care and Use of Laboratory Animals.

Abbreviations: GHS, GH secretagogue; GHS-R, GHS specific receptor; GPCR, G protein-coupled receptor; HPA, hypothalamo-pituitary-adrenal; icv, intracerebroventricular; NRS, normal rabbit serum; TP, total protein.

Exp 1

Time-course effects of icv ghrelin on AVP release. Rats were injected icv with ghrelin (100 pmol/rat) or vehicle, and blood samples were obtained by decapitation 5, 10, 20, or 60 min after the injection.

Exp 2

Dose-response effects of icv ghrelin on AVP release. Rats were injected icv with ghrelin (1, 10, 100, and 1000 pmol/rat) or vehicle, and blood samples were obtained by decapitation 20 min after the injection.

Exp 3

Effects of systemic administration of ghrelin on AVP release. Rats were anesthetized on the day before the experiment, and a polyethylene cannula was implanted into the right jugular vein for iv injection of ghrelin. On the experimental day, ghrelin (1, 10 nmol/rat) or vehicle was injected iv, and blood samples were obtained by decapitation 15 min after the injection.

Exp 4

Effects of icv injection of anti-NPY serum on ghrelin-induced AVP release. Rats were pretreated with an icv injection of anti-NPY serum (Yanaihara Institute Inc., Shizuoka, Japan) or normal rabbit serum (NRS) (Chemicon International Inc., Temecula, CA). Ghrelin (100 pmol/rat) or vehicle was injected icv 10 min after the injection of anti-NPY serum, and blood samples were obtained by decapitation 5 or 20 min after the ghrelin injection. Anti-NPY serum or NRS was injected icv at a dose of 10 μ l/rat.

Exp 5

Effects of ghrelin on blood pressure. Rats were anesthetized on the day before the experiment, and a polyethylene cannula was implanted into the right carotid artery for blood pressure measurement. On the experimental day, 1) ghrelin (100 pmol/rat) or vehicle was injected icv, and 2) ghrelin (1, 10 nmol/rat) or vehicle was injected iv and arterial blood pressure was recorded continuously for 20 min or 30 min after the injection. Baseline values were recorded for 10 min before the injection. Arterial blood pressure was measured with a blood pressure transducer (Gould Inc., Oxnard, CA) connected to the cannula implanted into the carotid artery.

Plasma AVP, Na⁺, and total protein (TP) measurement

Immediately following decapitation, trunk blood was collected in a tube-containing EDTA for the determination of plasma AVP, Na⁺, and TP. Immediately after separation, plasma AVP was extracted through a Sep-pak C18 cartridge (Waters Associates Inc., Milford, MA) and measured using a RIA kit (provided by Mitsubishi Chemical Co., Ltd., Tokyo, Japan) as previously described (25). Plasma Na⁺ was measured using an autoanalyzer (Hitachi, Tokyo, Japan) for estimation of the change in plasma osmolality. TP was also measured by the autoanalyzer for estimation of the change in plasma volume.

Statistics

All results are expressed as mean values $\pm$ SEM. Multiple comparisons were evaluated by a one-way ANOVA followed by a Fisher protected least significant difference test. Differences were considered statistically significant at $P < 0.05$. The group size was five in all experiments.

Results**Exp 1**

Time-course effects of icv ghrelin on AVP release. Icv injection of ghrelin (100 pmol/rat) significantly increased plasma AVP as early as 5 min after the injection and as late as 60 min thereafter. Plasma AVP peaked 20 min after the injection (2.51 ± 0.37 pg/ml *vs.* control, 0.98 ± 0.19 pg/ml, $P < 0.01$,

Fig. 1). Ghrelin did not affect plasma Na⁺ or TP, suggesting that plasma osmolality or plasma volume was not changed (Table 1). The rats demonstrated no apparent changes in drinking, grooming, activity, or other behavior modifications following icv administration of ghrelin.

Exp 2

Dose-response effects of icv ghrelin on AVP release. Icv injection of ghrelin increased plasma AVP dose dependently over the range from 1–1000 pmol/rat (100 pmol/rat; 2.49 ± 0.08 pg/ml *vs.* control, 1.03 ± 0.08 pg/ml; $P < 0.01$, Fig. 2). Ghrelin did not affect plasma Na⁺ or TP.

Exp 3

Effects of systemic administration of ghrelin on AVP release. Iv injection of ghrelin (10 nmol/rat) significantly increased plasma AVP 15 min after the injection (10 pmol/rat; 2.13 ± 0.18 pg/ml *vs.* control, 1.33 ± 0.08 pg/ml; $P < 0.05$, Fig. 3). There was no significant increase in plasma AVP after iv injection of the lower dose of ghrelin (1 nmol/rat). Ghrelin did not affect plasma Na⁺ or TP (Table 2).

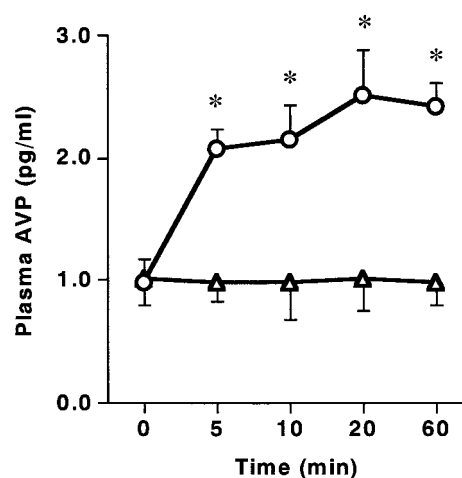


FIG. 1. Time-course effects of ghrelin icv on AVP release. Conscious rats were injected icv with ghrelin (100 pmol/rat; ○) or control (△). After the indicated periods, the animals were killed. AVP was extracted from plasma and quantified by RIA. Values are expressed as mean $\pm$ SEM ($n = 5$). *, $P < 0.05$ compared with control.

TABLE 1. Effects of central administration of ghrelin on plasma Na⁺ and TP

	Plasma Na ⁺ (mEq/liter)	
	Vehicle	Ghrelin (100 pmol/rat)
0 min	138.0 $\pm$ 1.3	138.1 $\pm$ 0.9
5 min	137.8 $\pm$ 1.1	138.0 $\pm$ 1.4
20 min	138.1 $\pm$ 0.8	137.8 $\pm$ 2.2

	Plasma TP (g/dl)	
	Vehicle	Ghrelin (100 pmol/rat)
0 min	5.80 $\pm$ 0.58	5.74 $\pm$ 0.83
5 min	5.91 $\pm$ 0.65	5.65 $\pm$ 0.99
20 min	5.78 $\pm$ 0.31	5.84 $\pm$ 0.45

Values are expressed as mean $\pm$ SEM ($n = 5$).

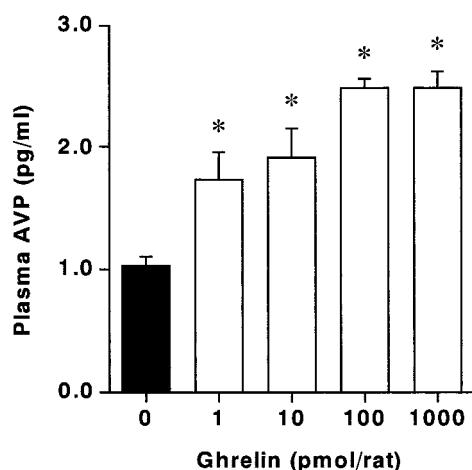


FIG. 2. Dose-response effects of ghrelin icv on AVP release. Conscious rats were injected icv with ghrelin (1–1000 pmol/rat) or control. The animals were killed 10 min after the injection. AVP was extracted from plasma and quantified by RIA. Values are expressed as mean $\pm$ SEM (n = 5). *, $P < 0.05$ compared with control.

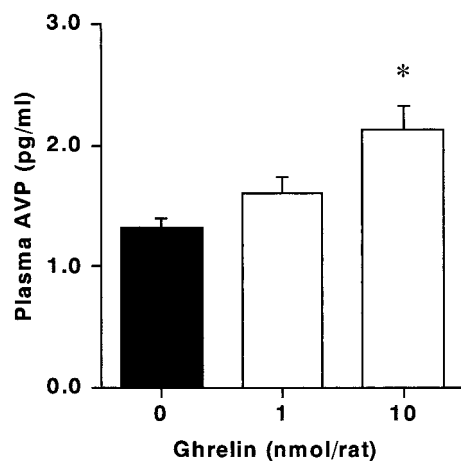


FIG. 3. Effects of systemic administration of ghrelin on AVP release. Conscious rats were injected iv with ghrelin (1, 10 nmol/rat) or control. The animals were killed 15 min after the injection. AVP was extracted from plasma and quantified by RIA. Values are expressed as mean $\pm$ SEM (n = 5). *, $P < 0.05$ compared with control.

Exp 4

Effects of anti-NPY serum on ghrelin-induced AVP release. To elucidate the possible involvement of NPY neurons in the AVP release-stimulating effect of ghrelin, we examined the effect of anti-NPY serum on ghrelin-induced AVP release. Pretreatment with anti-NPY serum significantly suppressed the ghrelin-induced AVP release both 5 min and 20 min after the ghrelin injection (Fig. 4). Icv injection of anti-NPY serum by itself did not cause significant changes in basal plasma AVP levels (data not shown).

Exp 5

Effects of ghrelin on blood pressure. Icv injection of ghrelin (100 pmol/rat) did not cause significant changes in mean arterial blood pressure until 20 min after the injection (Table 3). Iv injection of ghrelin (10 mol/rat) significantly decreased

TABLE 2. Effects of systemic administration of ghrelin on plasma Na^+ and TP

	Plasma Na^+ (mEq/liter)	
	Vehicle	Ghrelin (10 nmol/rat)
0 min	137.0 $\pm$ 1.3	136.7 $\pm$ 1.5
15 min	137.8 $\pm$ 0.9	137.7 $\pm$ 1.0

	Plasma TP (g/dl)	
	Vehicle	Ghrelin (10 nmol/rat)
0 min	5.44 $\pm$ 0.38	5.42 $\pm$ 0.20
5 min	5.51 $\pm$ 0.65	5.50 $\pm$ 0.13

Values are expressed as mean $\pm$ SEM (n = 5).

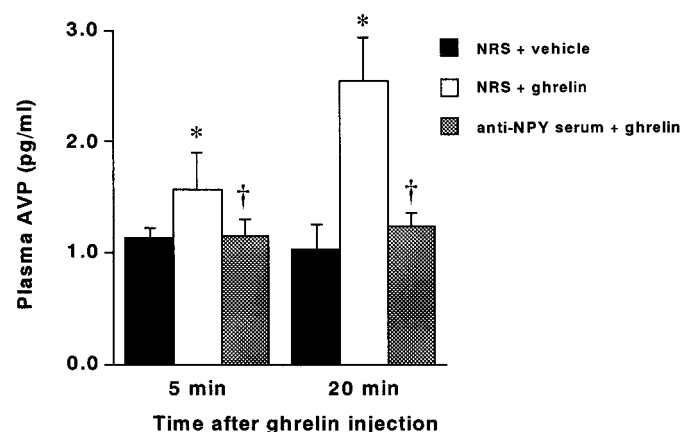


FIG. 4. Effects of anti-NPY serum on ghrelin-induced AVP release. Conscious rats were injected icv with anti-NPY serum or NRS. Ghrelin (100 pmol/rat) or vehicle was injected icv 10 min after the injection of antiserum. Rats were killed 5 and 20 min after the ghrelin injection. AVP was extracted from plasma and quantified by RIA. Values are expressed as mean $\pm$ SEM (n = 5). *, $P < 0.05$ compared with NRS + vehicle; †, $P < 0.05$ compared with NRS + ghrelin.

TABLE 3. Effects of central administration of ghrelin on mean arterial blood pressure

Ghrelin (pmol/rat)	Time after ghrelin icv.			
	0 min	5 min	10 min	20 min
0	109.4 $\pm$ 0.8	111.3 $\pm$ 4.3	109.2 $\pm$ 5.8	108.1 $\pm$ 3.1
100	110.1 $\pm$ 2.7	112.6 $\pm$ 5.3	111.0 $\pm$ 3.1	109.5 $\pm$ 2.8

Values are expressed as mean $\pm$ SEM (n = 5).

mean arterial blood pressure as early as 5 min after the injection and as late as 30 min after. Iv injection of a low dose of ghrelin (1 nmol/rat) also significantly decreased the blood pressure, although its effect was less potent than that of a high dose of ghrelin (Table 4).

Discussion

Ghrelin is a recently isolated peptide thought to be an endogenous ligand for GHS-R (6). It has been reported that ghrelin has a wide variety of functions such as its GH-releasing effect and orexigenic effect (7). However, its precise physiological roles remain to be elucidated. In the present study, we evaluated the effect of ghrelin on AVP release and clearly showed that central administration of ghrelin stim-

TABLE 4. Effects of systemic administration of ghrelin on mean arterial blood pressure

Ghrelin (nmol/rat)	Time after ghrelin iv					
	0 min	5 min	10 min	15 min	20 min	30 min
0	104.7 ± 6.8	103.1 ± 4.4	104.6 ± 5.2	104.9 ± 5.9	105.3 ± 5.9	102.3 ± 5.5
1	103.0 ± 3.8	102.2 ± 6.6	92.8 ± 4.7 ^a	90.0 ± 1.8 ^a	95.0 ± 1.9 ^a	101.4 ± 2.8
10	105.6 ± 6.3	94.6 ± 4.1 ^a	86.9 ± 2.9 ^b	81.1 ± 4.3 ^b	86.5 ± 1.9 ^b	86.9 ± 4.4 ^a

Values are expressed as mean ± SEM (n = 5).

^a *P* < 0.05 compared with control.

^b *P* < 0.01 compared with control.

ulated AVP release in conscious rats. This effect was so potent that even 1 pmol/rat of ghrelin significantly stimulated AVP release. The effect was also quick and long-lasting since ghrelin significantly stimulated AVP release as early as 5 min and as late as 60 min after the injection. This stimulating effect cannot be attributed to an increase in the level of osmotic or hypovolemic stimulation, because plasma Na and TP were not affected by the central injection of ghrelin. Moreover, icv injection of ghrelin caused no significant changes in blood pressure. Therefore, it is also unlikely that the changes in blood pressure caused plasma AVP changes by the central injection of ghrelin observed in this study. Furthermore, we also evaluated the effects of systemic administration of ghrelin on AVP release because ghrelin peptides are most abundant in the stomach (6). We showed that iv injection of ghrelin clearly stimulated AVP release. Because plasma Na and TP were not affected by the iv injection of ghrelin, the stimulating effect of ghrelin iv cannot be attributed to an increase in the level of osmotic or hypovolemic stimulation. However, iv injection of ghrelin caused significant decrease in arterial blood pressure. This is compatible with the report from another group indicating that an iv bolus of ghrelin (3.1 nmol/kg) significantly decreased mean arterial pressure without a significant change in heart rate in humans (26). Therefore, the possibility cannot be excluded that some part of the AVP-releasing effect of systemic administration of ghrelin may be caused by its hypotensive effect.

Ghrelin has been reported to increase food intake by activating NPY neurons (18). NPY is thought to be involved in the regulation of AVP release. Central injection of NPY stimulates AVP release in rats (23, 24). Such evidence leads us to the assumption that the AVP release-stimulating effect of ghrelin is possibly mediated by NPY neurons. Therefore, we tested the effect of the immunoneutralization of endogenous NPY by icv injection of anti-NPY serum on AVP release induced by ghrelin. Results showed that the AVP release-stimulating effects of ghrelin were markedly suppressed by pretreatment with NPY antiserum, suggesting that these effects of ghrelin are possibly mediated by NPY neurons. Because ghrelin effects are quick and long-lasting as mentioned above, one may assume that there is more than one mechanism involved in the AVP release-stimulating effect of ghrelin; for example, by acting directly on the hypothalamic AVP-producing neurons at in one phase and indirectly mediated by other neurons in another phase. Therefore, we tested the effect of anti-NPY serum in both early (5 min) and late (20 min) phases, and showed that the AVP release-stimulating effect of ghrelin was markedly attenuated in both

phases. A central injection of NPY reportedly causes a rapid and sustained increase in plasma AVP (26), which has a time course similar to that of the AVP-releasing effect of ghrelin in this study. Therefore, the duration of the effect of ghrelin itself is unclear, and even though it may be short, the action of NPY neurons can be long-lasting and cause a sustained stimulation of AVP release. However, considering a report that GHSs stimulate AVP release from acute hypothalamic explants *in vitro* (15), we still cannot exclude the possibility that ghrelin stimulates AVP release by directly acting on AVP-producing neurons in hypothalamic paraventricular nuclei or supraoptic nuclei, because there can be no efficient connection between arcuate nuclei and paraventricular nuclei or supraoptic nuclei in acute hypothalamic explants. Further studies are needed to clarify these issues.

The full physiological importance of the AVP release-stimulating effect of ghrelin is yet to be determined. However, ghrelin expression in the stomach is increased by both fasting and insulin-induced hypoglycemia (20). Hypoglycemia is a well-known stimulus for AVP release, but its mechanism still remains unclear. The results of the present study raise the possibility that ghrelin may be involved in the mechanism of hypoglycemia-induced AVP release. Hypoglycemia also dramatically increases plasma ACTH levels. In addition, GHS has been reported to increase ACTH, possibly through the stimulation of AVP release (14). Therefore, one may postulate that ghrelin may be involved in the hypoglycemia-induced AVP release, which could then stimulate ACTH release from the anterior pituitary.

Ghrelin peptides are located in the stomach and the hypothalamus (6), and it remains unclear from the present study which ghrelin is more important in the regulation of AVP release. These peptides are abundant in the stomach, and it seems possible that ghrelin secreted from the stomach acts on the brain to stimulate AVP release. Systemic administration of ghrelin reportedly induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus (27), indicating that circulating ghrelin peptides can penetrate the blood brain barrier and activate cells in the arcuate nucleus such as NPY neurons. Furthermore, we actually showed in the present study that peripherally administered ghrelin could increase AVP release. However, the concentration of ghrelin in the human circulation is low (6), and it remains to be determined whether ghrelin released from the stomach reaches the brain in high enough concentrations to stimulate AVP release. Another possibility is that hypothalamic ghrelin is working inside the brain to stimulate AVP release. Although the expression of ghrelin in the hypothalamus is small compared with that in the stomach, it may be enough to act inside the

brain. Much remains unknown about the regulation of ghrelin expressing in the hypothalamus or its physiological role, and further studies are needed to clarify these matters.

In conclusion, the present studies showed that central administration of ghrelin potently stimulated AVP release in conscious rats, and that this effect was markedly attenuated by the pretreatment with NPY antiserum. These results clearly suggest that ghrelin plays a stimulatory role in the regulation of AVP release, and that the effect is possibly mediated by NPY neurons.

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Address all correspondence and requests for reprints to: Takashi Murase, M.D., Ph.D., Department of Teratology and Genetics, Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan. E-mail: tmurase@riem.nagoya-u.ac.jp.

References

- Smith RG, Van der Ploeg LH, Howard AD, Feighner SD, Cheng K, Hickey GJ, Wyvrat Jr MJ, Fisher MH, Nargund RP, Patchett AA 1997 Peptidomimetic regulation of growth hormone secretion. *Endocr Rev* 18:621–45
- Bowers CY 1998 Growth hormone-releasing peptide (GHRP). *Cell Mol Life Sci* 54:1316–1329
- Torsello A, Luoni M, Schweiger F, Grilli R, Guidi M, Bresciani E, Deghenghi R, Muller EE, Locatelli V 1998 Novel hexarelin analogs stimulate feeding in the rat through a mechanism not involving growth hormone release. *Eur J Pharmacol* 360:123–129
- Torsello A, Locatelli V, Melis MR, Succu S, Spano MS, Deghenghi R, Muller EE, Argiolas A 2000 Differential orexigenic effects of hexarelin and its analogs in the rat hypothalamus: indication for multiple growth hormone secretagogue receptor subtypes. *Neuroendocrinology* 72:327–332
- Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van der Ploeg LH, Howard AD 1997 Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res* 48:23–29
- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402:656–660
- Kojima M, Hosoda H, Matsuo H, Kangawa K 2001 Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. *Trends Endocrinol Metab* 12:118–122
- Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M 2000 Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 141:4255–4261
- Peino R, Baldelli R, Rodriguez-Garcia J, Rodriguez-Segade S, Kojima M, Kangawa K, Arvat E, Ghigo E, Dieguez C, Casanueva FF 2000 Ghrelin-induced growth hormone secretion in humans. *Eur J Endocrinol* 143:R11–R14
- Seoane LM, Tovar S, Baldelli R, Arvat E, Ghigo E, Casanueva FF, Dieguez C 2000 Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. *Eur J Endocrinol* 143:R7–R9
- Arvat E, Maccario M, Di Vito L, Broglio F, Benso A, Gottero C, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Camanni F, Ghigo E 2001 Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *J Clin Endocrinol Metab* 86:1169–1174
- Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K 2000 Ghrelin strongly stimulates growth hormone release in humans. *J Clin Endocrinol Metab* 85:4908–4911
- Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR 2000 The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 141:4325–4328
- Korbonits M, Kaltsas G, Perry LA, Putignano P, Grossman AB, Besser GM, Trainer PJ 1999 The growth hormone secretagogue hexarelin stimulates the hypothalamo-pituitary-adrenal axis via arginine vasopressin. *J Clin Endocrinol Metab* 84:2489–2495
- Korbonits M, Little JA, Forsling ML, Tringali G, Costa A, Navarra P, Trainer PJ, Grossman AB 1999 The effect of growth hormone secretagogues and neuropeptide Y on hypothalamic hormone release from acute rat hypothalamic explants. *J Neuroendocrinol* 11:521–528
- Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiyama M, Nijijima A, Fujino MA, Kasuga M 2001 Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 120:337–345
- Inui A 2001 Ghrelin: an orexigenic and somatotrophic signal from the stomach. *Nat Rev Neurosci* 2:551–560
- Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S 2001 A role for ghrelin in the central regulation of feeding. *Nature* 409:194–198
- Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K 2001 Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes* 50:227–232
- Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, Kangawa K, Matsukura S 2001 Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem Biophys Res Commun* 281:1220–1225
- Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML 2001 Circulating ghrelin levels are decreased in human obesity. *Diabetes* 50:707–709
- Willesen MG, Kristensen P, Romer J 1999 Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* 70:306–316
- Harfstrand A 1987 Brain neuropeptide Y mechanisms. Basic aspects and involvement in cardiovascular and neuroendocrine regulation. *Acta Physiol Scand Suppl* 565:1–83
- Willoughby JO, Blessing WW 1987 Neuropeptide Y injected into the supraoptic nucleus causes secretion of vasopressin in the unanesthetized rat. *Neurosci Lett* 75:17–22
- Leibowitz SF, Sladek C, Spencer L, Tempel D 1988 Neuropeptide Y, epinephrine and norepinephrine in the paraventricular nucleus: stimulation of feeding and the release of corticosterone, vasopressin and glucose. *Brain Res Bull* 21:905–912
- Sato K, Crofton JT, Wang YX, Share L 1995 Effects of gender on the central actions of neuropeptide Y and norepinephrine on vasopressin and blood pressure in the rat. *Brain Res* 689:71–78
- Oiso Y, Iwasaki Y, Kondo K, Takatsuki K, Tomita A 1988 Effect of the opioid kappa-receptor agonist U50488H on the secretion of arginine vasopressin. Study on the mechanism of U50488H-induced diuresis. *Neuroendocrinology* 48:658–662
- Nagaya N, Kojima M, Uematsu M, Yamagishi M, Hosoda H, Oya H, Hayashi Y, Kangawa K 2001 Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. *Am J Physiol Regul Integr Comp Physiol* 280:R1483–R1487
- Hewson AK, Dickson SL 2000 Systemic administration of ghrelin induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats. *J Neuroendocrinol* 12:1047–1049