

Potential role of ghrelin on the micturition reflex in rats

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Introduction The aim of this study was to investigate the effects of intravenous ghrelin administration on the micturition reflex in rats.

Material and methods Continuous cystometrograms were performed with female Sprague-Dawley rats under urethane anesthesia. Stable micturition cycles were established, and ghrelin was then administered intravenously to evaluate changes in bladder activity. In this experiment investigating the role of opioid systems, ghrelin was administered intravenously at the time of first observed bladder contraction after intravenous administration of naloxone methiodide, an opioid receptor antagonist.

Results Intravenous administration of ghrelin increased intercontraction interval that was dose-dependent. These inhibitory effects returned to pre-controls level within 80 minutes of administration. Similarly, intravenous administration of ghrelin increased threshold pressure in a dose-dependent fashion. These inhibitory effects of ghrelin were antagonized by intravenous naloxone methiodide administration.

Conclusions These results show that ghrelin inhibits the micturition reflex through an opioid-dependent mechanism in rats under urethane anesthesia.

Key Words: ghrelin ◊ cystometrogram ◊ bladder ◊ rats

INTRODUCTION

Afferent pathways innervating the urinary bladder arise in the lumbosacral dorsal root ganglia and are carried in the pelvic and hypogastric nerves [1]. These fibers enter the spinal cord via the L6 and S1 dorsal root ganglia (DRG) and are responsible for initiating the micturition reflex in rats. Afferent pathways innervating the bladder consist of myelinated A δ - and unmyelinated C-fibers [1]. In normal rats, conscious voiding is dependent on A δ -fiber bladder afferents even though both A δ -fiber and C-fiber bladder afferents are mechanoreceptive; C-fiber afferents are responsible for bladder nociceptive responses [1]. Previous studies have suggested that hyperexcitability of C-fiber bladder afferents is involved in various conditions, such as overactive bladder and interstitial cystitis. Thus, targeting afferent hyperexcitability to treat detrusor overactivity and bladder pain symptoms may be useful [2].

Ghrelin is a 28 amino acid peptide in a fatty acid side chain and is mainly produced by endocrine cells of the stomach lining [3]. Ghrelin's role as a messenger molecule that promotes appetite and growth hormone release is well established [3, 4, 5]. However, ghrelin and its small molecule mimetics have a wide range of other functions, including suppression of inflammation and actions on autonomic effectors in the cardiovascular and digestive systems [6–9]. There is an increasing amount of literature indicating that ghrelin has antinociceptive effects in murine pain models [10–13]. It has been reported that intravenous ghrelin administration reduces visceral hypersensitivity via regulation of transient receptor potential vanilloid type 1 (TRPV1) and opioid receptors in a rat model of irritable bowel syndrome [14]. Interestingly, there is a delicate interaction between ghrelin and opioid systems. Ghrelin interacts with opioid receptors to regulate the mesolimbic dopaminergic system, which is associated with food

reward [15] and the antihyperalgesic effect of ghrelin is reversed by the μ opioid receptor antagonist, naloxone [11]. A recent study has shown that TRPV1 co-locates with the μ and κ opioid receptors on DRG neurons of the spinal cord, indicating crosstalk between the TRPV1 and opioid systems [16]. Furthermore, a previous study has demonstrated that intravenous administration of ghrelin receptor agonist in rats enhances bladder contractile activity [17]. Therefore, we hypothesized that ghrelin itself may also play a role in controlling the micturition reflex. This study aimed to investigate the effects of intravenous ghrelin administration on the micturition reflex in urethane-anesthetized rats.

MATERIAL AND METHODS

Animal care

Twelve-week-old female Sprague-Dawley rats weighing 228 to 252 g were used. The rats were maintained under standard laboratory conditions with a 12-h light/12-h dark cycle and free access to food pellets and tap water. All experiments were performed in accordance with institutional guidelines and approved by the animal ethics committees of the institution. Every effort was made to minimize animal suffering and pain.

Experimental procedure

Ghrelin (Tocris Bioscience, Ellisville, MO) was used. For intravenous administration, ghrelin was dissolved in saline (0.9% NaCl). Rats were anesthetized with isoflurane (2%) followed by urethane (1.2 g/kg subcutaneously) (Sigma Chemical Co., St. Louis, MO). Thereafter, the abdomen was opened through a midline incision and a polyethylene catheter (PE-50; Clay-Adams, Parsippany, NJ) connected to a pressure transducer and an amplifier was implanted into the bladder through the bladder dome. This catheter was used to record intravesical pressure during cystometry. The catheter was also used to fill the bladder by continuous saline infusion. Intravenous injections were made through a cannula (PE-10) inserted into the right jugular vein. The PowerLab system (ADInstruments Pty, Ltd., Castle Hill, New South Wales, Australia) was used for data acquisition and manipulation. After intravesical catheter insertion, saline was continuously infused for about 2 hours at a rate of 0.04 ml per minute to record cystometrograms during a control period. Ghrelin (300, 600, and 900 $\mu\text{g}/\text{kg}$, $n = 6$ per dose) was then administered intravenously and changes in bladder activity were monitored. Intravenous ghrelin was adminis-

tered at a dose of 0.1 ml/100 g body weight. To determine if the effect of ghrelin was mediated by the opioid system, ghrelin (900 $\mu\text{g}/\text{kg}$, $n = 6$) was administered intravenously in another group of animals and at the first observed bladder contraction, the opioid receptor antagonist naloxone hydrochloride (Tocris Bioscience, Ellisville, MO), was administered intravenously (3 mg/kg, $n = 6$). The intercontraction interval (ICI, time between two voiding cycles), maximum pressure (MP, highest pressure during voiding bladder contraction), threshold pressure (TP, pressure just prior to the initiation of voiding bladder contraction), and basal pressure (BP, pressure during urine collection) were measured before and after drug administration.

Statistical analysis

All data values are expressed as the mean \pm standard deviation. In experiments with intravenous administration of ghrelin or a placebo, ICI, MP, TP, and BP values 30 minutes before and after drug administration were averaged in each rat and these averages in each group of animals were then combined. One-way ANOVA followed by Dunnett's multiple comparison test was used for the statistical analysis between the placebo (control) and drug-treated rats. Student's paired t-test was used to compare cystometric variables before and after treatment. Statistical analyses were conducted by using SPSS 13.0 (SPSS Inc., Chicago, IL) and GraphPad Prism software (GraphPad Software, Inc., San Diego, CA). For all statistical tests, $p < 0.05$ was considered significant.

RESULTS

Intravenously administered ghrelin delayed the onset of micturition in urethane-anesthetized rats (Table 1;

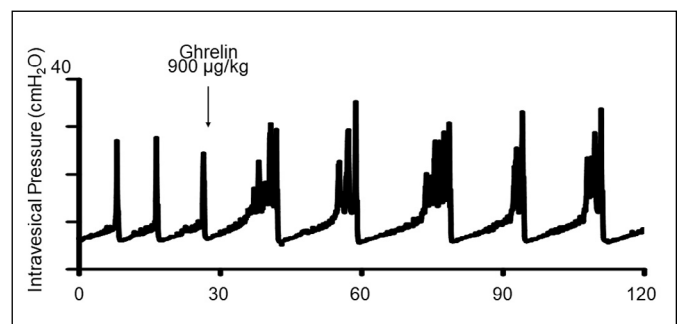


Figure 1. Representative cystometrograms showing the effects of intravenous ghrelin administration on bladder activity in urethane-anesthetized rats. The timing of the drug application is indicated by an arrow. The duration of drug application was less than 1 min.

Table 1. Changes in cystometric parameters after intravenous ghrelin administration in urethane-anesthetized rats

Variable	Placebo	Ghrelin (300 µg/kg)	Ghrelin (600 µg/kg)	Ghrelin (900 µg/kg)	Naloxone (3 mg/kg) and ghrelin (900 µg/kg)
Number of rats					
Mean ±SD					
ICI, %	98.9 ±1.3	121.0 ±3.4 [†]	143.3 ±3.4 [†]	157.4 ±11.5 [†]	101.8 ±4.2
BP, cmH ₂ O					
before treatment	2.84 ±0.47	2.85 ±0.37	2.78 ±0.27	2.96 ±0.47	3.11 ±0.33
after treatment	2.88 ±0.39	2.73 ±0.12	2.81 ±0.16	2.93 ±0.45	3.10 ±0.22
TP, cmH ₂ O					
before treatment	6.94 ±1.16	7.05 ±1.15	6.92 ±0.90	6.95 ±1.20	6.96 ±1.46
after treatment	6.99 ±1.06	11.1 ±0.76 ^{*†}	15.0 ±1.33 ^{*†}	18.3 ±2.07 ^{*†}	7.09 ±1.15
MP, cmH ₂ O					
before treatment	28.5 ±6.20	30.5 ±4.34	28.6 ±5.01	28.6 ±3.97	28.7 ±4.13
after treatment	28.5 ±5.95	30.1 ±2.83	28.3 ±3.66	29.7 ±.73	28.4 ±5.31

SD – standard deviation; ICI – intercontraction interval; BP – basal pressure; TP – threshold pressure; MP – maximum pressure
 p < 0.01 (paired t-test); [†]p < 0.05 vs placebo injection (Dunnett's multiple comparison test)

Figure 1). Ghrelin at 300, 600 and 900 µg/kg increased ICI in a dose dependent fashion to 121.0 ± 3.4%, 143.3 ± 3.4%, and 157.4 ± 11.5% of the control value, respectively (p < 0.05) (Table 1). This inhibitory effect was seen immediately after administration and returned to pre-control levels within 80 minutes. Intravenously administered ghrelin at 300, 600, and 900 µg/kg also increased TP in a dose-dependent fashion to 11.1 ± 0.76 cm H₂O, 15.0 ± 1.33 cm H₂O, and 18.3 ± 2.07 cm H₂O, respectively, from the control value of 6.99 ± 1.06 cm H₂O (p < 0.05) (Table 1). There were no significant changes in MP and BP at any of the tested drug doses (Table 1). Injection of a placebo (saline) had no effect on ICI, MP, TP, and BP (Table 1). When naloxone hydrochloride (3 mg/kg) was administered one voiding cycle before ghrelin (900 µg/kg) administration, there were no significant changes in ICI, MP, TP, and BP (Table 1; Figure 2).

DISCUSSION

This study aimed to assess the effects of intravenous ghrelin on the micturition reflex in urethane anesthetized rats. Our findings indicate that intravenous ghrelin has an inhibitory effect on the micturition reflex as intravenous ghrelin administration dose-dependently increased ICI and TP in urethane anesthetized rats. The effect of ghrelin seems to be mediated by modulation of afferent activity as ghrelin induced increases in ICI and TP without affecting MP or BP.

Consistent with its site of action at the preganglionic neurons, ghrelin receptor gene expression was local-

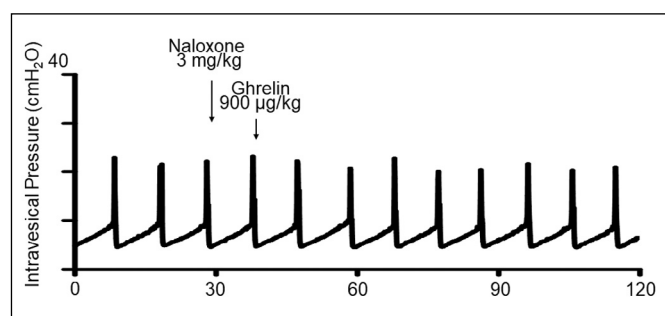


Figure 2. Representative cystometrograms showing the effects of intravenous administration of ghrelin (900 µg/kg) and naloxone hydrochloride (3 mg/kg) on bladder activity in urethane-anesthetized rats. The timing of the drug application is indicated by the arrows. The duration of drug application was less than 1 min.

ized to the cell bodies of parasympathetic neurons in the intermediolateral area (IML) of the lumbosacral spinal cord, and direct application of capromorelin to the lumbosacral cord caused bladder contractions [17]. The involvement of a subset of these neurons in bladder control has been confirmed by the induction of c-Fos in ghrelin receptor gene-expressing neurons of the IML when the bladder was perfused with saline to elicit micturition reflexes [17]. An increase in the rate of bladder perfusion was accompanied by an increase in numbers of c-Fos positive neurons and c-Fos-positive/ghrelin receptor gene-expressing neurons [17]. The distribution of neurons in which c-Fos was induced is very similar to a previous report when micturition reflexes were elicited in anesthetized rats [18].

A previous study showed that ghrelin agonists activate neurons involved in defecation reflexes [19]. Intravenous administration of ghrelin receptor agonists caused propulsive activity of the colon that was prevented by cutting spinal outflows at the lumbosacral level [19]. Injection into the fourth ventricle was ineffective and severing the spinal cord rostral to the lumbosacral spine had no effect on ghrelin receptor agonist-induced activation of defecation [19]. It was therefore postulated that ghrelin receptor agonists exert their colokinetic effect on autonomic nuclei within the lumbosacral spinal cord. In situ hybridization results indicate that ghrelin-responsive neurons controlling defecation reflexes are also most likely to be parasympathetic preganglionic neurons of the lumbosacral IML cell columns [17].

Our present study also indicates that the inhibitory effects of intravenous ghrelin is mediated by activation of the opioid system, as the ghrelin-induced increase in ICI or TP was prevented when naloxone hydrochloride was administered before ghrelin application. The interaction between ghrelin and the opioid system is supported by other studies. A previous study has shown that TRPV1, μ opioid receptor, and κ opioid receptor are co-expressed in the DRG, cerebral cortex and colon tissue in a rat model of irritable bowel syndrome [14]. Ghrelin showed marked antinociceptive effects in a maternal deprivation group that was accompanied by marked reduction of TRPV1 expression [14]. Rats treated with both ghrelin and naloxone showed higher abdominal withdrawal reflex scores in response to the same colonic pressure [14]. Moreover, an increase of TRPV1 was accompanied by opioid receptor blockade [14]. Sibilia et al demonstrated that ghrelin exerts an inhibitory role on inflammatory pain through an interaction with the opioid system [11]. Previous studies also have shown that ghrelin produces excitatory effects

on neurons of the ventromedial arcuate nucleus [20] where endogenous opioid containing neurons are located and are known to play a modulatory role on nociception in rats with carrageenan-induced inflammation [21]. It is unknown if ghrelin binds directly to opioid receptors or to other unknown receptors.

A recent study showed that intravenous administration of capromorelin, a ghrelin receptor agonist, enhances bladder contractile activity and disrupts normal micturition reflexes in rats [17]. In contrast, our study showed that intravenous ghrelin inhibited the micturition reflex through activation of the opioid system. There are two possible reasons for this difference in results. First, in the former study, the ghrelin receptor agonist capromorelin (10 mg/kg) was administered intravenously [17], in our study, ghrelin itself (300, 600, and 900 μ g/kg) was administered intravenously. The effects of ghrelin may depend on the applied dose and drug affinity. Second, the interaction between ghrelin, opioid receptors, and TRPV1 may influence the effects of intravenous ghrelin on the micturition reflex in urethane-anesthetized rats. It is well known that opioid receptors and TRPV1 play an important role in micturition and pain modulation. Further studies are needed to study this difference in lower urinary tract function.

CONCLUSIONS

Our study results suggest that ghrelin plays an important role in the control of the micturition reflex and that ghrelin can inhibit the micturition reflex through activation of the opioid system. Thus, ghrelin could be effective for the treatment of bladder dysfunction, such as overactive bladder.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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