Published in final edited form as:

Urology. 2013 November ; 82(5): 1188.e9–1188.13. doi:10.1016/j.urology.2013.06.056.

Inhibitory Role of the Spinal Galanin System in the Control of Micturition

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Abstract

OBJECTIVE—To investigate the effect of intrathecal galanin on the micturition reflex in rats.

METHODS—Continuous cystometrograms (0.04 mL/min infusion rate) were performed in female Sprague-Dawley rats (225–248 g) under urethane anesthesia. After stable micturition cycles were established, galanin was administered intrathecally to evaluate changes in bladder activity. Then, in order to examine the involvement of opioid systems in the galanin effects, galanin was administered intrathecally when the first bladder contraction was observed after intrathecal administration of naloxone, an opioid receptor antagonist.

RESULTS—Intrathecal administration of galanin (1 to 10 µg) increased intercontraction intervals in a dose-dependent fashion. Intrathecal administration of galanin (1 to 10 µg) also increased pressure threshold in a dose-dependent fashion. These inhibitory effects of galanin (10 µg) were partially antagonized by intrathecal administration of naloxone (10 µg).

CONCLUSION—These results indicate that in urethane-anesthetized rats galanin delays the onset of micturition through activation of the opioid mechanism, suggesting the inhibitory role of galanin system in the control of the micturition reflex.

INTRODUCTION

Afferent pathways innervating the urinary bladder arise in the lumbosacral dorsal root ganglia and are carried in two sets of nerves; pelvic and hypogastric nerves.¹ Afferent fibers passing in the pelvic nerve to the spinal cord are responsible for initiating the micturition reflex, and consist of myelinated Aδ- and unmyelinated C-fibers.¹ In normal rats, conscious voiding is dependent on Aδ-fiber bladder afferents even though both Aδ-fiber and C-fiber bladder afferents are mechanoreceptive; whereas C-fiber afferents are responsible for bladder

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nociceptive responses.¹ Previous studies have suggested that hyperexcitability of C-fiber bladder afferents, which are silent under normal conditions, is involved in the emergence of overactive bladder and bladder pain in various pathological conditions, such as spinal cord injury, bladder outlet obstruction or interstitial cystitis.² Thus, it has been postulated that targeting afferent activity could be effective for treating bladder overactivity and/or pain symptoms.²

Galanin, a 29 amino acid peptide, distributes widely in the peripheral and central nervous systems, including primary afferents, spinal dorsal horn interneurons and the descending bulbospinal tract.³ Many studies have demonstrated that galanin is an important messenger for intercellular communication.^{3,4} Recent studies have demonstrated that galanin is involved in nociceptive responses, especially in the transmission of nociceptive information in the spinal cord.^{5,6} In intact rats, the spinal antinociception induced by morphine is attenuated by galanin receptor antagonists.⁶ Thus, it is suggested that there may be an interaction of galanin and opioids at the spinal level in modulating the transmission of nociceptive information. Moreover, electrophysiological results show an inhibitory effect of galanin in the modulation of central sensitization in response to C-fiber stimulation in anesthetized mice with over-expression of galanin.⁷ However, to our knowledge, it is not known whether galanin is involved in the regulation of neural mechanisms controlling the micturition reflex. Therefore, we examined the effects of galanin on the micturition reflex in urethane-anesthetized rats. Furthermore, the involvement of opioid systems in the galanin-mediated regulation of micturition reflex was also studied.

MATERIAL AND METHODS

Animals

Adult female Sprague-Dawley rats weighing 225 to 248 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 conducted in accordance with NIH guidelines and approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Drugs

Galanin (Tocris Bioscience, Ellisville, MO) was used. For intrathecal (i.t.) administration galanin was dissolved in saline (0.9% NaCl).

Experimental Procedure

A i.t. polyethylene catheter (PE-10, Clay-Adams, Parsippany, NJ) was implanted with using isoflurane anesthesia via an incision in the dura at the Th11 vertebra 3 days before the experiments. The catheter was directed caudally into the spinal subarachnoid space and positioned at the level of the L6-S1 spinal cord. The volume of fluid in the catheter was kept constant at 6 μ l. Single doses of drugs were then administered in a volume of 2 μ l, followed by a 7 μ l flush with saline within 1 min. Injection sites in the spinal cord were confirmed by injecting dye (methylene blue) at the end of the experiments.

Under isoflurane anesthesia, the abdomen was opened through a midline incision and a PE-60 catheter (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 PowerLab (ADInstruments Pty, Ltd., Castle Hill, New South Wales, Australia) was used for data acquisition and manipulation. The catheter was also used to fill the bladder by continuous infusion of saline.

After the surgery, isoflurane anesthesia was turned off and replaced with urethane anesthesia (0.75 g/kg subcutaneously, Sigma Chemical Co., St. Louis, MO), and additional doses of anesthetic (0.1 g/kg/injection) were administered as required to obtain the sufficient level of anesthesia, which was confirmed by negative responses to the test for a pinch reflex. The final dose of urethane ranged from 1.00 to 1.20 g/kg in 30 animals tested. Rats were placed in a supine position for the experiments.

Physiological saline was continuously infused at room temperature into the bladder for about 2 hours at a rate of 0.04 ml per minute to record cystometrograms during a control period. Galanin (1, 3 and 10 μ g, $n = 6$ per dose) or vehicle (saline) was then administered intrathecally and changes in bladder activity were monitored. I.t. injections were made through a PE-10 catheter positioned at the level of the L6-S1 spinal cord. In another group of animals, galanin (10 μ g) was administered intrathecally when the first bladder contraction was observed after i.t. administration of naloxone, an opioid receptor antagonist (10 μ g, $n=6$), to determine whether the effect of galanin was mediated by the opioid system.

Intercontraction intervals (ICI, time between two voiding cycles), maximum voiding pressure (MVP, highest pressure during voiding bladder contraction), pressure threshold (PT, pressure just prior to the initiation of voiding bladder contraction), baseline pressure (BP, pressure during urine collection) were measured before and after drug administration. Post-void residual urine volume (PVR) was also measured in a separate group of 6 animals before galanin administration and at the end of 2 micturition cycles after drug administration. After constant voided volumes were collected, infusion was stopped and PVR was measured by withdrawing intravesical fluid through the catheter by gravity.

Statistical Analysis

All data values are expressed as the mean $\pm$ standard deviation. In experiments with i.t. administration of galanin ICI, MVP, PT, BP values during 30 minutes before and after drug administration were averaged in each rat and then the averages in a group of animals were combined. A one-way ANOVA followed by Dunnett's multiple comparison test was used for the statistical analysis between the vehicle and drug-treated groups. Student's paired t -test was used to compare cystometric variables before and after treatment. Student's unpaired t -test was used to compare cystometric variables between the galanin (10 μ g) administration group and the galanin (10 μ g) and naloxone (10 μ g) administration group. P values less than 0.05 were considered to indicate statistical significance.

RESULTS

I.t. applied galanin delayed the onset of micturition (Fig. 1). I.t. administration of galanin at 1, 3 and 10 μ g increased ICI in dose dependent fashion to $117.7 \pm 2.4\%$, $146.5 \pm 4.8\%$ and $141.1 \pm 12.5\%$ of the control value, respectively ($p<0.01$) (Fig. 2). These inhibitory effects were seen immediately after administration and returned to the pre-control level within 70 minutes. I.t. administration of galanin at 1, 3 and 10 μ g also increased PT in dose dependent fashion to 9.52 ± 0.98 cmH₂O, 11.2 ± 1.12 cmH₂O and 12.9 ± 1.67 cmH₂O, respectively, from the control value of 5.46 ± 0.88 cmH₂O ($p<0.01$) (Table 1). There were no significant changes in BP, MVP or PVR at any doses tested (Table 1). I.t. administration of vehicle (saline) failed to produce significant effects on ICI, MVP, PT and BP in rats (Table 1).

When naloxone was administered one voiding cycle before galanin administration, the increases in ICI and PT in rats with combined administration of naloxone (10 μ g) and galanin (10 μ g) were significantly smaller compared to rats with galanin administration (10 μ g) alone (Fig. 3 and Table 1). When naloxone was administered before galanin administration, there were no significant changes in BP, MVP or PVR (Fig. 3 and Table 1).

COMMENT

The goal of this study was to assess the effects of i.t. galanin on the micturition reflex in urethane anesthetized rats. Our findings indicate that i.t. galanin has an inhibitory effect on the micturition reflex. These were demonstrated by the data showing that i.t. administration of galanin dose-dependently increased ICI and PT in urethane anesthetized rats. The main function of galanin seems to be mediated by modulation of afferent activity because galanin induced increases in ICI and PT without affecting MVP or BP.

The neuropeptide galanin has a widespread distribution in the central and peripheral nervous system and modulates sensory transmission in the spinal cord after peripheral nerve injury or inflammation.⁸ Galanin co-exists with a number of classical neurotransmitters, including acetylcholine, serotonin, glutamate, gamma-aminobutyric acid, noradrenalin, and dopamine.⁹ Galanin also co-exists with other neuropeptides like enkephalin, neuropeptide Y, substance P (SP), vasopressin, calcitonin gene-regulated peptide (CGRP), and gonadotropin-releasing hormone.¹⁰ Galanin is upregulated in dorsal root ganglia (DRG) neurons,⁸ and galanin release is increased in the superficial dorsal horn after peripheral nerve injury.¹¹ A role for galanin in bladder-urethral function has been suggested based upon its anatomical distribution in lower urinary tract tissues.¹² Galanin expression has been documented in autonomic regions of the lumbosacral spinal cord, and a sexual dimorphism has been demonstrated in laminae VII and X.¹² Recent studies have demonstrated prominent upregulation of galanin in bladder afferent cells in DRG and in the L2 and S1 spinal cord after central injury.¹³ However, decreases in galanin expression have also been observed in the L1 spinal cord nearest the spinal transection site.¹³ Galanin was shown to influence the activity of smooth muscles of the rat bladder and modulate neural transmission both in autonomic ganglia and at neuromuscular junctions where galanin suppressed the cholinergic component of the response to electric field stimulation.¹⁴ Thus, an inhibitory action of galanin on neurotransmitter release has been suggested in smooth muscle tissues and this may also pertain to the urinary bladder.¹⁴ Moreover, some anti-inflammatory properties of galanin were suggested since it was shown to presynaptically inhibit the release of SP and CGRP from capsaicin-sensitive primary afferents.¹⁵ When administered intrathecally in higher doses, galanin also blocked the facilitatory effects of SP and CGRP on the excitability of the nociceptive flexor reflex in rats.¹⁶ This study further clarified that the spinal galanin system has an inhibitory role in the control of micturition in rats. Since it has been proposed that increased afferent activity is one of the pathophysiological basis of bladder overactivity², the present study raises the possibility that modulating the galanin mechanism in the spinal cord could be effective for treating overactive bladder.

The current study also indicated that the inhibitory effects of i.t. galanin is mediated at least in part by activation of the opioid system. In the present study, the galanin-induced increases in ICI and PT were significantly, but partially prevented when naloxone was administered prior to galanin application. It is well known that opioid peptides play an important role in micturition or pain modulation in the spinal cord.¹⁷ Moreover, the interaction of galanin and opioid systems are supported by other studies. Intracerebroventricular administration of galanin potentiated the morphine-induced antinociception in the rat.¹⁸ I.t. injection of the antagonist of galanin can attenuate the intraperitoneal morphine-induced antinociception.⁶ Previous studies also demonstrate an involvement of the opioid system in the galanin-induced nociceptive modulation in the spinal cord of rats with mononeuropathy.¹⁹ Hua et al. find that spinal galanin potentiated i.t. morphine-induced antinociception in rats with inflammation.²⁰ The intracellular transduction signaling pathways of galanin receptors are similar to opioid receptors. Activation of galanin receptor, via the G_i protein, inhibits the cAMP pathways, and therefore attenuates the neuronal activity.²¹ Therefore, it is assumed that galanin may synergize the

effect of endogenous or exogenous opioid at the second messenger level. Galanin may also interact with opioid receptors by stimulating phospholipase (PLC)-protein kinase C (PKC) pathways, because there is evidence that PKC can affect the function of mu-opioid receptor.²¹

The effects of galanin are mediated by the activation of one or more G-protein coupled galanin receptors (GalR1, GalR2 and GalR3).²² GalR1 is expressed in the central and peripheral nervous system, where it was detected in hippocampus, hypothalamus, amygdala, thalamus, cortex, brainstem, spinal cord, and DRG.²³ GalR2 was identified in rat hypothalamus, spinal cord, and DRG.²³ GalR2 is expressed in a wider pattern, compared to GalR1, as it is found in several peripheral tissues including the pituitary gland, gastrointestinal tract, skeletal muscle, heart, kidney, uterus, ovary, and testis as well as in regions in the central nervous system.²³ On the other hand, the distribution pattern of GalR3 is somewhat unclear but it is assumed that this receptor has a more restricted expression pattern in relation to the other two receptors.²³ Galanin receptors in DRG neurons have been analyzed with *in situ* hybridization and with blot techniques, which show high levels of expression of GalR1 and GalR2 and a lower level of GalR3 mRNAs in DRGs.^{24,25} The *in situ* studies have shown the presence of GalR1 mRNA mainly in medium-sized and large, often CGRP-positive neurons and GalR2 mRNA mostly in small CGRP-positive neurons.^{24,25} The inhibitory, but not excitatory, effect of i.t. galanin was reduced in rats treated with peptide nucleic acid antisense reagents against GalR1,²⁶ suggesting that GalR1 is responsible for the inhibitory effect of galanin. Recent study has also confirmed the involvement of GalR1 in galanin-mediated antinociception and in its interaction with opioids and SP²⁰. Thus, galanin may produce inhibitory effects on the micturition reflex through activation of GalR1, although the distribution pattern of galanin receptors in the urinary tract is unclear and the limited number of specific ligands to the galanin receptor subtypes has hindered the understanding of the individual effect of each receptor subtype. Further studies are needed to clarify the distribution pattern of galanin receptors in the urinary tract and the role of galanin receptor subtypes in the lower urinary tract function.

CONCLUSIONS

The results in this study suggest that galanin plays an important role in the control of the micturition reflex and that galanin can delay the onset of micturition reflex at least in part through activation the opioid system at the spinal level in rats.

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Reply

We agree with the Editorial comment that the findings are a long way removed from the clinical setting and that the use of anesthesia and the implantation of an intravesical catheter may have affected the results. However, because there is no previous study that examined the involvement of galanin system and its interaction with the opioid system in the control of micturition, this study is the first step to show that galanin plays an important role in the micturition reflex and that galanin can inhibit the micturition reflex through activation of the opioid system at the spinal level in rats. We plan to perform further studies to clarify the role of galanin and its interaction with the opioid system using animal models of various pathological conditions in the lower urinary tract, such as overactive and/or hypersensitive bladder disorders.

Galanin is widely expressed in the central and peripheral nervous system and co-exists with a number of classical neurotransmitters, including acetylcholine, serotonin, glutamate, γ -aminobutyric acid (GABA), noradrenalin and dopamine.¹ Thus, we also agree with the Editorial comment that galanin and opioid systems are widespread, and would be challenging to target with sufficient specificity for the lower urinary tract. However, recent studies including ours have shown that organ-specific local delivery of therapeutic genes such as nerve growth factor, GABA synthesizing enzyme, maxi-K channel and opioids using viral and non-viral vectors can be achieved to treat lower urinary tract dysfunction in animal models.² Therefore, there is a possibility that galanin gene therapy targeting the bladder and bladder afferent pathways is a viable, localized treatment option for lower urinary tract dysfunction such as such overactive bladder or genitourinary pain syndromes although future studies are needed to clarify this point.

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Figure 1.
Representative cystometrograms showing the effects of intrathecal (i.t.) administration of galanin on bladder activity in urethane-anesthetized rats. The timing of the drug application is indicated by an arrow. The duration of drug application was within 1 min.

Figure 2. Changes in intercontraction intervals (ICI) (% of control [Pre]) after intrathecal (i.t.) galanin (1-10 µg) and naloxone (10 µg) in urethane-anesthetized rats. * <0.01 vs. vehicle administration (Dunnett's multiple comparison test).

Figure 3.

Representative cystometrograms showing the effects of intrathecal (i.t.) of galanin (10 µg) and naloxone (10 µg) on bladder activity in urethane-anesthetized rats. The timing of the drug application is indicated by arrows. The duration of drug application was within 1 min.

TABLE 1

Changes in cystometric parameters after intrathecal (i.t.) galanin and naloxone administration in urethane-anesthetized rats.

Variable	Vehicle	galanin (1 µg)	galanin (3 µg)	galanin (10 µg)	naloxone (10 µg) and galanin (10 µg)
Number of rats	6	6	6	6	6
Mean ± SD					
ICI, min					
before treatment	11.1 ± 2.06	10.7 ± 2.56	10.1 ± 1.15	11.2 ± 1.97	10.4 ± 1.70
after treatment	10.9 ± 2.67	12.6 ± 1.76 *	14.8 ± 2.35 *	± 2.35 *	13.2 ± 2.01 *
% ICI, %	98.3 ± 2.2	117.7 ± 2.4 †	146.5 ± 4.8 †	141.1 ± 12.5 †	126.9 ± 3.1 †#
BP, cmH ₂ O					
before treatment	3.01 ± 0.89	2.65 ± 0.56	2.89 ± 0.54	2.80 ± 0.59	2.77 ± 0.76
after treatment	2.87 ± 0.42	2.59 ± 0.45	2.78 ± 0.50	2.91 ± 0.63	2.69 ± 0.65
PT, cmH ₂ O					
before treatment	5.79 ± 0.98	5.34 ± 0.45	6.23 ± 1.02	6.02 ± 0.89	6.45 ± 1.07
after treatment	5.46 ± 0.88	9.52 ± 0.98 *	11.2 ± 1.12 *	12.9 ± 1.67 *	9.67 ± 1.15 *
%PT, %	94.9 ± 1.0	176.5 ± 2.7 †	178.1 ± 3.3 †	213.7 ± 3.2 †	148.9 ± 2.9 †#
MVP, cmH ₂ O					
before treatment	25.8 ± 3.09	23.6 ± 1.89	20.5 ± 2.56	26.9 ± 2.89	22.9 ± 3.67
after treatment	24.9 ± 2.78	24.4 ± 2.11	22.8 ± 2.11	27.5 ± 3.01	23.1 ± 3.32
PVR, ml					
before treatment	0.11 ± 0.05	0.09 ± 0.03	0.10 ± 0.03	0.10 ± 0.05	0.09 ± 0.04
after treatment	0.08 ± 0.06	0.09 ± 0.04	0.08 ± 0.05	0.07 ± 0.04	0.10 ± 0.05

* P < 0.01 vs. (paired t-test) vs. before treatment

† P < 0.01 vs. vehicle injection (Dunnett's multiple comparison test).

P < 0.05 vs. galanin (10 µg) injection (unpaired t-test).