Inhibition Mechanism of Gosha-jinki-gan on the Micturition Reflex in Rats

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Abstract. We investigated the actions of Gosha-jinki-gan, a traditional Japanese medicine containing processed Aconiti tubers, on urinary bladder function in anesthetized rats. In cystometrical investigations, Gosha-jinki-gan (1.0 g/kg, i.d.) increased bladder capacity as well as micturition threshold pressure. In addition, it decreased the frequency of distension-induced rhythmic bladder contractions. However, it did not influence the amplitude of bladder contractions induced by electrical stimulation of the pontine micturition center. The inhibitory effect of Gosha-jinki-gan on bladder motility was abolished by pretreatment with nor-binaltorphimine (10 mg/kg, s.c.), and was diminished by the concomitant use of anti-dynorphin A antiserum (10 μg/g, i.t.), yohimbine (10 μg/g, i.t.), or methysergide (20 μg/g, i.t.). Processed Aconiti tuber extract (27 mg/kg, i.d.) also suppressed bladder motility, and the effect was abolished by nor-binaltorphimine. These results suggest that Gosha-jinki-gan attenuates bladder sensation via the kappa-opioid receptor-stimulating action of processed Aconiti tuber. Gosha-jinki-gan may be a useful anti-pollakiuria agent that does not influence bladder contractility at micturition.

Keywords: Gosha-jinki-gan, anti-pollakiuria effect, micturition reflex, kappa-opioid receptor, processed Aconiti tuber

Gosha-jinki-gan is a traditional Japanese medicine used for urinary disorders. Tokunaga et al. (1) administered the agent to patients with urinary disturbance and reported that it significantly decreased the frequency of urination, although dysuria did not improve. Gosha-jinki-gan decreases the amplitude of distension-induced rhythmic bladder contractions in dogs while increasing its frequency (2). In addition, Gosha-jinki-gan inhibits bladder contraction induced by electrical stimulation of the pelvic nerve or infusion of acetylcholine (3). These results suggest that Gosha-jinki-gan has anti-cholinergic activity similar to that of atropine. An increase in bladder capacity related to the above action plays an important role in the treatment of urinary frequency. However, these changes were induced by intravenously administered Gosha-jinki-gan. Although traditional Japanese medicines are always orally administered, the influence and mechanism of Gosha-jinki-gan administered to the digestive tract on bladder function have not been investigated. Gosha-jinki-gan is also used to treat lower limb/lumbar pain. The anti-nociceptive effects of the agent are partially associated with stimulation of spinal kappa-opioid receptors via dynorphin release. This is a result of the action of processed Aconiti tuber, a crude drug component of Gosha-jinki-gan (4). Recently, we found that activated spinal kappa-opioid receptors inhibited bladder motility (5). A clinical study showed that a putative mu antagonist and kappa agonist, nalbuphine, causes delayed full bladder sensation and increases bladder capacity (6). These findings suggest that the anti-pollakiuria effects of Gosha-jinki-gan involve activation of kappa-opioid receptors. In this study, we compared the effects of Gosha-jinki-gan administered
Materials and Methods

General procedure

All animal experiments were performed in accordance with our institutional guidelines after obtaining permission from the Laboratory Animal Committee. Male Wistar rats (210 – 290 g) were anesthetized with urethane (700 mg/kg, s.c.) and α-chloralose (35 mg/kg, s.c.). If necessary, catheters were inserted into the duodenum and femoral vein for drug administration. Through a midline incision of the abdomen, bilateral ureters were cut on the rostral side after being ligatured. PE-50 polyethylene tubing filled with saline was catheterized from a small incision of the apex of the bladder dome and secured in place by double ligatures around the incision. The intravesicular pressure of the bladder dome and secured in place by double ligatures around the incision. The intravesicular pressure of the bladder dome and secured in place by double ligatures around the incision. The intravesicular pressure of the bladder dome and secured in place by double ligatures around the incision. The intravesicular pressure of the bladder dome and secured in place by double ligatures around the incision. The intravesicular pressure of the bladder dome and secured in place by double ligatures around the incision.

Measurement of pontine micturition center stimulation-induced bladder contractions

After rats in which the urethra was double-ligated were placed in a stereotaxic apparatus, a 1-mm hole was drilled in the cranium dorsal to the pontine micturition center (9, 10). The coordinates for the placement of a bipolar stimulating electrode (tip diameter, 0.2 mm), derived from the atlas of Paxinos and Watson (11), were as follows: A –9.2 mm, L 1.0 mm, H –7.0 mm. The bladder was partially distended due to the infusion of saline through the catheter and the resting intravesicular pressure was kept at 3 – 6 mmHg. The dorsolateral
pontine tegmentum was electrically (1 ms, 50 Hz, 75 – 150 μA) stimulated for 10 s every 5 min in the quiescent bladder. After confirmation that the increase in intravesicular pressure due to 3 continual stimuli remained relatively constant, each drug was administered. The percent change in the evoked contraction amplitude was estimated based on the mean value of 3 stimuli before drug administration. The changes in the amplitude of bladder contractions in animals treated with administration of Gosha-jinki-gan, processed Aconiti tuber extract, or atropine were examined over 90 min after the administration of respective drugs.

**Measurement of distension-induced bladder contractions**

After double ligation of the urethra, the bladder was slowly filled with 37°C saline until spontaneous bladder contractions appeared. Only animals that developed bladder contractions at a stable frequency with an amplitude over 20 mmHg were selected when the baseline value of the intravesicular pressure was 8 – 13 mmHg. After confirmation of the appearance of rhythmic bladder contractions for at least 15 min, drug was administered. The percent change in the contraction frequency every 15 min was estimated based on the number of bladder contractions before drug administration. The changes in the frequency of bladder contractions after administration of Gosha-jinki-gan, or processed Aconiti tuber extract, were observed over 120 min. The changes in the contraction frequency in animals treated with i.t. injection of saline, anti-dynorphin A antiserum, yohimbine or methysergide, followed by Gosha-jinki-gan administration, were checked over 90 min thereafter. Furthermore, effects of independent i.t. administration of anti-dynorphin A antiserum, yohimbine or methysergide on the frequency of bladder contractions were also observed over 90 min.

**Statistical analyses**

The effects of the test agents on cystometrograms were evaluated based on differences in the mean values of bladder capacity, threshold pressure, and amplitude of micturition contraction after administration in comparison to pretreatment values. The inhibitory activities of the drugs on bladder contractions induced by distension or pontine micturition center stimulation were expressed as the area under the time-response curve (AUC), which was calculated by plotting the increase in inhibitory rate (%) on the ordinate and time interval (min) on the abscissa (5). The results were expressed as the mean ± S.E.M. The significance of differences was determined using a one-way analysis of variance (ANOVA) followed by Tukey’s test. For all cases, differences of P<0.05 were considered significant.

**Results**

**Effect of Gosha-jinki-gan on cystometrograms**

When intravesicular pressure reached 5 to 10 mmHg by infusion of saline at a constant rate, micturition with transient bladder contraction was observed. There were no significant differences in the mean values of bladder capacity, threshold pressure, or maximum bladder contraction pressure before administration among the groups (data not shown).

In animals treated with Gosha-jinki-gan (0.3, 1.0 g/kg, i.d.), the interval between the start of saline infusion and micturition was prolonged. Gosha-jinki-gan increased not only bladder capacity but micturition threshold pressure. However, there were no changes in the maximum bladder contraction pressure (Table 1). Processed Aconiti tuber extract (27 mg/kg, i.d.) corresponding to 1.0 g/kg of Gosha-jinki-gan showed similar effects. Atropine (1 mg/kg, i.d.) increased bladder capacity, but did not influence threshold pressure. The agent reduced the maximum bladder contraction pressure during micturition.

**Table 1.** Effects of Gosha-jinki-gan, processed Aconiti tuber extract, and atropine on the cystometrograms in anesthetized rats

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
<th>n</th>
<th>Bladder capacity (μl)</th>
<th>Threshold pressure (mmHg)</th>
<th>Maximum bladder contraction pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5 ml/kg</td>
<td>6</td>
<td>0.00 ± 0.01</td>
<td>0.1 ± 0.1</td>
<td>−0.2 ± 0.5</td>
</tr>
<tr>
<td>Gosha-jinki-gan</td>
<td>0.3 g/kg</td>
<td>6</td>
<td>0.17 ± 0.06</td>
<td>2.4 ± 0.7</td>
<td>−0.3 ± 0.7</td>
</tr>
<tr>
<td>Gosha-jinki-gan</td>
<td>1.0 g/kg</td>
<td>6</td>
<td>0.36 ± 0.06**</td>
<td>4.2 ± 1.0**</td>
<td>0.1 ± 1.7</td>
</tr>
<tr>
<td>Processed Aconiti tuber extract</td>
<td>27 mg/kg</td>
<td>6</td>
<td>0.19 ± 0.02*</td>
<td>3.0 ± 0.9*</td>
<td>−0.5 ± 1.0</td>
</tr>
<tr>
<td>Atropine</td>
<td>1 mg/kg</td>
<td>6</td>
<td>0.21 ± 0.03*</td>
<td>0.4 ± 0.1</td>
<td>−8.5 ± 0.8**</td>
</tr>
</tbody>
</table>

Each cystometric parameter was evaluated based on the difference between the mean of values measured before and after drug administration. Each value represents the mean ± S.E.M. *P<0.05, **P<0.01, compared with the value of distilled water-treated group.
Effect of Gosha-jinki-gan on pontine micturition center stimulation-induced bladder contractions

Electrical stimulation of the dorsolateral pontine tegmentum produced an increase in intravesicular pressure from 21 to 47 mmHg (28.5 ± 0.8 mmHg, n = 36). The amplitude of evoked bladder contractions was decreased by atropine (1 mg/kg, i.d.). However, neither Gosha-jinki-gan (1.0 g/kg, i.d.) nor processed Aconiti tuber extract (27 mg/kg, i.d.) influenced this parameter during the observation period for 90 min after administration (Figs. 1 and 2). In contrast to i.d. Gosha-jinki-gan, it inhibited the contraction amplitude when administered to a femoral vein at 0.1 g/kg (Fig. 3).

Effect of Gosha-jinki-gan on distension-induced bladder contractions

When the resting intravesicular pressure was adjusted to 8 – 13 mmHg by infusion of an appropriate amount of saline, spontaneous bladder contractions were observed from 9 to 35 times per 15 min (21.2 ± 0.6, n = 100). There was no significant difference in the contraction frequency between the groups in the 15 min before drug administration (data not shown).

Distension-induced rhythmic bladder contractions were not affected by distilled water (5 ml/kg, i.d.); however, they were suppressed by Gosha-jinki-gan (0.3, 1.0 g/kg, i.d.) (Figs. 4 and 5). Pretreatment with nor-BNI (10 mg/kg, s.c.), a selective kappa antagonist, abolished the effects of Gosha-jinki-gan (1.0 g/kg, i.d.) on the frequency of distension-induced bladder contractions (Fig. 5). In contrast, atropine (1 mg/kg, i.d.) increased the number of bladder contractions per 15 min (AUC = –4399.5 ± 869.9, n = 7; P<0.01 vs distilled water-treated group). Processed Aconiti tuber extract (27 mg/kg, i.d.) also inhibited spontaneous bladder contractions (Fig. 6), although the duration of action was shorter than that of Gosha-jinki-gan at 1.0 g/kg. The action of processed Aconiti tuber extract was also abolished by nor-BNI (Fig. 6). The suppression of bladder motility after i.d. administration of Gosha-jinki-gan (1.0 g/kg) was also confirmed in animals treated with saline (10 µl, i.t.). However, the concurrent use of anti-dynorphin A antiserum (10 µg, i.t.), yohimbine (10 µg, i.t.), a noradrenaline antagonist, or methysergide (20 µg, i.t.), a serotonin antagonist, diminished the inhibitory effect (Fig. 7). In another experiment, it was confirmed that anti-dynorphin A antiserum, yohimbine or methysergide did not affect the frequency of spontaneous bladder contractions, which were observed for 90 min (data not shown).

Discussion

In this study, the actions of Gosha-jinki-gan administered to the duodenum on bladder function were markedly different from those of atropine. A decrease in the amplitude of bladder contractions and an increase in the frequency of rhythmic bladder contractions after...
systemic administration of atropine may be associated with blockade of excitatory (at detrusor muscles) and inhibitory (at vesical pelvic ganglia) muscarinic receptors (12–14). In contrast to atropine, i.d. Gosha-jinki-gan suppressed distension-induced bladder contractions without influencing the amplitude of bladder contractions. Intravenously administered Gosha-jinki-gan inhibited vesical contractions in an experiment involving...
electrical stimulation of the pontine micturition center, as reported by Suzuki et al. (2, 3). Therefore, Gosha-jinki-gan may contain an anti-cholinergic substance that is not absorbed from the digestive tract. However, traditional Japanese medicines are always orally administered, and we cannot conclude that the clinical effects of Gosha-jinki-gan on urinary frequency (1) are associated with anti-cholinergic actions.

Pretreatment with nor-BNI eliminated the inhibitory effects of Gosha-jinki-gan on bladder motility. This finding suggests that kappa-opioid receptors are closely involved in the actions of Gosha-jinki-gan. Gosha-jinki-gan activates spinal kappa-opioid receptors by releasing dynorphin in the spinal cord (4). Stimulation of peripheral kappa-opioid receptors induces hyperactivity of the detrusor muscle (15). In contrast, activation of spinal kappa-opioid receptors inhibits the micturition reflex via blunting of bladder sensation (5). I.d. Gosha-jinki-gan increased bladder capacity and threshold pressure to micturition, but decreased the frequency of distension-induced bladder contractions. This finding may be explained by inhibition of an afferent pathway from the urinary bladder to the micturition center. Gosha-jinki-gan administered to the digestive tract did not influence pontine micturition center stimulation-induced bladder contractions. Therefore, the possibility that the agent acts on an efferent pathway of micturition can be ruled out.

I.t. administration of anti-dynorphin A antiserum attenuated the action of Gosha-jinki-gan on rhythmic bladder contractions. The result supports the theory that activation of spinal kappa-opioid receptors after administration of Gosha-jinki-gan is mediated by an increase in dynorphin release in the spinal cord. We previously reported that spinal kappa-opioid receptors not only in the lumbo-sacral region but also in the thoracic region inhibit the micturition reflex (5). However, a compound injected intrathecally to the lumbo-sacral region through a catheter which was inserted from a slit in the dura mater between L1 and L2 failed to diffuse to the thoracic region (5). It is likely that anti-dynorphin A antiserum injected at the L5 or L6 spine level in this study could not inhibit the activity of dynorphin in the thoracic region. Therefore, the inhibitory effect of Gosha-jinki-gan might be partially reduced by i.t. injection of anti-dynorphin A antiserum, differing from systemically administered nor-BNI.

Activation of the descending noradrenergic and serotoninergic systems is closely involved in anti-nociception related to stimulation of spinal kappa-opioid receptors (16, 17). These activated descending monoaminergic systems inhibit not only pain but also bladder motility (5, 18, 19). The results that the activity of Gosha-jinki-gan was decreased by i.t. administration of yohimbine...
or methysergide, at the same doses which prevented the inhibitory effect of a kappa agonist U-50488H on the bladder motility (5), suggested that both noradrenergic and serotonergic mechanisms play important roles also in the inhibitory effects of Gosha-jinki-gan on the micturition reflex. Primary afferent fibers from the urinary bladder use various neuropeptides (substance P, somatostatin, and vasoactive intestinal polypeptide, etc.)
as sensory neurotransmitters and/or neuromodulators in the dorsal horn (20–23). The activated descending inhibitory systems may have diminished the bladder sensation by inhibiting the release of these neuropeptides through noradrenaline α2 and methysergide-sensitive serotonin receptors as well as analgesia-producing mechanisms (24, 25).

Gosha-jinki-gan activates the descending monoaminergic systems by releasing spinal cord dynorphin. This action is mediated by the activity of one of the crude drug components of Gosha-jinki-gan, processed Aconiti tuber (4, 17). Administration of processed Aconiti tuber extract alone also decreased the frequency of distension-induced bladder contractions, suggesting the importance of processed Aconiti tuber in the inhibitory effects of Gosha-jinki-gan on the micturition reflex. However, when the effect of processed Aconiti tuber extract on bladder motility was evaluated using AUC, the activity was less potent than that of Gosha-jinki-gan at 1.0 g/kg. This finding suggests that Gosha-jinki-gan contains an ingredient derived from a galenical component other than processed Aconiti tuber that prolongs or exacerbates the action of processed Aconiti tuber.

In conclusion, these results suggest that the effects of Gosha-jinki-gan on urinary frequency are associated not with anti-cholinergic action but with diminished bladder sensation via spinal kappa-opioid receptors. Gosha-jinki-gan does not inhibit the amplitude of bladder contractions and may be useful for treating urinary frequency in patients with prostatic enlargement.

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References


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