EFFECTS OF INTRATHECAL GALANIN ON NOCICEPTIVE RESPONSES IN RATS WITH MONONEUROPATHY

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Summary

The present study was performed on rats with experimental mononeuropathy induced by left common sciatic nerve loose ligation. Unilateral sciatic nerve loose ligation induced decreases of the hindpaw withdrawal latency to the hot-plate test, cold-plate test and the Randall Selitto test. Sciatic nerve loose ligation induced hyperresponsiveness to touch at room temperature also. Intrathecal administration of either 3 or 6 nmol of galanin, but not 1 nmol, induced significant bilateral increases in hindpaw withdrawal latencies to the hot-plate test, cold-plate test and the Randall Selitto tests in rats with left mononeuropathy. The results indicate that galanin may play important roles in transmission of presumed nociceptive information in the spinal cord of mononeuropathic rats.

Key Words: galanin, sciatic nerve loose ligation, mononeuropathy, hindpaw withdrawal latency, hot-plate test, cold-plate test, Randall Selitto test

Since the discovery of galanin in 1983 (1), many studies have demonstrated that the peptide is an important messenger for intercellular communication within the central nervous system (2, 3). Galanin is found in primary afferents as well as dorsal root ganglia cells (4, 5) and in laminae I and II of the spinal cord dorsal horn (6). The effects of exogenously administered galanin on nociception are contradictory. Using intact rats Kuraishi and co-workers reported that intrathecal injection of galanin decreased the nociceptive threshold for mechanical stimulation but was without effect on thermal nociceptive responses, while galanin antiserum markedly increased the nociceptive threshold to mechanical stimulation (7). On the other hand, Post et al. reported that intrathecal galanin increased the latency in the tail-flick and hot-plate tests in mice (8). Wiesenfeld-Hallin et al. reported that intrathecal galanin at the low dose of 0.0316 nmol (0.1 μg)
increased rat spinal reflex excitability to thermal stimulation more than to mechanical stimulation (9). At the high dose of 0.316 nmol (1 μg) galanin inhibited the nociceptive flexor reflex, an effect which was enhanced after sciatic nerve injury (10). In behaviour studies Verge et al. reported that administration of a galanin antagonist and a galanin antisense oligonucleotide induced a higher rate of autotomy after axotomy, so they suggested that endogenous galanin has an anti-nociceptive effect (11). The discrepancies between the different results on galanin may very well be related to differences in technique and/or to the different models of nociception used (12). The present study was performed to unravel the role of intrathecal galanin in the mononeuropathic rat model.

**Methods**

*Animal preparation*

All experiments were performed on freely moving male Sprague-Dawley rats (250-300 g; Experimental Animal Center of Beijing Medical University, Beijing, China). The rats were housed in cages with free access to food and water, and maintained in a room temperature of 24 +2°C with a 12 h light/dark cycle. Rats were accustomed to the testing conditions before starting the experiments in order to stabilize response latencies and to decrease the stress caused by handling and measurements. All experiments were conducted according to the guideline of the animal ethical committee of Karolinska Institutet and every effort was made to minimize animal suffering.

*Left common sciatic nerve ligation*

The mononeuropathy model employed was that according to Bennett and Xie (13). Briefly, the rats were anesthetized with intraperitoneal pentobarbital sodium (40 mg/kg). The left common sciatic nerve was exposed for about 8-10 mm at the level of the mid thigh. Four loose ligatures (4.0 chromic gut), 1 mm apart, were tied around the nerve under microscope guidance and carefully manipulated so that the nerve was barely constricted. Rats in control group received the same operation without sciatic nerve loose ligation. The incision was closed in layers with 4-0 silk sutures.

*Intrathecal catheter implantation and intrathecal injection*

Five days after sciatic nerve loose ligation, the intrathecal catheter was implanted. A long term polyethylene catheter (Intramedic PE 10) was implanted with the inner tip at L3 to L5 according to Yaksh and Rudy (14). Two days after the implantation, intrathecal injections were performed only on rats which showed no disorder of movements after implantation. Intrathecal administrations were performed between 7 to 14 days after sciatic nerve ligation. Ten microliters of solution (see below) were injected intrathecally followed by a 10 μl of 0.9% saline flush of the catheter.

*Nociceptive tests*

The latency to hindpaw withdrawal during heat, cold or mechanical stimulation was measured (12, 15-18). The hindpaw withdrawal latency (HWL) to heat stimulation was assessed by the hot-plate test. The entire ventral surface of the rat’s left or right hindpaw was placed on the hot-plate which was maintained at a temperature of 52°C (51.8-52.4°C). The HWL to cold stimulation was
similarly assessed by the cold-plate at a temperature of 4 °C (4.0-4.5 °C). The time to hindpaw withdrawal was measured and is referred to as HWL. The Randall Selitto Test (UGO Basile, Type 7200, Italy) was used to assess the HWL to mechanical stimulation. A wedged-shaped pusher with a loading rate of 48 g/second was applied to the dorsal surface of the manually handled hindpaw and the pressure required to initiate a struggle response was assessed. The HWL induced by mechanical stimulation is expressed in seconds, i.e., latency to withdrawal from the start of stimulation. The data measured before intrathecal injection were regarded as the basal HWL to heat, cold and mechanical stimulation. The HWL recorded during subsequent experiments were expressed as % change of the basal HWL for each rat (15-18). The three behavioural tests were initiated before intrathecal injection and repeated at 5, 15, 30 and 60 min after the injection. In order to familiarize the testing conditions and to minimize the differences in response the rats were trained for 5 days before the experiment was performed.

**Chemicals**

Solutions for intrathecal administration were prepared with sterilized saline (0.9%), each with a volume of 10 μl: 1, 3 or 6 nmol of galanin (rat-galanin, Sigma Chemical Company, St. Louis, Mo). Ten μl of 0.9% saline was injected intrathecally as a control.

**Statistical analysis**

Data from hindpaw withdrawal tests were presented as mean ± SEM. The difference between groups was determined by either two-way analysis of variance (ANOVA) for the repeated measures or Student's t-test (two tailed) where applicable. *P<0.05, **P<0.01 and ***P<0.001 were considered as significant differences.

**Results**

*Effects of left common sciatic nerve ligation on HWLs to heat, cold and mechanical stimulation*

Ten rats of control group (sham operation) and ten rats with left common sciatic nerve loose ligation were tested by the hot-plate test, the cold-plate test and the Randall Selitto test. The results are shown in TABLE I.

<table>
<thead>
<tr>
<th>Nociceptive tests</th>
<th>treatments</th>
<th>n</th>
<th>left side</th>
<th>right side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot-plate test</td>
<td>intact rats</td>
<td>10</td>
<td>5.28 ± 0.20s</td>
<td>5.29 ± 0.22s</td>
</tr>
<tr>
<td></td>
<td>ligated rats</td>
<td>10</td>
<td>3.04 ± 0.13s***</td>
<td>4.25 ± 0.08s**</td>
</tr>
<tr>
<td>Cold-plate test</td>
<td>intact rats</td>
<td>10</td>
<td>2.81 ± 0.05s</td>
<td>2.81 ± 0.06s</td>
</tr>
<tr>
<td></td>
<td>ligated rats</td>
<td>10</td>
<td>1.77 ± 0.08s***</td>
<td>2.71 ± 0.05s</td>
</tr>
<tr>
<td>Randall Selitto test</td>
<td>intact rats</td>
<td>10</td>
<td>5.20 ± 0.20s</td>
<td>5.49 ± 0.19s</td>
</tr>
<tr>
<td></td>
<td>ligated rats</td>
<td>10</td>
<td>3.20 ± 0.13s***</td>
<td>4.10 ± 0.12s**</td>
</tr>
</tbody>
</table>

Student t-test, **P<0.01 and ***P<0.001 compared to the group of intact rats; s: seconds.
No significant difference was seen between left and right HWLs to heat stimulation in control group \((t_{left/right}=0.28, P=0.78)\), but those in rats with mononeuropathy were decreased bilaterally \((t_{left/left}=17.1, P<0.001; t_{right/right}=9.57, P<0.001)\) compared with the control group. The HWL was significantly shorter on the ligated side than on the contralateral side \((t_{left/right}=8.63, P<0.001)\). In control group, rats showed no significant difference between left and right HWLs to cold stimulation \((t_{left/right}=0.56, P=0.59)\). The decrease in the left HWL of mononeuropathic rats was significant but that on the right was not \((t_{left/left}=10.65, P<0.001; t_{right/right}=1.43, P=0.19)\) compared with that of control group. Similarly in the Randall Selitto test there was no significant difference between left and right HWLs to mechanical stimulation in control group \((t_{left/right}=1.38, P=0.19)\), but there were comparative bilateral decreases in HWLs in rats with mononeuropathy \((t_{left/left}=12.94, P<0.001; t_{right/right}=11.33, P<0.001)\). Again HWL to mechanical stimulation was significantly shorter on the ligated side than on the contralateral side \((t_{left/right}=10.5, P<0.001)\) in rats with left mononeuropathy. The left (ligated side) HWLs decreased more significantly than the right (contralateral side) HWLs tested by hot-plate \((t_{left/right}=10.67, P<0.001)\), cold-plate \((t_{left/right}=12.53, P<0.001)\) and Randall Selitto test \((t_{right/right}=7.82, P<0.01)\) in rats with left sciatic nerve loose ligation.

**Hyper responsiveness to touch induced by sciatic nerve ligation**

Rats in control group \((n=10, \text{sham operation})\) and rats with mononeuropathy \((n=10)\) were tested by the cold-plate test \((4^\circ C, \text{as shown in Fig. 1A})\), the room temperature-plate test \((24^\circ C, \text{as shown in Fig. 1B})\) and the hot-plate test \((52^\circ C, \text{as shown in Fig. 1C})\).

![Fig. 1](image)

HWL to cold \((4^\circ C, \text{Fig. 1A})\), room temperature \((24^\circ C, \text{Fig. 1B})\) and heat \((52^\circ C, \text{Fig. 1C})\) stimulation in control group \((n=10)\) and in rats with left common sciatic nerve ligation \((n=10)\). The left sides are shown by the shaded columns and the right sides by the open columns. The short bar indicates standard error of the mean. Student's t-test (two-tails), \(^*^*^*P<0.01\) and \(^*^*^*^*P<0.001\) compared with control group.

In the cold-plate test \((4^\circ C)\) the HWLs of both sides of control group were very similar \((\text{left: } 2.81 + 0.05s, \text{right: } 2.81 + 0.06s)\). But there was a significant decrease in the left HWL \((\text{left side: } P<0.001)\) in rats after left sciatic nerve ligation as shown in Fig. 1A. In the hot-plate test the HWLs of both sides of control group were also very similar \((\text{left: } 5.28 + 0.20s, \text{right: } 5.29 + 0.22s)\). However, significant bilateral decreases of HWLs to heat stimulation \((\text{left: } P<0.001;\)
Effect of Galanin in Mononeuropathic Rat

Effect of intrathecal administration of galanin on HWLs in rats with mononeuropathy

Twenty-four rats with mononeuropathy were divided into four groups receiving intrathecal injections of: (1) 10 μl of 0.9% saline as the control (n=6), (2) 1 nmol of galanin (n=6), (3) 3 nmol of galanin (n=6) or (4) 6 nmol of galanin (n=6). Fig. 2 shows the results of the hot-plate test. Compared to the control group, there was significant increase in left and right HWLs in the groups receiving galanin: 1 nmol (Fig. 2A: Fleft/left=17.82, P<0.01; Fig. 2B: Fright/right=8.71, P<0.01), 3 nmol (Fig. 2A: Fleft/left=25.38, P<0.001; Fig. 2B: Fright/right=23.44, P<0.001) and 6 nmol (Fig. 2A: Fleft/left=24.62, P<0.001; Fig. 2B: Fright/right=24.38, P<0.001).

Fig. 3 shows the results of the cold-plate test. Compared to the control group, there was no significant increase in HWLs in the group receiving 1 nmol of galanin (Fig. 3A: Fleft/left=3.27, P=0.08; Fig. 3B: Fright/right=0.35, P=0.56), but in those received 3 or 6 nmol of galanin there were significant increases in HWLs (3 nmol: Fleft/left=12.55, P<0.001; Fright/right=18.35, P<0.001. 6 nmol: Fleft/left=14.36, P<0.001; Fright/right=18.41, P<0.001) compared with the control group, as shown in Fig. 3A and 3B.

Fig. 4 shows the results of the Randall Selitto test. Compared to the control group, there was no significant increase in the left HWL in the group receiving 1 nmol of galanin (Fleft/left=2.50, P=0.12; Fig. 4A), but there was a significant increase in right HWL (Fright/right=10.29, P<0.01; Fig. 4B). In the groups received 3 or 6 nmol of galanin there were significant increase in HWLs (3 nmol: Fleft/left=9.19, P<0.01; Fright/right=20.25, P<0.001. 6 nmol: Fleft/left=39.96, P<0.001; Fright/right=30.01, P<0.001) compared to control group, as shown in Fig. 4A and 4B.

Fig. 2 Effects of intrathecal injection of galanin on hindpaw withdrawal latencies to heat stimulation in rats with left common sciatic nerve ligation.

Time=0: intrathecal injection of galanin or 10 μl of 0.9% saline as a control. Vertical bars indicate S.E.M. Two-way ANOVA, *P<0.05, **P<0.01 and ***P<0.001 compared to control group.
Effect of Galanin in Mononeuropathic Rat

Fig. 3
Effects of intrathecal injection of galanin on hindpaw withdrawal latencies to cold stimulation in rats with left common sciatic nerve ligation. Time=0: intrathecal injection of galanin or 10 µl of 0.9% saline as a control. Vertical bars indicate S.E.M. Two-way ANOVA, **P<0.01 and ***P<0.001 compared to control group.

Fig. 4
Effects of intrathecal injection of galanin on hindpaw withdrawal latencies to mechanical stimulation in rats with left common sciatic nerve ligation. Time=0: intrathecal injection of galanin or 10 µl of 0.9% saline as a control. Vertical bars indicate S.E.M. Two-way ANOVA, **P<0.01 and ***P<0.001 compared to control group.

Discussion
Chronic constriction injury produced by loosely tying four ligatures around the sciatic nerve is used as an animal model of neuropathic pain resulting from partial nerve injury (13, 16). Large myelinated fibres degenerate after constriction injury, but thinly myelinated and unmyelinated fibres are less severely damaged (19). The damage and loss of axonal fibres is a slow and
gradual process in partial nerve injury. In the present study in both heat and mechanical stimulation tests we found bilateral decreases in hindpaw withdrawal responses in rats with unilateral mononeuropathy (TABLE I) when tested by the hot-plate and Randall Selitto tests. These findings confirmed the earlier observations in this model by Attal et al. (20) and Bennett and Xie (13), and are indicative of spinal cord hyperexcitability which is not restricted to the side of common sciatic nerve ligation. That unilateral nociceptive input may activate neurons of both dorsal horns of the spinal cord has been demonstrated earlier (21, 22). Such activation of the contralateral dorsal horn might result from dorsal root reflexes (23), direct segmental projections (24-26) or indirectly via a supraspinal loop (27).

In the present study the cold-plate test was also used. As shown in TABLE I the decrease in the HWL to both heat and mechanical stimulation was bilateral, but to cold stimulation only significant in left HWL in rats with mononeuropathy (TABLE I). Neurogenic pain tests for cold “allodynia” and “hyperalgesia” in rats with experimentally induced neuropathic pain have proved to be of interest (13, 20, 28). As a decrease in HWL to cold stimulation was found only on the nerve ligated side in the present study, it may be suggested that cold stimulation may possibly serve as a test allowing for differentiation between nociceptive and neurogenic pain in rats (20, 28).

Touch is one of the somatosensory functions whose receptors are low-threshold mechanoreceptors. Clinically, touch has been employed as a parameter for examination in patients with neuropathic pain where there is an increased touch threshold (29, 30). The present study found hyperresponsiveness of touch only in the ipsilateral hindpaws of rats with left side mononeuropathy.

Numerous studies have demonstrated that sciatic nerve injury induces significant up-regulation of galanin peptide and galanin mRNA levels in dorsal root ganglion neurons (31, 32), which contrasts with the down-regulation of substance P level in the dorsal horn and dorsal root ganglion (26, 33-36). Ma and Bisby have reported that partial sciatic nerve injuries induced greater galanin up-regulation in medium- and large-sized dorsal root ganglion neurons than complete sciatic nerve section, suggesting that more severe neuropathic pain was caused by partial sciatic nerve injury than by complete sciatic nerve section, thus leading to a higher level of galanin expression as a compensatory response (37).

As stated previously, discrepancies between the different results on exogenously administered galanin are likely due to differences in technique and/or to the different models of nociception used (7, 8, 12, 38). Also, the differences in results may be explained by dose-dependent effects. This suggestion is supported by Wiesenfeld-Hallin et al. who have reported that lower doses of intrathecal galanin increased the excitability of the flexor reflex, while higher doses produced a prolonged depression of this reflex and blocked the facilitatory effects of substance P and CGRP upon it (9, 39, 40). In rats with sciatic nerve axotomy, Wiesenfeld-Hallin et al. reported that galantide, the antagonist of galanin, enhanced the nociceptive reflex in spinalized rats with much greater effect in axotomized than in intact rats (40). The results from sciatic nerve loose ligation (sciatic nerve chronic constriction injury) are differ from the results from sciatic nerve tight ligation or axotomy. Recently, Goff et al. (1998) have demonstrated that there were different changes in behavioural nociceptive tests and in central reorganisation after sciatic nerve chronic constriction injury and tight ligation (41). In rats with sciatic nerve loose ligation, the present study showed that intrathecal administration of 3 and 6 nmol of galanin produced significant increases in hindpaw withdrawal latency to both heat and mechanical tests. In dorsal horn of the spinal cord, galanin is found to coexist with substance P, calcitonin gene-
related peptide and other neuritransmitters (40, 42). It is well known that in dorsal horn of the spinal cord substance and calcitonin gene-related peptide released from peripheral afferents to transmit the presumed nociceptive information (43). Galanin is a neuropeptide with inhibitory action (44). Intrathecal administration of galanin produce an inhibitory effect on the transmission of presumed nociceptive information in the spinal cord. These results suggested that galanin might exert an antinociceptive action following peripheral nerve chronic constriction injury.

In summary, the results of the present study demonstrated that unilateral sciatic nerve loose ligation induced decreases in the hindpaw withdrawal latency to the hot-plate test, cold-plate test and the Randall Selitto test. Sciatic nerve loose ligation induced hyperresponsiveness to touch at room temperature also. Intrathecal administration of either 3 or 6 nmol of galanin, but not 1 nmol, induced significant bilateral increases in hindpaw withdrawal latencies to the hot-plate test, cold-plate test and the Randall Selitto tests in rats with left mononeuropathy. The results indicate that galanin may play important roles in transmission of presumed nociceptive information in the spinal cord of mononeuropathic rats.

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