ANTINOCICEPTIVE ROLE OF GALANIN IN PERIAQUEDUCTAL GREY OF RATS WITH EXPERIMENTALLY INDUCED MONONEUROPATHY

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Abstract—The present study was performed in rats with experimentally induced mononeuropathy after left common sciatic nerve ligation. The hindpaw withdrawal latencies to thermal and mechanical stimulation increased significantly after intra-periaqueductal grey injection of 2 or 3 nmol, but not 1 nmol of galanin in rats with mononeuropathy. Intraperitoneal administration of 4.5 mg/kg morphine induced significant increases in hindpaw withdrawal latencies to both noxious stimulation, which were attenuated by following intra-periaqueductal grey injection of 2 nmol of the galanin antagonist galantide. Furthermore, the antinociceptive effect induced by intra-periaqueductal grey injection of 26.6 nmol of morphine was attenuated significantly by following intra-periaqueductal grey administration of 2 nmol of galantide.

The results demonstrated that in periaqueductal grey galanin plays an antinociceptive role in rats with mononeuropathy and galanin is involved in the mechanisms of opioid-induced antinociception. © 2000 IBRO. Published by Elsevier Science Ltd.

Key words: galanin, opioid peptides, periaqueductal grey, mononeuropathy, antinociception, hindpaw withdrawal latency.

Opioid binding sites in the superficial dorsal horn of the spinal cord in rats also changed after nerve chronic constriction injury. A significant up-regulation in μ binding sites was found at the spinal cord level following nerve ligation. The up-regulation appeared to be bilateral although, on the ipsilateral side, this effect might be masked by fiber degeneration.

Recent studies indicated that there may be a close interaction between galanin and opioids in the mechanisms of endogenous antinociception in intact rats. Recent studies in our laboratory demonstrated that intrathecal injection 1 or 3 nmol of galantide, an antagonist of galanin, attenuated the antinociceptive effects induced by intrathecal injection of morphine in rats with mononeuropathy.

Moreover, our study demonstrated that intra-periaqueductal grey (PAG) administration of galanin induced marked antinociceptive effects in intact rats. The present study was performed to investigate the role of intra-PAG injection of galanin on nociception and the presumable interaction between galanin and opioids in PAG in mononeuropathic rats.

EXPERIMENTAL PROCEDURES

Animal preparation

Freely moving male Sprague–Dawley rats weighing 250–300 g (Experimental Animal Center of Beijing Medical University, Beijing, China) were used in the present experiments, which were conducted according to the guidelines of the animal ethical committee of Karolinska Institutet and every effort was made to minimize both the animal suffering and the number of animals used.

Surgical procedures

Rats were anaesthetized with intraperitoneal sodium pentobarbital (45 mg/kg). To produce a mononeuropathic model, the rat’s left sciatic nerve was exposed for 8–10 mm at the level of the mid thigh. Four loose ligatures (4.0 chronic gut) were made around the dissected nerve with a 1.0–1.5-mm interval between each of them. The ligation was
carefully manipulated so that the nerve was barely constricted. The skin incision was closed with 4-0 silk sutures.

Intra-periaqueductal grey injection

The animals were anesthetized by intraperitoneal sodium pentobarbital (45 mg/kg) and were mounted on a stereotaxic instrument. A stainless steel guide cannula of 0.8 mm o.d. was directed to PAG (AP 5.5, L 0.5, H 6.0 mm from the surface of the skull) according to Paxinos and Watson and was fixed to the skull by dental acrylic. On the experimental day a stainless steel needle with 0.4 mm diameter was directly inserted into the guide cannula, with 1 mm beyond the tip of the latter. One microliter of solution was thereafter infused into PAG over 1 min.

Nociceptive tests

All rats were accustomed to the testing conditions for five days before the experiment was conducted. The latencies to hindpaw withdrawal during thermal and mechanical stimulation were measured. The thermal response was assessed by the hot-plate test. The entire ventral surface of the rat’s hindpaw was placed manually on the hot-plate which was maintained at a temperature of 52°C (51.8–52.4°C). The time to hindpaw withdrawal was measured in seconds to be referred to as the hindpaw withdrawal latency (HWL). The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess the HWL. A wedge-shaped pusher at a loading rate of 30 g/s was applied to the dorsal surface of the manually handled hind-paw and the latency required to initiate the struggle response was measured and expressed in seconds. A microliter of solution was thereafter infused into PAG area were used for statistical analysis.

Histological test

At the end of the experiment rats were killed with intraperitoneal high dose of sodium pentobarbital (80 mg/kg) and the rat’s heads were fixed in 10% formalin for one week with the injecting tube placed within PAG. The injecting tube was verified in serial 50-μm crystal sections. Only the results from nociceptive tests with the tip of the injecting tube placed within PAG area were used for statistical analysis.

Chemicals

Morphine hydrochloride (morphine hydrochloride, Shenyang First Pharmaceutical Factory, Shenyang, China) was diluted to 1 mg/ml with sterilized saline and was administered intraperitoneally at a dose of 4.5 mg/kg. Solutions for intra-PAG administration were prepared with sterilized saline, each with a volume of 1 μl: (i) 1, 2 or 3 nmol of galanin (galanin, Bachem, Feinchemikalien AG, Switzerland), respectively; (ii) 2 nmol of galantide [Galanin (1–13)-Substance P (5–11) amide, Bachem, Feinchemikalien AG, Switzerland]; (iii) 26.6 nmol of morphine. Control groups were given 1 μl of 0.9% saline injected into PAG.

Statistical analysis

Data from nociceptive tests were presented as mean ± S.E.M. The difference between groups was determined by two-way analysis of variance (ANOVA) for repeated measures. *P < 0.05, **P < 0.01 and ***P < 0.001 were considered as significant differences.

RESULTS

Effects of intra-periaqueductal grey administration of galanin on hindpaw withdrawal latencies to noxious stimulation in rats with mononeuropathy

Rats received intra-PAG injection of 1 nmol (n = 9), 2 nmol (n = 8) or 3 nmol (n = 9) of galanin, or 1 μl of 0.9% saline (n = 14) as the control group. As shown in Fig. 1, the HWLs to thermal and mechanical stimulation increased significantly after intra-PAG injection of 2 nmol (Thermal test: Fleft/left = 45.43, P < 0.001; Fright/right = 13.73, P < 0.001. Mechanical test: Fleft/left = 18.39, P < 0.001; Fright/right = 7.49, P < 0.01) or 3 nmol of galanin (Thermal test: Fleft/left = 65.23, P < 0.001; Fright/right = 71.70, P < 0.001. Mechanical test: Fleft/left = 44.11, P < 0.001; Fright/right = 44.00, P < 0.001) compared with the control group. In the group receiving intra-PAG injection of 1 nmol of galanin there were no significant changes in HWLs in comparison with the control group (Thermal test: Fleft/left = 2.18, P = 0.14; Fright/right = 3.28, P = 0.07. Mechanical test: Fleft/left = 2.26, P = 0.14; Fright/right = 0.15).
The effects of intra-PAG administration of 3 nmol of galanin reached the peak between 10–20 min after the injection and then began to recover from 30 min.

Effects of intra-periaqueductal grey injection of galantide on intraperitoneal morphine-induced increases in hindpaw withdrawal latency

Rats with mononeuropathy received intraperitoneal injection of 4.5 mg/kg morphine, followed 10 min later, by intra-PAG injection of 2 nmol of galantide \((n = 8)\) or 1 \(\mu\)l of 0.9% saline \((n = 8)\) as the control group. The results are shown in Fig. 2. In the control group, the HWLs to both thermal and mechanical stimulation increased and lasted for more than 60 min. In the group receiving intraperitoneal morphine followed by intra-PAG injection of 2 nmol of galantide, the increased HWLs were attenuated significantly in both tests (Thermal test: \(F_{\text{left/right}} = 70.61, P < 0.001; F_{\text{right/right}} = 132.40, P < 0.001\). Mechanical test: \(F_{\text{left/left}} = 37.02, P < 0.001; F_{\text{right/right}} = 49.05, P < 0.001\)) compared with the control group. In another group, rats receiving intraperitoneal administration of 1 ml of 0.9% saline, followed 10 min later, by intra-PAG administration of 2 nmol of galantide \((n = 6)\), there were no marked changes in the HWL to thermal and mechanical stimulation, as shown in Fig. 2.

Effects of intra-periaqueductal grey injection of galantide on the increased hindpaw withdrawal latency induced by intra-periaqueductal grey injection of morphine

Rats with mononeuropathy received intra-PAG injection of 26.6 nmol of morphine, followed 10 min later, by 2 nmol of galantide \((n = 6)\) or 1 \(\mu\)l of 0.9% saline \((n = 6)\) as a control. The results are shown in Fig. 3. After intra-PAG injection of morphine, the HWL to both noxious stimulation increased. In the group receiving intra-PAG injection of morphine, followed by 2 nmol galantide, the increased HWLs were attenuated significantly (Thermal test: \(F_{\text{left/left}} = 37.18, P < 0.001; F_{\text{right/right}} = 25.64, P < 0.001\). Mechanical test: \(F_{\text{left/left}} = 19.08, P < 0.001; F_{\text{right/right}} = 28.21, P < 0.001\)) as compared with the control group. In another group, rats receiving intra-PAG administration of 1 \(\mu\)l of 0.9% saline, followed 10 min later, by intra-PAG administration of 2 nmol of galantide \((n = 6)\), there were no marked changes in HWLs to both noxious thermal and mechanical stimulation.

DISCUSSION

The results of the present study show that intra-PAG administration of 2 or 3 nmol of galanin induced a dose-dependent antinociceptive effect in rats with mononeuropathy, while 1 nmol of galanin did not. Intraperitoneal injection of 4.5 mg/kg of morphine induced significant increases in HWLs to thermal and mechanical stimulation, and the effects were attenuated by intra-PAG injection of 2 nmol of the galanin receptor antagonist galantide. Furthermore, intra-PAG administration of 2 nmol of galantide attenuated the antinociceptive effects induced by intra-PAG injection of 26.6 nmol of morphine. The results indicate that galanin may have an antinociceptive role in the PAG in mononeuropathic rats, and that galanin interacted with opioid peptides in this brain area.

Galanin is a neuropeptide with mainly inhibitory effects in central nervous system.\(^{1,9,10}\) There was a marked increase of galanin-immunoreactivity in primary sensory neuron and DRG after loose sciatic nerve ligation.\(^{6,12}\) Interestingly, the change was paralleled by changes in nociceptive behaviors.\(^{15}\) It has, therefore, been suggested that galanin may be involved in the endogenous analgesic system, especially after peripheral nerve injury.\(^{6,24,25}\) Nerve injury, such as loose sciatic nerve ligation and axotomy, led to hyperactivity in
the somatosensory system and spontaneous firing of some primary afferent fibers. It is possible that the release of galanin is due to the abnormal impulses of these afferents. Another reason could be diffusion and/or rapid enzymatic breakdown of accumulated peptide. It is also possible that there may be a slowing down of peripheral axonal transport. Furthermore, Carlton et al. found that many galanin terminals no longer co-localized with CGRP after peripheral nerve lesion, possibly indicating there was an increased antinociceptive activity after nerve lesions. In conclusion, the up-regulation of galanin might respond to the increased nociceptive input induced by partial nerve injury.

Another possible and important role of galanin after nerve injury may be to re-establish the neural target contact. The cytokine leukemia inhibitory factor produced by the degenerated nerve possibly up-regulates the galanin in the DRG large neurons in chronic constriction injury rats. This is in line with the finding that galanin has trophic effects on axotomized neurons which are temporarily deprived of their target-derived trophic factors. This may partly explain why some axotomized afferents cease synthesizing their “normal” neuropeptides (e.g. substance P and CGRP) and start synthesizing others such as galanin. Also, Ma and Bisby reported that partial sciatic nerve injury induced greater galanin up-regulation in medium- and large-size DRG neurons than complete sciatic nerve injury. Moreover, according to the report of Munglani et al., the level of galanin-immunoreactivity was down-regulated 100–120 days after sciatic nerve lesion, by which time resolution of the hyperalgesia and peripheral nerve injury has occurred. All the above results indicate that the change in galanin expression observed in peripheral neurons after nerve injury could be characterized as reflecting a shift in the “focus” of the neuron from synaptic transmission to regeneration.

Previous studies showed that galanin co-exists with several other neurotransmitters in the CNS in animals, including opioids. Both galanin receptors and opioid receptors, including μ- and κ-receptors are found in rat PAG, an essential structure for opioid analgesia. Galanin has been proposed to interact with opioids in modulation of the transmission of nociceptive information at the spinal cord level and in PAG in intact rats. In the present study, the increased HWL induced by intra-PAG or intra-peritoneal administration of morphine was significantly attenuated by intra-PAG administration of the galanin receptor antagonist galantide, indicating a possible interaction in antinociception between galanin and opioids in PAG in rats with mononeuropathy.

Interestingly, μ-opioid receptor binding sites were also up-regulated in the spinal cord in chronic constriction injury rats, possibly due to the activation of descending control systems in response to increased nociceptive inputs following sciatic nerve lesion. After binding to its receptor, galanin may facilitate the inhibitory effects of opioid peptides, and/or enhance the affinity of opioid peptides to their own receptors. When galantide, the antagonist of galanin receptors, was applied, it prevented galanin from binding to its receptors, and thus indirectly attenuating the opioid analgesia, as we observed in the present study. Also, in support of our findings is the study by Reimann et al. who investigated whether endogenous galanin, by the use of the galanin antagonist galantide, interacts with exogenously administered morphine, in the rat spinal cord. They reported that in the rat tail-flick test, intrathecal injection of 3 μg of morphine had a significant antinociceptive effect which was almost completely antagonized by co-injection of 2 μg of galantide. Also, this would
suggest that galantide may have a pro-nociceptive effect, a suggestion supported by the finding that galantide induced a higher rate of autotomy after axotomy.\(^2\)

**CONCLUSION**

Intra-PAG injection of galanin induced a significant anti-nociceptive effect in rats with sciatic nerve ligation. The increased HWLs induced by intraperitoneal administration of morphine were significantly attenuated by following intra-PAG injection of galanin, the antagonist of galanin receptors, indicating that PAG is one of the key sites of the interaction between galanin and opioids in rats with mononeuropathy. Furthermore, intra-PAG injection of galanin also reversed the antinociceptive effects induced by intra-PAG administration of morphine. The data suggest an antinociceptive role of galanin in the PAG of mononeuropathic rats, and a possible interaction between galanin and opioid peptides in this brain area.

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**REFERENCES**


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