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Short communication

Endogenous noradrenergic tone controls symptoms of allodynia in the spinal nerve ligation model of neuropathic pain

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Abstract

Endogenous inhibitory controls were studied in the spinal nerve ligation model of neuropathic pain. Atipamezole, a selective α_2-adrenoceptor antagonist, produced both mechanical and cold allodynia in those rats which had not developed clear neuropathic symptoms. The same doses (50 μg i.t. or 1 mg/kg s.c.) did not increase the severity of symptoms in rats which had developed them. The opioid receptor antagonist naloxone (20 μg i.t. or 1 mg/kg s.c.) had no effect on the neuropathic symptoms. These results indicate that mechanical and cold allodynia are under endogenous noradrenergic rather than opioidergic control in this model of neuropathic pain.

Keywords: α_2-Adrenoceptor antagonist; Atipamezole; Neuropathic pain; Allodynia; Animal model

1. Introduction

Peripheral nerve injury can trigger a series of events leading to hyperalgesia and allodynia which may be resistant to the antinociceptive actions of opioids (Arnér and Meyerson, 1988; Kontinen et al., 1998a). Tricyclic antidepressant drugs are commonly used to relieve neuropathic pain by inhibiting the reuptake of noradrenaline and serotonin (Dubner and Max, 1992). An increasing number of studies indicate that α_2-adrenoceptor agonists are effective in attenuating neuropathic pain (Yaksh et al., 1995; Yamamoto and Nozaki-Taguchi, 1996). Neuropathic pain can be sympathetically maintained or at least partly controlled by the activity of the sympathetic nervous system. Several mechanisms in the periphery, along the nerve or at the level of the dorsal root ganglion, could explain the coupling between sympathetic and sensory afferent fibers in an injured nerve (Jänig and McLachlan, 1994). The spinal nerve ligation neuropathy model is considered to represent sympathetically maintained pain (Kim and Chung, 1991; Kim et al., 1993). The results would indicate that the spinal nerve ligation neuropathy is not sensitive to opioids (Yaksh et al., 1995; Kontinen et al., 1998a). Naloxone has so far not been studied in this neuropathy model to assess the role of endogenous opioidergic controls. In the present study we tested the effects of atipamezole and naloxone to assess the role of endogenous noradrenergic and opioidergic activity in the spinal nerve ligation model of neuropathic pain.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Bkl:SD, B&K Universal Ab, Sollentuna, Sweden) weighing 150–175 g in the beginning of the experiment were used. Rats were housed in groups of 5 in plastic cages in artificial lighting with a fixed 12-h light–dark cycle. Lab chow and water were available ad libitum. Guidelines for animal research by local authorities and IASP (International Association for the Study of Pain) (Zimmermann, 1983) were adhered to and the study protocol was approved by the institutional animal investigation committee.

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2.2. Neuropathic pain model

The L_5–L_6 spinal nerves were tightly ligated to produce the spinal nerve ligation model of neuropathic pain (Kim and Chung, 1992). The animals were anaesthetised with halothane (0.5–1%, Trothane®, ISC Chemicals, Bristol, UK) in N_2O:O_2 70%:30%. The left L_5 and L_6 spinal nerves were exposed by removing a small piece of the paravertebral muscle and a part of the left spinous process of the L_5 lumbar vertebra. The L_5 and L_6 spinal nerves were then carefully isolated and tightly ligated with 6-0 silk. After checking haemostasis, the muscle and the adjacent fascia were closed with sutures and the skin was closed with metal clips.

2.3. Intrathecal cannulation

For the insertion of the intrathecal cannula, rats were anaesthetised with a s.c. injection of midazolam 5.0 mg/kg (Dormicum®, Roche, Basle, Switzerland) and 1.0 ml/kg of Hypnorm® (fentanyl 0.2 mg/ml and fluanisone 10 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). A thin polyethene cannula (PE-10, Meadox Surgimed, Stenløse, Denmark) was inserted through the cisterna magna into the lumbar subarachnoid space, 8 cm from the insertion, and fixed with a suture to the paravertebral muscles (Yaksh and Rudy, 1976; Kontinen and Kalso, 1995). After cannulation, the animals were housed individually in standard plexiglass cages. To verify the proper placement of the cannula, 10 μl of 5% hyperbaric lidocaine (Lidocain Pond®, Medipolar, Oulu, Finland) was injected. Only rats which developed reversible symmetrical paralysis of both hind limbs and the tail after the injection of lidocaine were used in the experiments.

2.4. Nociceptive tests

For the assessment of mechanical and cold allodynia, the rats were placed on a metal mesh covered with a plastic dome and they were allowed to habituate until the exploratory behaviour diminished. Threshold for mechanical allodynia was measured with a series of von Frey hairs (Semmes-Weinstein monofilaments, Stoelting, IL, USA) (Ren and Dubner, 1993; Kontinen et al., 1998b). The ventral surface of the paw was touched with different von Frey hairs with a bending force from 0.217 to 12.5 g until the threshold force that induced paw withdrawal in more than half of the stimuli was found. If the rat responded to

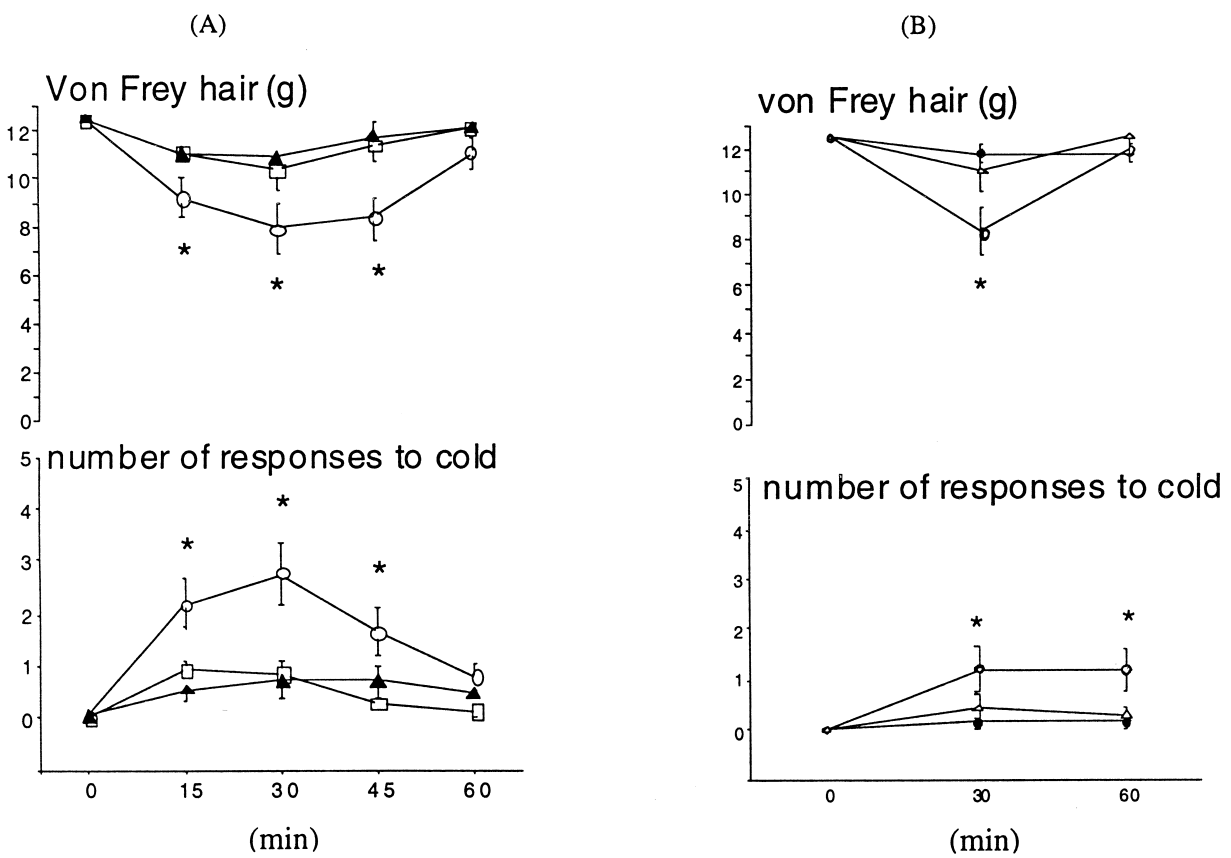

Fig. 1. The effect of s.c. injection of atipamezole 1.0 mg/kg (○, $n = 14$), naloxone 1.0 mg/kg (□, $n = 14$) and saline 1 ml/kg (▲, $n = 14$) (A) or i.t. administration of atipamezole 50 μg (○, $n = 9$), naloxone 20 μg (△, $n = 7$) and saline 10 μl (●, $n = 14$) (B) on mechanical (upper panel) and cold (lower panel) allodynia in rats that did not develop signs of neuropathic pain after spinal nerve ligation injury. Mean (S.E.M.) von Frey hair force that induced paw withdrawal and the mean (S.E.M.) number of paw withdrawals to five consecutive acetone stimuli are shown. ∗ $P < 0.05$.

the stimulation with a paw withdrawal the next lighter hair was used until the threshold was found. In order to avoid excessive stimulation the probing was started in the following testing sessions with the weakest hair that had elicited withdrawal responses in the previous session. If the strongest hair did not give a response, 12.5 g was recorded as the threshold.

Cold allodynia was measured as the foot withdrawal response after application of acetone to the plantar surface of the paw (Choi et al., 1994). A drop of acetone was gently applied to the heel of the rat with a syringe connected to a thin polyethylene tube. A brisk foot withdrawal response after the spread of acetone over the plantar surface of the paw was considered as a sign of cold allodynia. The testing was started with the paw contralateral to the nerve injury and repeated 5 times for both paws with an interval of approximately 2 min between each test.

After 2 weeks from the ligation of the spinal nerves, the rats were divided into the 'neuropathic' and 'non-neuropathic' groups. The animals that responded to a von Frey hair force < 12.5 g and with at least one positive response in the acetone test were considered neuropathic. The rats which responded to a von Frey hair force ≥ 12.5 g and had no response in the acetone test were considered non-neuropathic. This division was made pre hoc and it was based on the results from previous studies (Kontinen et al., 1998a,b).

2.5. Drugs

Atipamezole hydrochloride (Orion, Turku, Finland) was given i.t. to the rats at a dose of 50 μg in 10 μl, or as a s.c injection of 1 mg/kg. Naloxone hydrochloride (RBI, Natick, MA, USA) was given as an i.t. injection of 20 μg in 10 μl or as a s.c. injection of 1 mg/kg. Saline served as control.

2.6. Statistical analysis

Analysis of variance for repeated measurements, followed by *t*-test with Bonferroni's correction when appropriate, was used for the statistical analysis of the behavioural symptoms over time and between the treatment groups.

3. Results

After standard ligation of spinal nerves L_5–L_6, about 70% of the rats developed neuropathic symptoms. There were 7–14 rats in each group. In the rats which had not

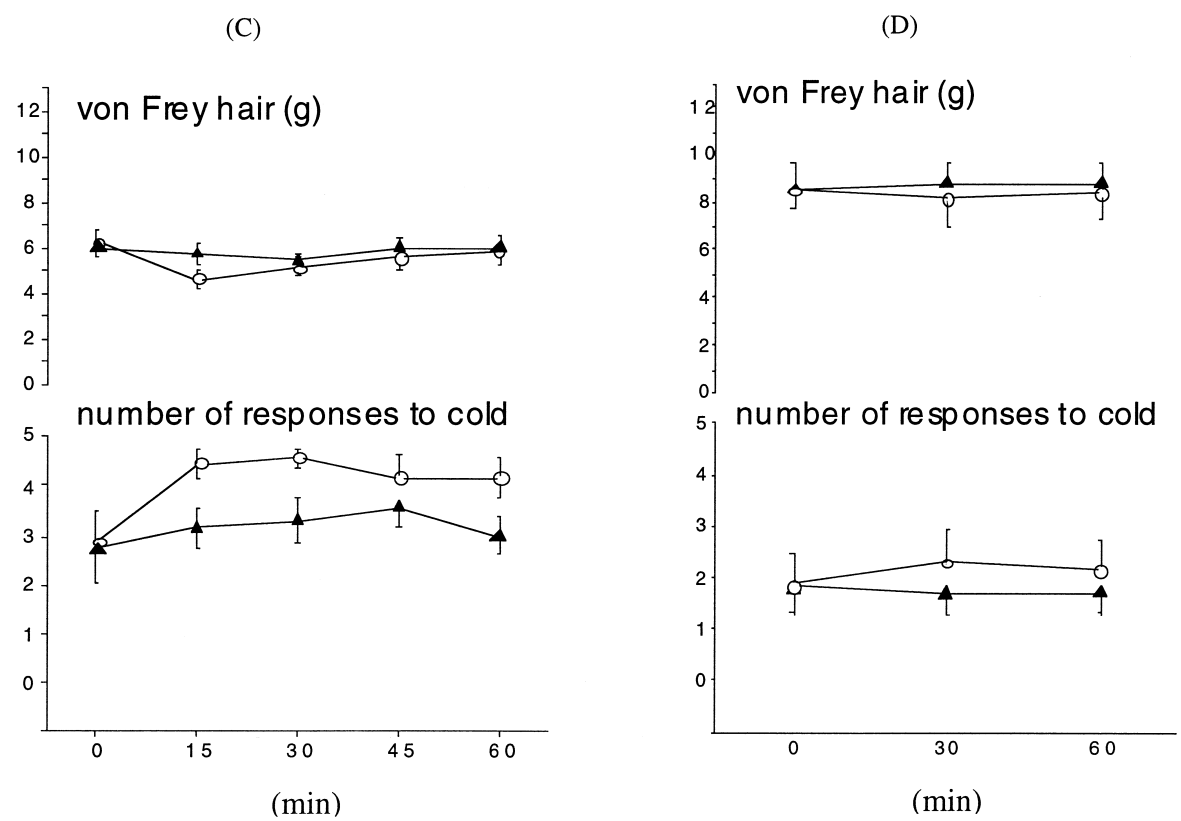

Fig. 2. The effect of s.c. injection of atipamezole 1.0 mg/kg (○, $n = 7$), and saline 1 ml/kg (▲, $n = 7$) (C) or i.t. administration of atipamezole 50 μg (○, $n = 7$) and saline 10 μl (▲, $n = 12$) (D) on mechanical (upper panel) and cold (lower panel) allodynia in rats that developed signs of neuropathic pain after spinal nerve ligation injury. Mean (S.E.M.) von Frey hair force that induced paw withdrawal and the mean (S.E.M.) number of paw withdrawals to five consecutive acetone stimuli are shown.

developed neuropathic symptoms, s.c. injection of atipamezole (1 mg/kg) significantly increased both mechanical and cold allodynia compared with naloxone or saline ($P < 0.05$), while neither s.c. nor i.t. administration of naloxone brought out neuropathic symptoms ($P > 0.05$) (Fig. 1A and B). I.t. administration of atipamezole (50 μg/10 μl) significantly increased both mechanical and cold allodynia ($P < 0.05$) (Fig. 1B). Most of the allodynic symptoms returned to baseline in about 60 min.

In the rats which developed neuropathic symptoms after the spinal nerve ligation of L_5–L_6, s.c. or i.t. administration of atipamezole (1 mg/kg) did not significantly increase either mechanical or cold allodynia ($P > 0.05$; Fig. 2).

4. Discussion

Peripheral nerve injury may result in neuropathic pain which is characterised by hyperalgesia and allodynia (Richards, 1967; Devor, 1983). In recent years, several experimental models of neuropathic pain have been developed (Bennett and Xie, 1988; Seltzer et al., 1990; Kim and Chung, 1992). The sensory abnormalities produced by the different models vary to some extent (Kim et al., 1997). In the spinal nerve ligation model (Kim and Chung, 1992), mechanical and cold allodynia are the most constant symptoms. The neuropathic symptoms produced by this model are most clearly under sympathetic control as they are significantly attenuated by sympathectomy (Kim et al., 1997). A high degree of sympathetic sprouting in the sensory ganglia has been shown in this model after the injury (Lee et al., 1998). In our hands the spinal nerve ligation model produces clear neuropathic pain behaviour characterised by mechanical and cold allodynia in about 70% of the rats (von Frey hair force < 12.5 g and more than one responses in the acetone test, respectively) after 2 weeks of standard nerve injury. The remaining 30% of the rats show no cold allodynia measurable with the method used, but could exhibit mechanical allodynia if von Frey hair forces over 12.5 g were used.

Neuropathic symptoms are less likely to respond to the antinociceptive actions of opioids (Arnér and Meyerson, 1988; Kontinen et al., 1998a) than nociceptive pain conditions. Tricyclic antidepressant drugs are commonly used to relieve neuropathic pain (Dubner and Max, 1992). Their analgesic action is at least partly mediated by inhibition of the reuptake of noradrenaline. Yaksh et al. (1995) showed that various α_2-adrenergic agonists administered intrathecally to the lumbar spinal cord could dose dependently attenuate spinal nerve injury induced mechanical allodynia in the spinal nerve ligation model in the rat. The authors concluded that the action of α_2-adrenoceptor agonists was based on the ability to diminish the sympathetic out flow from spinal preganglionic neurones. However, continuous systemic administration of dexmedetomidine using s.c. osmotic pumps did not alleviate allodynia in this model when doses that do not cause significant sedation were used (Kontinen et al., 1998b). In the present study atipamezole increased both mechanical and cold allodynia in the rats which did not previously present neuropathic symptoms. This could indicate that there was higher endogenous inhibitory noradrenergic activity in those rats.

At least four (Bian et al., 1995; Lee et al., 1995; Nichols et al., 1995; Yaksh et al., 1995) studies have tested morphine in the spinal nerve ligation model of neuropathic pain. All four studies indicate that i.t morphine is virtually without effect. However, systemic (Bian et al., 1995; Lee et al., 1995) and supraspinal morphine (Lee et al., 1995) have been shown to reduce mechanical allodynia in this model. Based on these results our assumption was that the endogenous opioidergic system might not be important in controlling the allodynic symptoms in this pain model. The fact that naloxone did not modify these symptoms in the present study would support this hypothesis. Naloxone has not been tested in the spinal ligation induced neuropathy before. Systemic naloxone has been reported to induce typical allodynia in rats which did not develop the allodynia-like symptoms after photochemical spinal cord lesion (Xu et al., 1994). Interestingly, this model of neuropathic pain is sensitive to intrathecal morphine (Yu et al., 1997). In the chronic constriction injury model of neuropathic pain naloxone induced antinociceptive effects at very low doses and hyperalgesia with high doses when administered intravenously 1 week after surgery. Two weeks after sciatic nerve ligation low doses of naloxone produced antinociception. A high dose of naloxone elicited hyperalgesia but only in those rats which had already recovered from hyperalgesia (Attal et al., 1990). In this neuropathy model there is relatively little sympathetic sprouting in the sensory ganglia after the injury (Lee et al., 1998).

In conclusion, atipamezole but not naloxone brought out both mechanical and cold allodynia in rats which did not develop neuropathic symptoms after ligation of spinal nerves L_5–L_6. This could be an indication of the role of endogenous noradrenergic tone in the inhibitory control of allodynia in these rats. The endogenous opioid control does not seem to be important in this model. These results may have both clinical and theoretical implications. Testing antagonists to endogenous modulators of nociception can give us insights to the mechanisms of various painful conditions. They also suggest that enforcing these endogenous systems may be effective in both the treatment and prevention of chronic pains.

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