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Spinal and pontine α_2-adrenoceptors have opposite effects on pain-related behavior in the neuropathic rat

Hong Wei, Antti Pertovaara *

Biomedicum Helsinki, Institute of Biomedicine/Physiology, POB 63, 00014 University of Helsinki, Finland

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Abstract

Descending noradrenergic pathways contribute to feedback inhibition of pain by releasing norepinephrine in the spinal cord. Noradrenergic nuclei in the pons contain abundant α_2-adrenoceptors. We assessed the contribution of pontine α_2-adrenoceptors to endogenous regulation of pain in nerve-injured rats. Tactile allodynia and mechanical hyperalgesia were assessed in the injured dermatome and heat nociception in an uninjured dermatome. Atipamezole, an α_2-adrenoceptor antagonist, or saline was administered systemically or microinjected into the locus coeruleus, the lateral parabrachial nucleus, the central nucleus of the amygdala, the midbrain periaqueductal gray, and/or through an intrathecal (i.t.) catheter to the spinal cord. Atipamezole administered systemically, into the amygdala or the periaqueductal gray had no significant effects on pain behavior. Atipamezole (0.3–5 μg) microinjected into the pons, the locus coeruleus or the lateral parabrachial nucleus, produced a selective and dose-related antiallodynia, which was reversed by i.t. administration of atipamezole (5 μg). I.t. administration of atipamezole alone (5 μg) produced thermal hypersensitivity in the non-neuropathic segment (tail) of nerve-injured animals. In sham-operated controls, i.t. administration of atipamezole had no effect. Suppression of heat nociception in uninjured dermatomes of nerve-injured but not the control animals following i.t. administration of atipamezole indicates that nerve injury produced a tonic activation of noradrenergic feedback inhibition acting on spinal α_2-adrenoceptors. In parallel, antiallodynia induced by pontine administration of atipamezole indicates that nerve injury induces a tonic activation of pontine α_2-adrenoceptors that promotes neuropathic hypersensitivity by attenuating descending inhibition. Thus, spinal and pontine α_2-adrenoceptors have opposite effects on pain-related behavior in neuropathic animals.

Keywords: α_2-Adrenoceptor; Atipamezole; Descending inhibition; Locus coeruleus; Neuropathic pain; Noradrenergic antinociception

1. Introduction

The noradrenergic locus coeruleus (A6) and the cell groups A5 and A7 in the pons are the main sources of noradrenergic innervation of the spinal cord (Kwiat and Basbaum, 1992; Proudfit, 1988). These pontine noradrenergic nuclei have an important role in descending modulation of pain through action on spinal α_2-adrenoceptors. This is shown by several studies in which chemical or electrical activation of the locus coeruleus, A5 or A7 produced antinociception that was reduced by spinal administration of α_2-adrenoceptor antagonists (Jones, 1991; Proudfit, 1988). Of these nuclei, the locus coeruleus is most extensively studied. It has been shown that the density of α_2-adrenoceptors is high in the locus coeruleus (Chamba et al., 1991), and particularly its subtype 2A is expressed in the locus coeruleus (Scheinin et al., 1994). α_2-Adrenoceptors in the locus coeruleus are involved in regulation of vigilance. This is indicated by the finding that microinjections of α_2-adrenoceptor agonists into the locus coeruleus produce sedation and anesthesia (Correa-Sales et al., 1992; DeSarro et al., 1987). Additionally, α_2-adrenoceptors in the locus coeruleus of healthy animals may partly mediate the spinal antinociceptive action induced by α_2-adrenoceptor agonists in some (Guo et al., 1996) but not all experimental conditions (Pertovaara et al., 1994; Xu et al., 2000b).

The role of pontine α_2-adrenoceptors in endogenous regulation of neuropathic hypersensitivity still remains to be studied. Administration of a selective receptor antagonist into the region of interest blocks the action of endogenous ligands on the receptor and thus, provides a method for assessing the pain

* Corresponding author. Tel.: +358 9 191 25280; fax: +358 9 191 25302.
E-mail address: Antti.Pertovaara@helsinki.fi (A. Pertovaara).

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regulatory role of the endogenous ligand acting in the treated site. In the present study we attempted to determine the role of pontine α_2-adrenoceptors in endogenous regulation of neuropathic hypersensitivity by assessing pain-related behavior in nerve-injured animals following microinjection of a selective α_2-adrenoceptor antagonist into two pontine sites, the locus coeruleus and the lateral parabrachial nucleus, close to the cell group A7. Control sites were the spinal cord and two suprapontine nuclei, the periaqueductal gray and the amygdala.

2. Material and methods

2.1. Experimental animals

The experiments were performed in adult, male Hannover–Wistar rats (weight: 200–300 g; Harlan, Horst, The Netherlands). The experimental protocol was accepted by the Institutional Ethics Committee of the University of Helsinki and the regional government of Southern Finland. The experiments were performed according to the guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.2. Techniques for producing neuropathy

The unilateral ligation of two spinal nerves (L_5 and L_6) was performed under pentobarbitone anesthesia (50 mg/kg i.p.) as described in detail earlier (Kim and Chung, 1992). Briefly, the left L_5 and L_6 spinal nerves were isolated and tightly ligated with 6-0 silk thread. Except for two groups of control animals, only animals with tactile allodynia (hind limb withdrawal thresholds in the operated side <2 g) were selected for this study. One of the control groups consisted of sham-operated animals; i.e., the operation was identical, except that spinal nerves were not injured. Another of the control groups consisted of animals that did not develop tactile allodynia following spinal nerve ligation (15–20%). Animals were tested 2 to 3 weeks after the operation.

2.3. Techniques for microinjection

For intrathecal (i.t.) drug injections a catheter (PE-10) was administered into the lumbar level of the spinal cord under pentobarbital anesthesia (50 mg/kg i.p.) as described in detail elsewhere (Størkson et al., 1996). Following recovery from anesthesia, the correct placing of the catheter was verified by administering lidocaine (4%, 7–10 μl followed by a 10 μl of saline for flushing) with a 50 μl Hamilton syringe. Only those rats that had no motor impairment before lidocaine injection but had a bilateral paralysis of hind limbs following i.t. administration of lidocaine were studied further. The lidocaine test was performed 10 days prior to the start of the drug testing sessions. For i.t. administration the drugs were microinjected with a 50 μl Hamilton microsyringe in a volume of 5–7 μl followed by a saline flush in a volume of 15 μl.

For intracerebral drug injections into the locus coeruleus ipsilateral to the nerve injury, the rats were implanted with a chronic guide cannula made of stainless steel (26 gauge) in a standard stereotaxic frame under general anesthesia. The chronic guide cannula was positioned 1.0 mm above the desired injection site in the locus coeruleus (AP −0.68 mm, ML 1.33 mm, DV 2.88 mm; Paxinos and Watson, 1986). The lateral parabrachial nucleus that is involved in relaying nociceptive signals (Bernard et al., 1996) was chosen as another pontine injection site (AP −0.68 mm, ML 2.33mm, DV 3.2 mm; Paxinos and Watson, 1986). The periaqueductal gray (AP 1.7 mm, ML 0.7 mm, DV 5.1 mm; Paxinos and Watson, 1986), was chosen as a control site in the midbrain, since it has an important role in descending regulation of pain (Pertovaara and Almeida, 2006), and it has reciprocal connections with pontine noradrenergic nuclei (Bajic and Proudfit, 1999; Kwiat and Basbaum, 1990). The central nucleus of the amygdala ipsilateral to the nerve lesion was chosen as a supraspinal control site outside of the pontomesencephalic area (AP 6.7 mm, ML 4.2 mm, DV 8.2 mm; Paxinos and Watson, 1986). This structure was chosen, because it is involved in mediating pain-related responses (Bernard et al., 1996) and it contains α_2-adrenoceptors (Scheinin et al., 1994). The chronic guide cannula was fixed into the skull using a dental screw and dental cement. A dummy cannula was placed into the guide cannula until the test session. Before behavioral testing, the animals were allowed to recover from surgery for 3–5 days. When the drug was administered into the brain, it was microinjected through a 33-gauge stainless steel injection cannula inserted through and protruding 1 mm beyond the tip of the guide cannula. The intracerebral microinjection was made using a 10 μl Hamilton syringe that was connected to the injection cannula by a length of a polyethylene (PE-10) tubing. The volume of intracerebral injections was 0.5 μl. The efficacy of injection was monitored by watching the movement of a small air bubble through the tubing. The injection lasted 30 s and the injection cannula was left in place for an additional 30 s to minimize flow of the drug solution back up the injector track. During the injections, the animal was held by one of the experimenters.

2.4. Behavioral testing

Observations by clinical neurologists indicate that nerve injury often produces mechanical hypersensitivity and only occasionally heat hyperalgesia (Scadding and Koltzenburg, 2006). In line with this, spinal nerve injury in the rat has produced a robust and highly reproducible mechanical hypersensitivity, while the change in heat sensitivity has been variable (Kim and Chung, 1992; Röyttä et al., 1999). Therefore, we assessed neuropathic hypersensitivity by determining a limb withdrawal response evoked by mechanical stimulation of the injured dermatome. To find out whether drug treatments produced a more wide-spread influence on nociception, heat nociception was assessed in an uninjured dermatome.

Prior to any testing, the rats were habituated to the experimental conditions by allowing them to spend 1–2 h daily in the laboratory during 2 to 3 days. For assessment of tactile