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Histamine in the locus coeruleus promotes descending noradrenergic inhibition of neuropathic hypersensitivity

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Cimetidine (PubChem CID: 2756)
Fadolmidine HCl (PubChem CID: 6433097)
Histamine dihydrochloride (PubChem CID: 5818)
Prazosin HCl (PubChem CID: 68546)
Pyrilamine maleate (PubChem CID: 5284451)
Zolantidine (PubChem CID: 11957725)

ABSTRACT

Among brain structures receiving efferent projections from the histaminergic tuberomammillary nucleus is the pontine locus coeruleus (LC) involved in descending noradrenergic control of pain. Here we studied whether histamine in the LC is involved in descending regulation of neuropathic hypersensitivity. Peripheral neuropathy was induced by unilateral spinal nerve ligation in the rat with a chronic intracerebral and intrathecal catheter for drug administrations. Mechanical hypersensitivity in the injured limb was assessed by monofilaments. Heat nociception was assessed by determining radiant heat-induced paw flick. Histamine in the LC produced a dose-related (1–10 μg) mechanical antihypersensitivity effect (maximum effect at 15 min and duration of effect 30 min), without influence on heat nociception. Pretreatment of LC with zolantidine (histamine H_2 receptor antagonist), but not with pyrilamine (histamine H_1 receptor antagonist), and spinal administration of atipamezole (an α_2-adrenoceptor antagonist), prazosine (an α_1-adrenoceptor antagonist) or bicuculline (a $GABA_A$ receptor antagonist) attenuated the antihypersensitivity effect of histamine. The histamine-induced antihypersensitivity effect was also reduced by pretreatment of LC with fadolmidine, an α_2-adrenoceptor agonist inducing autoinhibition of noradrenergic cell bodies. Zolantidine or pyrilamine alone in the LC failed to influence pain behavior, while A-960656 (histamine H_3 receptor antagonist) suppressed hypersensitivity. A plausible explanation for these findings is that histamine, due to excitatory action mediated by the histamine H_2 receptor on noradrenergic cell bodies, promotes descending spinal $\alpha_{1/2}$-adrenoceptor-mediated inhibition of neuropathic hypersensitivity. Blocking the autoinhibitory histamine H_3 receptor on histaminergic nerve terminals in the LC facilitates release of histamine and thereby, increases descending noradrenergic pain inhibition.

Introduction

The brain histaminergic system originating in the tuberomammillary nucleus contributes to the regulation of basic body functions, including the sleep–waking cycle, energy and endocrine homeostasis, synaptic plasticity and learning [1]. Among brain structures receiving histaminergic innervation from the tuberomammillary nucleus is the noradrenergic locus coeruleus (LC) in the pons. In the LC, histamine, through action mediated by histamine H_1 and H_2 receptors, increased firing of noradrenergic neurons, which was considered to reflect histamine-induced arousal [2]. However, the noradrenergic LC is known to be involved in regulation of pain as well as vigilance [3] suggesting that histaminergic innervation of the LC might contribute to pain control. In line with this, a recent electrophysiological study performed in anesthetized animals indicated that blocking the autoinhibitory histamine H_3 receptor on histaminergic nerve endings [4,5], which presumably promotes release of histamine in LC, decreased both evoked and spontaneous firing of spinal pain-relay neurons in neuropathic but not sham control animals [6]. A recent optogenetic study demonstrated that while one population of noradrenergic LC neurons induced spinal antinociception, another population of noradrenergic neurons promotes spinal nociception [7]. Therefore, even if increased LC histamine level were expected to increase

Abbreviations: ANOVA, analysis of variance; GABA, gamma-amino butyric acid; i.c.v., intracerebroventricular; i.t., intrathecal; LC, locus coeruleus.

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firing of noradrenergic LC neurons, the net effect of LC histamine on pain behavior, particularly under pathophysiological conditions, may not be predicted but needs to be determined experimentally.

Here we assessed whether histamine in the LC attenuates neuropathic pain hypersensitivity in awake behaving animals, and whether the antihypersensitivity effect is mediated by noradrenergic neurons with descending projections acting on spinal α_1- or α_2-adrenoceptors. Moreover, we assessed the contributions of different histamine receptor subtypes in the LC to the histamine-induced pain regulatory effect.

The experiments were performed in rats that had a spinal nerve ligation-induced model of peripheral neuropathy and a chronic guide cannula and intrathecal (i.t.) catheter for injections of drugs into the brain and spinal cord, respectively.

Materials and methods

Experimental animals

The experiments were performed in adult, male Hannover–Wistar rats (weight: 180–230 g; Harlan, Horst, The Netherlands). The experimental protocol was accepted by the Ethical Committee on Animal Experiments of the regional government of Southern Finland. The experiments were performed according to the guidelines of European Communities Council Directive 2010/63/EU on the use of animals for scientific purposes. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Techniques for producing neuropathy

There are a number of surgically induced models of peripheral neuropathy [8], of which we chose for this study the spinal nerve ligation (SNL) model. The unilateral ligation of two spinal nerves (L5 and L6) was performed under pentobarbitone anesthesia (60 mg/kg intraperitoneally) as described in detail earlier [9]. Briefly, the left L5 and L6 spinal nerves were isolated and tightly ligated with 6-0 silk thread. Only animals with tactile allodynia-like hypersensitivity (hind limb withdrawal threshold to monofilament stimulation in the operated side <2 g, which is below the lower 95% confidence limit of the threshold in unoperated control animals) were selected for this study. Animals were tested two to three weeks after the operation.

Techniques for microinjections

For drug injections into the LC, the rats were implanted with a chronic guide cannula made of stainless steel (26 gauge; PlasticsOne, Roanoke, VA) in a standard stereotaxic frame under general anesthesia. The chronic guide cannula was positioned according to the atlas of Paxinos and Watson [10] 1.0 mm above the desired injection site. The desired injection site was in the LC (AP −0.68 mm, ML 1.30 mm, DV 7.0 mm). While the LC cannula in most of the animals was ipsilateral to the peripheral nerve injury, in one group of animals the LC cannula was contralateral to the peripheral nerve injury. This, because earlier anatomical studies indicate that depending on the rat strain, LC may have descending projections not only ipsilaterally but also contralaterally [11]. Additionally, in one control group the injection site was in the cerebellum (AP −2.0 mm, ML 2.0 mm, DV 4.0 mm). 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 (PlasticsOne) inserted through and protruding 1 mm beyond the tip of the guide cannula. The intracerebral microinjection was made using a 10 μl Hamilton syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) that was connected to the injection cannula by a length of a polyethylene (PE-10) tubing (Becton Dickinson and Company, Sparks, MD). 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.

For intrathecal (i.t.) drug injections, a catheter (PE-10) was administered into the lumbar level of the spinal cord under pentobarbitone anesthesia (60 mg/kg intraperitoneally) as described in detail elsewhere [12]. Following recovery from anesthesia, the correct placing of the catheter was verified by administering lidocaine (4%, 7–10 μl followed by a 15 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 at least 3 days prior to the start of the drug testing sessions. For i.t. administration the drugs were microinjected with a 50 μl Hamilton microsyringe at a volume of 5–7 μl followed by a saline flush at a volume of 15 μl.

Behavioral testing

Observations by clinical neurologists indicate that nerve injury often produces tactile allodynia or hypersensitivity to mechanical stimulation of the skin (*e.g.*, with brush) and only occasionally heat hyperalgesia [13]. Therefore, the focus of this study was in the assessment of tactile allodynia-like hypersensitivity by determining a limb withdrawal response evoked by monofilament stimulation of the injured dermatome. To find out whether histamine in LC produced a more wide-spread influence on nociception, heat nociception was assessed in one experiment.

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 2–3 days. For assessment of tactile allodynia-like hypersensitivity, the hind limb withdrawal threshold evoked by stimulation of the hind paw with monofilaments (von Frey-hairs) was determined while the rat was standing on a metal grid. At each time point, the paw ipsilateral to the spinal nerve ligation was stimulated five times with an ascending series of calibrated monofilaments (1–26 g; North Coast Medical, Inc., Morgan Hill, CA). At each stimulus force, the withdrawal response frequency was determined. An increase in the withdrawal response rate was considered to represent mechanical hypersensitivity effect. When compared with the traditional determination of the withdrawal threshold value, the currently used method has the advantage that it allows assessing separately drug effects on withdrawal responses evoked by stimulus forces of threshold and suprathreshold levels.

For assessment of thermal nociception in the plantar skin of the hindpaw ipsilateral to nerve injury, the latency of the heat-induced limb withdrawal response was determined using a radiant heat device (Plantar test model 7370, Ugo Basile, Varese, Italy). To avoid tissue damage, two consecutive measurements at each time point were made at one min intervals. The mean latency at each time point was used in further calculations. The stimulus intensity was adjusted so that the mean baseline latency was 8–9 s and the cut-off latency was 15 s.

Course of the study

Animals were tested two to three weeks after spinal nerve ligation and administration of intrathecal catheter; *i.e.*, testing was