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Descending effect on spinal nociception by amygdaloid glutamate varies with the submodality of noxious test stimulation

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HIGHLIGHTS

- Glutamate in the central amygdala induced spinal antinociception in healthy rats.
- Mechanical antinociceptive effect was predominantly contralateral.
- Heat antinociception was of shorter duration and bilateral.
- Amygdaloid NMDA receptor mediated mechanical but not heat antinociception.
- Spinal antinociceptive effect did not vary with the brain hemisphere.

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ABSTRACT

Amygdala has an important role in the processing of primary emotions, such as fear. Additionally, amygdala is involved in processing and modulation of pain. While the amygdala, particularly its central nucleus (CeA), has been shown to contribute to pain control, the descending pain regulation by the CeA is still only partly characterized. Here heat and mechanical nociception was tested in both hind limbs of healthy rats with a chronic guide cannula for microinjection of glutamate into the CeA of the left or right hemisphere. The aim was to assess whether the descending pain regulatory effect by glutamate in the amygdala varies with the submodality or the body side of nociceptive testing, brain hemisphere or the amygdaloid glutamate receptor. Motor performance was assessed with the Rotarod test. Amygdaloid glutamate, independent of the treated hemisphere, produced a dose-related heat and mechanical antinociception that varied with the submodality of testing. Heat antinociception was short lasting (minutes), bilateral and not reversed by blocking the amygdaloid NMDA receptor with MK-801. In contrast, mechanical antinociception lasted longer (>20 min), was predominantly contralateral and reversed by blocking the amygdaloid NMDA receptor. At an antinociceptive dose, amygdaloid glutamate failed to influence motor performance. The results indicate that independent of the brain hemisphere, the spatial extent and duration of the descending antinociceptive effect induced by amygdaloid glutamate varies with the amygdaloid glutamate receptor and the submodality of pain.

1. Introduction

The amygdala exerts an important role in processing of primary emotions, such as fear. Amygdala, particularly its central nucleus (CeA), is also known to be involved in processing of emotional aspects of pain and in regulation of spinal nociception through its efferent connections to brainstem relay nuclei that project caudally [11,16]. Glutamatergic amygdaloid receptors are known to be involved in processing of pain-related signals [5,10]. The descending influence by glutamatergic receptors of the CeA has varied from facilitation to inhibition of spinal nociception depending on the type of the amygdaloid glutamate receptor, the behavioral assay and the pathophysiological condition of the animal [1,2,7,8,13,14,17]. The contribution of the CeA to nociceptive processing has varied with the brain hemisphere, the right CeA playing a major role in many [3,4,6,7] although not all experimental conditions [17]. While previous studies indicate that the glutamatergic system in the CeA plays a role in descending modulation of pain, many characteristics of the descending pain modulatory effect

Abbreviations: CeA, central nucleus of the amygdala; NMDA, N-methyl-D-aspartate.

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induced by glutamate in the amygdala of healthy control animals are still only partly known.

The aim of this study was to determine whether glutamate in the amygdala of healthy control animals modulates spinal nociception bi- or unilaterally, whether the descending effect varies with the submodality of noxious test stimulation or brain hemisphere, and whether amygdaloid NMDA and non-NMDA receptors have variable roles in the glutamate-induced descending effects.

2. Materials and methods

2.1. Animals

The experiments were performed with adult male Hannover-Wistar rats (Harland, Horst, The Netherlands) weighing 200–350 g. The experimental protocols were approved by the Experimental Animal Ethics Committee of the Provincial Government of Southern Finland (Hämeenlinna, Finland), and 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 limit distress and to use only the number of animals necessary to produce reliable scientific data. Rats were housed in a 12-hour light/dark cycle with food and water access *ad libitum*.

2.2. Cannula insertion and drug injection procedure

The animals had a guide cannula for drug administration either into the right or left amygdala as described in detail earlier [3]. For placement of the guide cannula (26 gauge; PlasticsOne, Roanoke, VA, USA), the skull was exposed and a hole drilled for its placement under pentobarbitone anesthesia (50 ml/kg i.p.). The desired injection target in the left or right amygdala was in the capsule lateral of the central nucleus of amygdala (CeA): 2.1 mm posterior from the bregma, 4.3 mm lateral from the midline, and 7.8 mm ventral from the dura mater [15]. The control injection site was in the right internal capsule: 2.1 mm posterior from bregma, 3.6 mm lateral from the midline, and 5.0 mm ventral from the dura mater. The tip of the guide cannula was positioned 2 mm above the desired injection site. The cannula was fixed into the skull using a dental screw and dental cement. Drug administration to the brain and experimental protocols started 1 week after fixation of the guide cannula to the skull. A dummy cannula was placed in the guide cannula, except when drug administrations were performed.

2.3. Drugs and their administration procedure

Glutamate (L-glutamic acid monosodium salt) and the NMDA receptor antagonist (+)-MK-801 maleate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Glutamate was administered at the dose of 32 μg or 100 μg, while MK-801 was administered at the dose of 2 μg. Physiological saline (OrionPharma, Espoo, Finland) was used for control injections.

Unilateral infusions of drugs, or an equivalent volume of saline, were made by using 33 gauge injection needles (PlasticsOne) connected to a 10 μl Hamilton microsyringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) by polyethylene (PE-10) tubing. The injection needles protruded 2.0 mm beyond the cannula tips and a 0.5 μl volume was injected. The animals were gently restrained during the infusion procedure. The duration of injection was 30 s. Injection needle was retained within the cannula for an additional 20 s after drug infusion to maximize diffusion and to prevent backflow of the drug into the cannula. At the currently used injection volume, the spread of injection may be close to 1 mm [9], due to which the drug application method allows concluding whether the drug-induced effect originates in the amygdala rather than pinpointing the effect to one of its subnuclei. However, since the CeA is involved in nociception and provides efferent connections of the amygdala to the brainstem [11], it may be argued that the present results on descending regulation of nociception reflect drug-induced actions on the CeA rather than on its other subnuclei.

2.4. Assessment of pain behavior

Before assessment of pain behavior, the rats were habituated to the testing conditions at least in three one hour sessions during three consecutive days. Mechanical pain behavior was assessed using a calibrated series of monofilaments that in the current experiment produced forces ranging from 1 to 300 g (North Coast Medical, Inc., Morgan Hill, CA, USA). During testing, rats were on a grid, free to move inside a transparent box. The monofilaments were applied below the grid to the foot pad with increasing force until the rat withdrew its hind limb. The lowest force producing a withdrawal response to all of its five consecutive presentations was considered the threshold. In each condition, the monofilament-induced withdrawal threshold was assessed separately in both hind limbs.

Heat nociception was assessed by determining limb withdrawal latency induced by heat applied to the plantar skin using radiant heat equipment (Plantar test model 7370, Ugo Basile, Varese, Italy). The cut-off point was set at 15 s. Rats were free to move inside a transparent box of thermal plantar test device.

2.5. Rotarod test

To exclude a motor effect of the glutamate injection into the left or right CeA, motor activity of the rats was assessed in the Rotarod test. After starting the Rotarod test device (Ugo Basile), it increased its speed each second by 2 revolutions per minute (rpm). The maximum revolution speed at which the rats were able to stay on the drum was determined one minute after saline and glutamate administrations.

2.6. Course of study

Experiments were performed at least a week following unilateral installation of the brain cannula into the left or the right CeA. Mechanical pain threshold, noxious heat-evoked withdrawal latency and motor performance were evaluated on separate days. In half of the animals, the experiment started with assessment of heat nociception and in half with assessment of mechanical nociception. The Rotarod test was always performed as the last test. Mechanical and heat nociception was tested in the following drug-treatment conditions: saline, glutamate at the dose of 32 μg or 100 μg, MK-801 at the dose of 2 μg followed 5 min later by glutamate at the dose of 100 μg, or MK-801 at the dose of 2 μg alone. Pain behavior was assessed 1, 10, 20, 40 and 60 min after drug administration as well as before it. The order of testing the drug conditions was randomized using Quickcalcs software of Graph Pad (http://www.graphpad.com/quickcalcs/randMenu/). The interval between testing the different experimental conditions in the same animal was two days.

2.7. Histology

At the end of the experiment, rats were sacrificed by an overdose of pentobarbital and the brain was removed and immersed in 4% formaldehyde. Coronal sections of the brain were cut to verify the site of injection according to the atlas of Paxinos and Watson [15] (Fig. 1).