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Antinociceptive effects of sensory stimulation involve dynorphin B supraspinally in rats

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ABSTRACT

The aim was to investigate the mechanisms behind sensory stimulation which can be used to desensitize CNS in patients with atypical orofacial pain. Earlier studies have shown that the kappa-receptor in the periaqueductal gray (PAG) is involved in sensory stimulation induced antinociception. A possible antinociceptive role for dynorphin B (DynB) in supraspinal regions was tested. The behavioral effect of sensory stimulation in conscious rats, by stroking the fur, was tested using the nociceptive test hotplate and the hindpaw withdrawal latency (HWL) was measured. In anesthetized rats sensory stimulation during different modalities, stroking or pinching was performed and the microdialysis technique was used to determine the extra cellular level of DynB in the ventrolateral PAG. To evaluate the antinociception after sensory stimulation DynB was microinjected into the PAG and the effect was measured with the HWL to heat. The results showed that sensory stimulation in conscious rats significantly increased the HWL as an antinociceptive effect. Innocuous sensory stimulation such as stroking the fore paw significantly elevated the DynB level in the PAG compared to internal control. After pinching a tendency to delayed release of DynB was seen and a possible discharge of the nerve terminals could be speculated upon. The blood pressure did significantly increase after pinching but not after stroking. An intra-PAG injection of DynB into the PAG increased the HWL to heat after 24 h compared to basal level of HWL and to saline treated animals. In conclusion, DynB is involved in the antinociception that is triggered by sensory stimulation.

1. Introduction

Atypical orofacial pain is a condition with a wide variety of disorders. The pain is a combination of nociceptive and neuropatic pain but is unexplained. Pharmacological treatment has until today failed to change the daily life for patients suffering of severe orofacial pain. There is scientific evidence that central sensitization, defined as – an augmentation of responsiveness of central neurons to input from unimodal and polymodal receptors, could be the reason [1]. Nijs et al. have stated that central sensitization is an increasing problem and should be a treatment target [2]. Sensory stimulation is a suggested treatment for desensitizing the central nervous system [2]. Transcutaneous nerve stimulation or electroacupuncture is known to activate opioids in the descending pain inhibitory system [3–5]. Sensory stimulation in different modalities has been shown to activate the endogenous antinociceptive pathways [6]. Massage-like stroking induces increased hind paw withdrawal latency (HWL) to thermal and mechanical stimuli interpreted as antinociceptive effects [7]. Mechanical stimulation to cutaneous and subcutaneous tissue activates inhibitory neurons in the superficial layers of the dorsal spinal cord, explained as the Gate Control Theory. The effects of cutaneous mechanical sensory stimulation can be measured in the brain by the microdialysis technique [8,9]. This technique has earlier been useful to measure substance P (SP) in the PAG and SP has been proposed to contribute to opioid antinociception in this area [10,11].

Dynorphin B (DynB) is an opioid derived from the prohormone prodynorphin and is an agonist to the κ-receptor, with which involvement in antinociception has been proposed [12–14]. Numerous reports describe the antinociceptive effect elicited by the opioid dynorphin [15–17], while others have suggested nociceptive action [18,19]. Elevated tissue concentration of DynB in the spinal cord after nerve injury is supported in several studies [20–23]. The Periaqueductal gray (PAG) is known to play a central role in endogenous antinociception [24,25]. The major descending outflow from the ventrolateral PAG runs via the nucleus raphe

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magnus down to the dorsal spinal cord where the inhibition of noxious stimuli takes place. In a study, increased tissue levels of DynB were shown in the PAG after induced inflammation [23].

In the present study, the main objective was to investigate the mechanisms behind sensory stimulation, which is supposed to target central pain processing, which can be a contribution to the development of methods to desensitize CNS in patient with atypical orofacial pain. The subgoals were to investigate which effects, such as stroking (innoculous stimuli) and pinching (noxious stimuli) have on antinociceptive thresholds in rats with special emphasize on the opioid DynB and its action in the PAG.

2. Materials and methods

Animals

Sprague–Dawley male rats with a body weight of 350–450 g were housed in cages in groups of five animals at constant room temperature (22 ± 2 °C) and exposed to a 12-h light:12-h dark cycle (light onset at 0600 h). All experiments were performed in the daytime (1000–1500 h). The experimental protocols were approved by the local Ethical Committee for Animal, Karolinska Institute, Stockholm, Sweden. Food and water were provided ad libitum. Following arrival to the laboratory, the animals were allowed to adapt to the environment for at least 1 week before the experiments started. When the experiments were finished, the rats were sacrificed by either an overdose of sodium pentobarbital or decapitation.

2.1. Part I

2.1.1. Sensory stimulation, stroking, in conscious animals

Sensory stimulation in conscious rats ($n = 10$) comprised manual stroking for 5 min of an approximately 10-cm^2 area on the ventral side with a low speed of approximately 20 cm/s and a frequency of 0.67 Hz (i.e. stroking every 1.5 s or at 40 strokes/min) and with an estimated pressure of 100 mm H_2O ($n = 10$). The reproducibility of the stroking was tested and confirmed by similar applications of pressure to a small pressure gauge [26]. During the massage-like stroking, the rats were held across the scapula and neck region as described by Kanetake [27]. The rats were naive to the massage-like stimulation before the experiment started.

2.1.2. Measurements of nociceptive thresholds

All rats were accustomed to the test conditions for 5 days before the experiment was conducted to decrease stress caused by handling and measuring. The latencies in seconds during thermal stimulation show the antinociceptive response of the intervention which has been described before [11].

Thermal response was assessed by the hot-plate test. The entire plantar surface of the rat's hindpaw was placed, without force, on the hot plate which was maintained at a temperature of 52 °C (51.8–52.4 °C). HWL was measured in seconds. A 50 s cutoff time was chosen to avoid tissue damage.

2.2. Part II

2.2.1. Sensory stimulation, stroking and pinching in anesthetized animals

Different kinds of sensory stimulation, stroking or pinching, of segmental skin areas (face, forepaw, abdomen, hind paw) were used ($n = 20$). Innocuous mechanical stimulation was performed by gently stroking a 5–10 cm^2 area of skin with a toothbrush. The speed of stroking was about 3 cm/s with a frequency of around 1 Hz. Noxious mechanical stimulation was performed by pinching of an approximately 1-cm^2 area of the skin with a surgical clamp (about 3-kg force). All stimulation was delivered for 15 min every 45 min in anesthetized rats during the collection of extra cellular liquid from the ventrolateral PAG.

2.2.2. Microdialysis experiments

The microdialysis method was used as previously described [10,11]. The rats in the present study were initially anesthetized with sodium pentobarbital (60 mg/ml, 0.1 ml/100 g body weight) and thereafter i.v. during the experiments. Local anesthetic was administered to the skin during the operation (bupivacain 5 mg/ml). The trachea was intubated for artificial ventilation using a respirator, and care was taken to maintain respiratory gases at a constant level. The animal's body temperature was maintained at approximately 37 °C with the aid of a thermostatically regulated heating pad. The right jugular artery and vein were cannulated for the measurement of systemic arterial blood pressure and the administration of drugs. The animals were mounted in a prone position on the stereotactic instrument (David Kopf instruments, Tujunga, CA, USA). A hole (1.5 mm) drilled in the skull exposed the dura and a microdialysis probe (CMA/12, CMA Microdialysis AB, Stockholm, Sweden) with an external membrane diameter of 0.5 mm and 2-mm membrane length was implanted in an angulated position with the dialyzing part in the ventromedial PAG. Stereotactic coordinates from Bregma were anterior/posterior (AP) = −7.3; latero/medial (LM) = +2.5 and dorso/ventral (DV) = −6.6 mm, measured from the surface of the dura according to the atlas of Paxinos and Watson [28]. The probe was inserted into the brain at an angle of 22° from the sagital plane. The probe was perfused with the following modified Krebs-Ringer solution (in mM): NaCl, 148; KCl, 2.7; $CaCl_2$, 1.2; $MgCl_2$, 0.85 (pH 7.4). The flow rate was maintained at 7 μl/min with a CMA/100 microinjection pump. Collection of 15-min samples (105 μl) was begun 1 h after probe implantation, and continued for a total of 4 h. The DynB release was stimulated with potassium (100 mM in the perfusion fluid) at the end of the experiment. The samples were immediately frozen and stored at −80 °C until assayed for DynB-like immunoreactivity (DynB-LI) [39].

2.2.3. Methodological studies in the microdialysis technique

The basal level of DynB was measured in the ventrolateral PAG. The microdialysis flow rate was maintained at 7 μl/min during the experiments. Ten 15-min samples were collected 1 h after probe implantation. The DynB level was measured in the ventrolateral PAG using modified Krebs-Ringer solution with ($n = 5$) or without ($n = 5$, controls) the addition of peptidase inhibitor (bacitracin). Potassium (100 mM) stimulated DynB release in fraction numbers 5 and 9. Toward the end of the experiments, the animals were injected with an overdose of sodium pentobarbital intraperitoneal (i.p.) and then decapitated. The whole brain was dissected out and frozen for later verification of the probe position. Each brain was sectioned in 14-μm frontal slices on a freezing microtome (Microtom, HM 500M), stained with toluidine blue, and examined microscopically to verify the probe.

2.2.4. Radioimmunoassays

The detection limit for the assay was sensitive to 0.01 fmol/ml of DynB or less. Samples and standards diluted in the perfusion medium were incubated with 50 μl of antiserum (113+) and tracer peptide (labeled with ^{125}I; 4500 cpm in 100 μl gelatin buffer) for 24 h at 4 °C. Samples without antiserum (to determine non-specific binding) and samples without unlabeled peptide (to determine maximal tracer binding) were simultaneously incubated. The antiserum and the labeled peptide were dissolved in 0.05 M phosphate buffer containing 0.15 M NaCl, 0.1% gelatin, 0.1% bovine serum albumin, 0.02% sodium acid, and 0.1% Triton X-100. After incubation, antibody-bound and free tracers were separated by incubation for 1 h at 4 °C with 100 μl of sheep antirabbit-antiserum (Pharmacia