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Kinin and opioid receptors in the paratrigeminal nucleus modulate the somatosensory reflex to rat sciatic nerve stimulation

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Abstract

The influence of kinin and opioid receptor blockade in the paratrigeminal nucleus (Pa5) on the somatosensory reflex (SSR) to sciatic nerve stimulation (SNS) was assessed in anaesthetized–paralyzed rats. SNS (square 1 ms pulses at 0.6 mA and 20 Hz for 10 s) increased mean arterial pressure from 87 ± 3 to 106 ± 3 mmHg. Pressor responses to SNS were reduced 40–60% by HOE-140 and LF 16-0687 (B_2 receptor antagonists; 20 and 100 pmol respectively), CTOP or nor-binaltorphimine (mu and kappa opioid receptor antagonists, respectively; 1 μg) but potentiated by naltrindole (delta opioid receptor antagonist) receptor antagonist microinjections into the contralateral (but not ipsilateral) Pa5. The SSR to sciatic nerve stimulation was not changed by B_1 kinin receptor or NK_1, NK_2 and NK_3 tachykinin receptor antagonists administered to the Pa5. Capsaicin pretreatment (40 mg/kg/day, 3 days) abolished the effects of the opioid receptor antagonists, but did not change the effect of kinin B_2 receptor blockade on the SSR. Thus, the activity of B_2 and opioid receptor-operated mechanisms in the Pa5 contribute to the SSR in the rat, suggesting a role for these endogenous peptides in the cardiovascular responses to SNS.

Keywords: Bradykinin; Blood pressure; Capsaicin-sensitive fibers; Cardiovascular reflex; Sciatic nerve; Nociception

1. Introduction

Sciatic nerve stimulation (SNS) evokes a somatosensory reflex (SSR), which consists of pronounced alterations in cardiovascular and other autonomic function parameters due to activation of supraspinal sites by nociceptive sensory input [1]. Recent evidence points to a role for the paratrigeminal nucleus (Pa5), a small medullary structure in the mediation of the SSR to SNS. This nucleus receives direct sensory input from the dorsal cervical spinal roots, vagus, glossopharyngeal and trigeminal nerves [2–4] and emits projections to several brain structures important for cardiovascular and respiratory control and processing of nociceptive information [5].

Firstly, contralateral Pa5 lesion markedly reduces both the pressor response to SNS [6] (Caous et al., unpublished data) and nociceptive responses to formalin injection into the hind paw of the rat (Koepp et al., unpublished data). Secondly, there is neuronal tract tracing and electrophysiological evidence [5,7,8] that Pa5 neurons project to the rostroventrolateral reticular nucleus (RVL), which harbors pre-motor sympathetic neurons and is also pivotal for manifestation of the cardiovascular responses to SNS [9]. Other relevant aspects concerning the cardiovascular functions of the Pa5 include its role in mediating the pressor response to centrally-administered bradykinin [10] and its participation in the baroreflex arch [11,12]. Indeed, the discharge patterns of most Pa5 neurons are closely locked to the cardiac cycle [12].

To date, very little is known about the neurotransmitters that mediate the SSR in the Pa5. Nonetheless, bradykinin appears to be of particular importance since this peptide causes prolonged pressor response and tachycardia when microinjected into the Pa5 [10], where the B_2 receptor is clearly expressed [13]. The Pa5 also expresses significant

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levels of preprodynorphin [14] and neuropeptide Y [15] although their functions within the structure remain to be disclosed. Considering the nociceptive component involved in the SNS-induced SSR, the present study aimed to assess, via microinjections of receptor antagonists into the Pa5, the possible contributions of bradykinin, neurokinins and opioids to the role played by this nucleus in the reflex.

2. Materials and methods

2.1. General procedures

Studies were conducted on male normotensive Wistar rats weighing 280–350 g, under isothane gas anesthesia (1.5–2%). A polyethylene catheter (PE10 connected to PE60) was placed in the abdominal aorta through the right femoral artery and connected to a pressure transducer (TP-1, Ampére Eletro-Eletrônica, São Paulo, SP, Brazil) to record the mean arterial blood pressure (MAP) and heart rate (HR) by CED 1401 (Cambridge Electronics Design, Cambridge, UK). Following tracheostomy, the animal was placed in the stereotaxic apparatus (David Kopf Instruments, Tucanja, USA), with the bite bar set at −11 mm, the rat was paralyzed with D-tubocurarine (0.15 ml/100 g, i.p.) and artificially ventilated (Inspira-ASV, Harvard Apparatus, South Natick, MA) with 70% oxygen at 30 strokes min^{-1}. Body temperature was maintained at 37 °C with a thermostatically controlled heating pad (Harvard Apparatus).

2.2. Sciatic nerve stimulation

The sciatic nerve was exposed in the lateral aspect of the limb contralateral to that with the MAP recording catheter and was covered with mineral oil and isolated from surrounding muscle with parafilm paper. A bipolar gold platted stimulation electrode was gently placed onto the sciatic nerve and 10 s trains of cathodal square-wave 1 ms pulses of 0.1 or 0.6 mA, generated at 20, 10 or 2 Hz by a Grass S88 stimulator, were applied.

2.3. Central microinjections

For antagonist microinjections into the Pa5, an occipital craniotomy was performed. After exposing the caudal portion of the fourth ventricle, the posterior vermis of the cerebellum was gently retracted to enable visualization of the *calamus scriptorium* that was used as a reference point. The cannula was then displaced to reach the Pa5 (AP = −4.3 mm, LAT = 2.4 mm, VERT = −0.5 mm referenced to stereotaxic zero according to Paxinos and Watson [16]. A 30G gingival needle connected to a 1 μl microsyringe (Hamilton Company, Nevada, USA) through a PE 10 polyethylene tube (Intramedic, Becton Dickinson, USA) was used to deliver all microinjections (0.1 μl min^{-1}).

2.4. Experimental protocol

All rats were subjected to three SNS trials, delivered at intervals of at least 15 min, the minimal time for response reproducibility. For each rat, the SSR produced by the first SNS trial was taken as reference for the control response, antagonist microinjection into the Pa5 was performed 10 min before the second SNS trial. The last SNS trial was performed 20–40 min later to verify reversal of antagonist administration effect. At the end of the experiment, the rats were transcardially perfused with 4% paraformaldehyde in PBS and the microinjection sites were verified, under light microscopy analysis, in 30 μm Klüver–Barrera stained sections (Fig. 1).

2.5. Drugs and doses

The following receptor antagonists were used: Des-Arg^9-Leu^8-bradykinin, a B_1 kinin receptor antagonist (100 pmol), HOE-140 a peptidic B_2 kinin receptor antagonist (20 pmol), LF16-0687 a non-peptidic B_2 kinin receptor antagonist (100 pmol), LY306740 a NK_1 receptor antagonist (100 pmol), SR 48968 a NK_2 receptor antagonist (100 pmol), RMP-786 a NK_3 receptor antagonist (100 pmol), Cys^2-Tyr^3-Orn^5-Pen^7-amide (CTOP a mu opioid receptor antagonist, 1 μg), naltrindole (delta opioid receptor antagonist, 1 μg) and

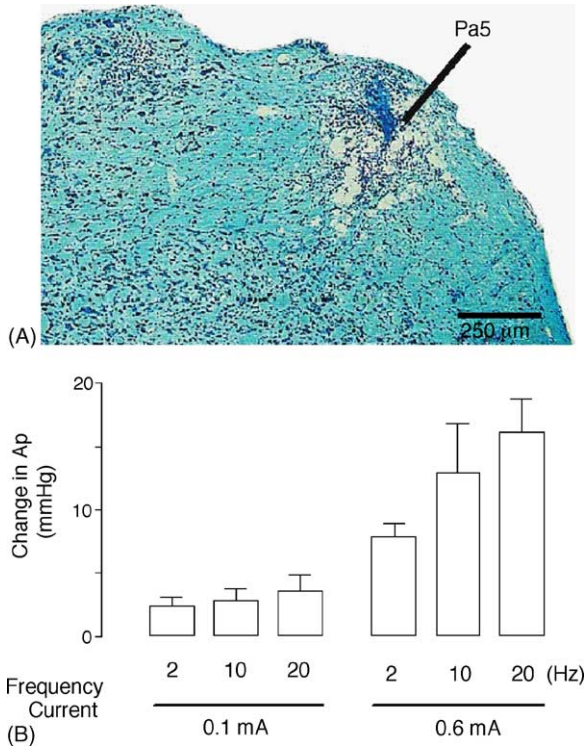

Fig. 1. (A) Transversal section of the rat medulla showing the injection site into the Pa5; scale bar = 250 μm. (B) Effects of stimuli intensity and frequency on arterial pressure changes caused by sciatic nerve stimulation in anaesthetized and paralyzed vehicle injected. All stimuli consisted of 10 s trains of 1 ms-wave pulses delivered at 0.1 or 0.6 mA and 2, 10 or 20 Hz. Values are mean ± S.E.M. of six preparations.