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Original Article

Role of calcitonin gene-related peptide in nociception resulting from hind paw incision in rats

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ABSTRACT

Introduction: The superficial laminae of the spinal cord are crucial sites for the transmission of incoming noxious information. Calcitonin gene-related peptide (CGRP) is released from the presynaptic nerve terminals in these laminae. One of the objectives was to evaluate the temporospatial pattern of expression of CGRP following paw incision in rats. Paw incision-induced nociception mimics postoperative pain in humans. The next objective was to administer a specific CGRP receptor antagonist directly into the intrathecal space and observe the antinociceptive effect, which was then compared to morphine.

Material and methods: Sprague Dawley rats were subjected to incision on the right hind paw. The related spinal cord segments (L4-5) were isolated at different time intervals after incision and immunostained for CGRP. A different set of rats were implanted with intrathecal catheter and administered saline (control) or BIBN 4096 (CGRP antagonist) or morphine (10 μg/10 μl) and then subjected to paw incision. Nociception was evaluated at different time intervals up to day 7.

Results: Expression of CGRP was observed over laminae I and outer part of lamina II. Synaptic terminals could be discerned containing CGRP. Following incision, the expression decreased abruptly at 2 h. However, at 12 h, the expression had increased. Between days 1–5, the expression decreased again towards basal levels. The antinociceptive effect of BIBN was comparatively less than morphine, which robustly inhibited all three pain parameters at 2 h after incision.

Discussion: Immunohistochemistry revealed that CGRP was involved in the transmission of nociception. However, blocking its action did not produce a robust antinociceptive effect.

1. Introduction

The spinal cord and the brainstem are key sites of transfer of information from the periphery to the central nervous system. Not only that, the neural signals are modulated before onward transmission to the brain by both excitatory and inhibitory interneurons as well as by descending nerve fibers from supra-spinal centers like the rostroventral medulla.[1] For example, interneurons containing γ-Amino butyric acid (GABA) can inhibit the transmission of pain by the "Gate control mechanism". Also, enkephalinergic interneurons can do the same by the release of endogenous opioids. Interestingly, GABAergic neurons constitute almost 25–30% of the neurons in Rexed's laminae I–II, also known as superficial laminae of the spinal cord.[2]

Aδ (thinly myelinated) and C (unmyelinated) groups of peripheral nerve fibers carry nociceptive information to the spinal cord. The central terminals of these nerve fibers contain neuro-transmitters like glutamate and neuropeptides like calcitonin gene-related peptide (CGRP) and substance P (SP).[1] Following tissue damage, these are released into the synaptic cleft, where they bind to specific receptors expressed by the dorsal horn neurons and trigger action potentials, which passes along the lateral spinothalamic tract to the thalamus.

CGRP, a 37 amino acid peptide, is derived from alternative splicing of the mRNA, originating from the calcitonin gene.[3] It is almost exclusively expressed in neurons and referred to as αCGRP or more commonly as CGRP. In contrast, the β isoform is derived from a different gene and is present in the enteric nervous system.[4] CGRP is extensively expressed in perivascular nerve fibers around the cerebral blood vessels, where it produces vasodilatation. Its role in migraine is well established.[5] The corresponding CGRP receptor is a prototypical G protein-coupled receptor (calcitonin-like receptor), which is associated with two other subunits

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(Receptor activity-modifying protein 1 and receptor component protein).

CGRP is expressed at high density over the superficial laminae in the rat, monkey and human spinal cords.[6,7] The corresponding CGRP receptor is expressed adjacent to CGRP containing nerve terminals.[8] CGRP expression has been noted to increase, decrease or even remain unchanged under different pain conditions.[9–12] These conflicting results could be due to the different time intervals at which the spinal cords were examined after the noxious injury. For example, Ishida et al. examined the spinal cord at the end of day 1 whereas Wang et al. examined it on day 18.[10–11] Importantly, none of the existing studies have investigated the temporospatial pattern of expression over a period of time.

The animal model for the present study was the same as that of Ishida et al. and involves a surgical incision on the plantar aspect of the right hind paw under anesthesia.[11] This postincisional model was first described in 1996 and has been extensively utilized for understanding the mechanism of postoperative pain. Postoperative nociception is characterized by spontaneously occurring pain during resting condition (guarding behaviour), mechanical allodynia (pain due to non-noxious stimuli) and thermal hyperalgesia (exaggerated response to a mildly noxious stimulus).[13] Despite occurring in a hospital setting, management of postoperative pain continues to remain suboptimal.[14]

2. Material and methods

2.1. Experimental subjects

The experiment was conducted on young adult (9–10 weeks old; 250–300 g) male Sprague Dawley rats (n = 54) (Fig. 1). Permission for experimental work was obtained from the Institutional Animal Ethics Committee (903/IAEC/15 dated 19-2-16). ARRIVE guidelines were followed during the experimental work. Food and water were available *ad libitum*. After intrathecal catheterization, animals were housed singly in each cage, which contained clean bedding (ALPHA-dri, Shepherd Speciality Papers, USA). 12 h light:dark cycles were maintained and temperature varied between 22 and 25 °C. The observer performing the behavioural assessment of nociception was blinded to the exact drug administered to the animals.

2.2. Drugs

BIBN 4096 (henceforth referred to as BIBN), as known as Olcegepant, is a potent and selective antagonist of the CGRP receptor (Tocris Bioscience, UK). It was dissolved in 1 M HCL and then diluted with 1 M NaOH to a pH of 6.8. This was further diluted with isotonic saline to a final concentration of 10 μg/10 μl. Ampoules containing morphine sulphate (15 mg/ml) were purchased from a government agency after obtaining permission from the Drug Controller. It was diluted with isotonic saline to the same concentration as BIBN. Control group was injected saline instead of the drug.

2.3. Immunohistochemistry

The procedure has been reported previously.[15] Briefly, animals (n = 36) were divided equally into six groups. The first group was the control group (without incision) and the remaining was subjected to paw incision (Section 2.6 for details). Among the incised rats, each group was sacrificed at a different time point (2 h, 12 h, day 1, day 3 and day 5 after incision). Rats were anaesthetized with pentobarbitone (100 mg/kg i.p) and perfused with cold 0.1 M phosphate buffer saline (PBS) by the transcardiac route (Masterflex animal perfusion pump, Cole Parmer, USA). It was followed by perfusion with 4% Paraformaldehyde in PBS. The lumbar enlargement of the spinal cord was identified and the region

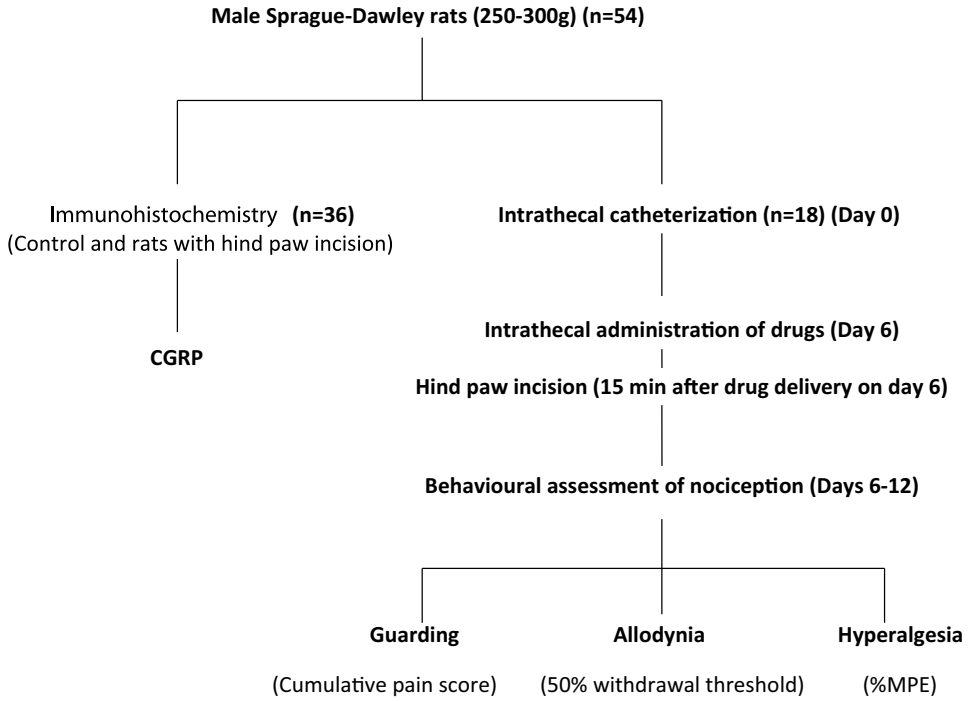

Fig. 1. Flow diagram of the experimental work. The study was divided into two parts – (1) study of the expression of CGRP by immunohistochemistry in the spinal cord and (2) the behavioural assessment of nociception after intrathecal administration of BIBN and Morphine. For the first part, rats were divided into two groups – with or without (control) paw incision. Those with paw incision were examined for CGRP at 2 and 12 h and days 1, 3 and 5. The second part of the study involved the surgical implantation of catheters in the intrathecal space (day 0) followed by drug administration (saline/BIBN/morphine) through the catheter (day 6; 8 a.m.) and paw incision (day 6, 8:15 a.m.). Subsequently, nociception was evaluated by three different tests at 2 and 12 h and days 1–7 (days 1–4 for guarding).