Opioids modulate the calcitonin gene-related peptide$_{8-37}$-mediated hindpaw withdrawal latency increase in thermally injured rats

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Summary The present study was performed to explore the modulatory potential of different endogenous opioid systems on transmission of presumed nociceptive information at the spinal cord level in thermally injured rats. Thermal injury was performed by dipping the left paw into water 60°C for 20 s. This induced a significant bilateral decrease in hindpaw withdrawal latency (HWL) to pressure. Intrathecal administration of 10 nmol of CGRP$_{8-37}$ induced a significant bilateral increase in HWL in the thermally injured group and in the intact controls. The effect of different opioid receptor antagonists on the increased latency to withdrawal response induced by intrathecal injection of 10 nmol of CGRP$_{8-37}$ was explored in the thermally injured rats. The effect was reversed by intrathecal injection of 40 and 80 nmol of: β-funaltrexamine (μ opioid receptor antagonist) and naltrindole (δ opioid receptor antagonist), but not by norbinaltorphimine (κ opioid receptor antagonist). The results of the present study show that intrathecal CGRP$_{8-37}$ increases hindpaw withdrawal latency in thermally injured rats, an effect reduced by a μ as well as by a δ opioid receptor antagonist.

INTRODUCTION

Calcitonin gene-related peptide (CGRP) is present in the peripheral nervous system and it has been shown that CGRP is involved in the transmission of presumed nociceptive information in the spinal cord. It has been reported that intrathecal administration of CGRP$_{8-37}$, a selective antagonist of CGRP receptors, induced a significant increase in latency to hindpaw withdrawal responses in intact rats evoked by mechanical stimulation, as well as a reversal of the substance P-induced decrease in latency to withdrawal responses. These results indicate that CGRP and its receptor are, to some extent, involved in the transmission of presumed nociceptive information in the spinal cord of intact rats. At the spinal level, it is known that opioid agonists exert a modulatory role on nociceptive transmission by pre- and postsynaptic inhibition. In this respect, it has also been shown that nociceptive stimulation results in release of CGRP and substance P from primary afferent fibres and that morphine and other opioid peptides reduce this release. Furthermore, supporting a close interaction between endogenous opioids and CGRP are the studies by Ménard and coworkers showing that CGRP$_{8-37}$ interacts with the development of tolerance to morphine.

Recently, we have shown that unilateral thermal injury of the hindpaw of rats results in a marked increase in the paw volume, leaving the contralateral hindpaw volume unaffected. On the other hand, there was a bilateral decrease in hindpaw withdrawal latencies (HWLs) and an increased release of CGRP into the cerebrospinal fluid. Intrathecal administration of CGRP$_{8-37}$ induced a significant increase in HWLs and these effects were partially reversed by naloxone. Taken together our results...
indicate that CGRP plays a role in the transmission of presumed noceptive information in the spinal cord of rats with a thermal injury and that its release and/or effects are subjected to opioid modulation.

The present study was performed to investigate the modulatory potential of different endogenous opioid systems on transmission of presumed noceptive information at the spinal cord level in thermally injured rats. This was employed by assessing the effect of intrathecal administration of different types of opioid antagonists; b-funaltrexamine (b-FNA), a μ opioid receptor antagonist;^26 nor-binaltorphimine (nor-BNI), a κ opioid receptor antagonist;^27 naltrindole, a δ opioid receptor antagonist,^28 on the increase in hindpaw withdrawal latency to pressure.

**MATERIALS AND METHODS**

**Animal preparation and intrathecal injection**

All experiments were performed on freely moving male Sprague-Dawley rats (250–300 g: ALAB, Stockholm, Sweden). The rats were housed in cages with free access to food and water, and maintained in a room temperature of 24°C with a 12 h light/dark cycle. Intrathecal administration was performed after 5 days of behavioural training (see below). Before experimentation, 2% lidocaine was injected subcutaneously into the area later penetrated to allow intrathecal injection. A stainless steel needle, with an outer diameter of 0.5 mm, was then directly inserted into the subarachnoid space between the L4–L5 or L3–L4 vertebrae. Ten μl of solution (see below) were thereafter infused intrathecally over a period of 1 min. The experimental protocol used was approved by the local ethical animal committee at Karolinska Institutet. All efforts were made to avoid excessive or unnecessary suffering.

**Testing hindpaw withdrawal latency**

All rats were accustomed to the testing conditions 5 days before the experiment was run. The thermal injury was produced by dipping the left paw into hot water at (60°C) for 20 s under ether anaesthesia. Four hours after immersion, the intrathecal injection was given and the hindpaw withdrawal tests started. The pressure force exerted to induce hindpaw withdrawal was measured. The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess withdrawal latencies to mechanical stimulation. A wedge-shaped pusher with a loading rate of 48 g/s was applied to the dorsal surface of the manually handled hindpaw and the pressure required to initiate the struggle response assessed and expressed in seconds (s). The average value of three repeated stimulations before intrathecal injection was regarded as the basal hindpaw withdrawal latency (HWL). The HWLs recorded during subsequent experiments were expressed as percentage change of the mean basal level for each rat (% change of HWL).

The tests were performed before intrathecal injection as basal HWL and repeated at 5, 15, 30 and 60 min after the injection. In order to confirm the effect of thermal injury on hindpaw withdrawal latency and changes following intrathecal injection of 10 nmol CGRP_α-37 18 rats were divided into two groups: thermally injured (n=9) and intact controls (n=9).

In order to investigate the possible role of opioid receptors in modulating the CGRP_α-37-induced effect on HWL in thermally injured rats, three selective opioid antagonists were used. Sixty-three rats received intrathecal injections of 10 nmol of CGRP_α-37 followed 15 min later by either 40 or 80 nmol of b-FNA (a μ opioid antagonist; n=9 + 9), 40 or 80 nmol of naltrindole (a δ opioid antagonist; n=9 + 9) 40 or 80 nmol of nor-BNI (a κ opioid antagonist; n=9 + 9) or 10 μl of 0.9% saline (n=9) as a control. Finally, 18 rats received an intrathecal injection of 10 μl of 0.9% saline followed 15 min later by either 40 nmol of b-FNA, nor-BNI or naltrindole to study the effects of these selective opioid antagonists alone.

When studying the interaction between CGRP_α-37 and opioids the tests were performed before intrathecal injection of CGRP_α-37, and 5 and 15 min afterwards; then a second intrathecal injection was given using the opioid antagonists and tests were continued at 20, 35 and 50 min after the first injection.

**Chemicals**

Solutions for intrathecal administration were prepared with sterilized saline, each with a volume of 10 μl: (1) 10 nmol of CGRP_α-37 (hCGRP_α-37; Peninsula Labs Inc, Europe LIT); (2) 40 or 80 nmol of b-funaltrexamine (b-FNA; Research Biochemicals Incorporated, MA, USA); (3) 40 or 80 nmol of nor-binaltorphimine (nor-BNI; Research Biochemicals Incorporated, MA, USA); (4) 40 or 80 nmol of naltrindole (naltrindole hydrochloride, Research Biochemicals Incorporated).

**Statistical analysis**

Data from hindpaw withdrawal tests are presented as mean ± SEM. The difference between the groups was determined by one-way analysis of variance (ANOVA). P<0.05, P<0.01 and P<0.001 were considered significant differences.

**RESULTS**

**Effects of intrathecal administration of 10 nmol CGRP_α-37 on hindpaw withdrawal latency**

To confirm the effects of intrathecal injection of 10 nmol CGRP_α-37 in rats with thermally injured left hindpaw...
inflammation, nine rats with thermally injured left hindpaw and nine intact controls received intrathecal injections of 10 nmol of CGRP_37. Figure 1 depicts the changes in withdrawal latency to pressure in both groups of rats. In the Randall Selitto test, there was no significant difference in withdrawal latency to pressure between left and right hindpaws in the intact control rats (P=0.72). In rats with left hindpaw inflammation due to thermal injury the withdrawal latency to pressure decreased bilaterally compared to intact rats (left, P<0.001; right, P<0.01). The decrease in HWL was significantly more pronounced in the thermally injured left hindpaw as compared to the non-injured right (Fig. 1: P<0.001).

The hindpaw withdrawal latency to pressure in rats with inflammation increased bilaterally after CGRP_37 injection, but the increase was less pronounced than in the control rats (Fleft/left=379.53, P<0.001; Fright/right=198.09, P<0.001).

**Effect of intrathecal administration of b-FNA, nor-BNI and naltrindole on the CGRP_37-induced effect on the hindpaw withdrawal latency**

After intrathecal injection of 10 nmol of CGRP_37 the HWL to mechanical stimulation was significantly increased and lasted for >50 min. This increase was unaffected by 10 μl of 0.9% saline administered intrathecally. There was no significant difference in HWL (Fleft/right=0.74, P=0.66) between left and right hindpaws in this group during the period of observation. After intrathecal injection of 40 or 80 nmol of b-FNA or naltrindole, the increased HWLs to mechanical stimulation, following intrathecal injection of CGRP_37, were significantly reduced compared to the control group (b-FNA group, Fleft/left=143.48, P<0.001; Fright/right=87.69, P<0.001; naltrindole group, Fleft/left=42.91, P<0.001; Fright/right=64.11, P<0.001). In the group receiving 40 or 80 nmol of nor-BNI no significant difference was found in HWLs compared to the control group (Fleft/left=0.03, P=0.95; Fright/right=0.43, P=0.83).

In order to study the effect of intrathecal injection of b-FNA or naltrindole, alone, in thermally injured rats, 18 rats were given an intrathecal injection of 10 μl of 0.9% saline followed 15 min later by 40 nmol of b-FNA (n=9) or 40 nmol of naltrindole (n=9). No significant change in HWL to mechanical stimulation was found during the period of observation, as shown in Figure 2.

**DISCUSSION**

The results of the present study show that thermal injury of one hindpaw induces a bilateral decrease in hindpaw withdrawal latency (HWL) and that intrathecal administration of CGRP_37 significantly increases it again bilaterally. This effect of CGRP_37 was dose-relatedly reduced by intrathecal injection of the μ receptor antagonist b-FNA and by the δ receptor antagonist naltrindole, but not by the κ opioid antagonist nor-BNI.

The present and previous results suggest that the effects of CGRP_37 seen in the present study are due to interaction with opioid systems at the spinal cord level. Endogenous opioid peptides have been shown to exert an inhibitory effect on transmission of nociceptive input to the dorsal horn of the spinal cord,
especially in laminae I–II and V which are known to receive terminations of primary nociceptive afferents.\textsuperscript{15, 16, 30–33} It has been reported that opioids control the release of CGRP from primary afferent fibres in rat spinal cord slices.\textsuperscript{16, 31} Pohl and co-workers found that \( \mu \) and \( \delta \) agonists inhibited the release of CGRP via presynaptic opioid receptors, thereby possibly contributing to the analgesic action of opioids.\textsuperscript{16} Recently, Collin et al\textsuperscript{32} demonstrated that CGRP-like material was spontaneously released from the spinal cord in anaesthetized rats and that this release was under a tonic inhibitory control by endogenous opioid peptides, acting at \( \mu \) and \( \kappa \) receptors. In the present study, the increase in HWL obtained after intrathecal administration of CGRP\textsubscript{3-37} was reduced by intrathecal injection of the opioid antagonists, b-FNA (a \( \mu \) opioid antagonist) and naltrindole (a \( \delta \) opioid antagonist).

A direct effect of CGRP\textsubscript{3-37} on primary or secondary nociceptive neurons is questionable as intrathecal CGRP\textsubscript{3-37} has been shown to facilitate the spinal nociceptive flexor reflex in decerebrated rats.\textsuperscript{29} On the other hand, in rats with intact descending opioid systems CGRP\textsubscript{3-37} increases the hindpaw withdrawal latency suggesting that the effects obtained are mediated through an interaction with these systems. One feasible effect of CGRP\textsubscript{3-37} could be an action on CGRP receptors located on opioid-containing neurons resulting in opioid release.

Taken together, the present and previous studies indicate that thermal injury results in the release of CGRP at the spinal cord level. This release facilitates the transmission of nociceptive information which is under control of endogenous opioids acting at \( \mu \) and \( \delta \) receptors.

ACKNOWLEDGEMENTS

This study was supported by funds from the Anna-Greta Crafoord’s Foundation, the Gustav Vth 80-year Anniversary Foundation, the Karolinska Institute Foundation, the Magnus Bergvall Foundation, the Nanna Svaertz Foundation and the Swedish Society against Rheumatism (RMR).

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