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Intrathecal $CGRP_{8-37}$-induced bilateral increase in hindpaw withdrawal latency in rats with unilateral inflammation

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1 Recent work in our laboratory has demonstrated that intrathecal administration of a selective antagonist of calcitonin gene-related peptide (CGRP), $CGRP_{8-37}$, increased the hindpaw withdrawal latency (HWL) to thermal stimulation and hindpaw withdrawal threshold (HWT) to pressure in normal rats, and that these effects were more pronounced than in rats with mononeuropathy.

2 The present study was performed to investigate the effects of intrathecal administration of $CGRP_{8-37}$ on the HWL and HWT in rats with unilateral hindpaw inflammation induced by subcutaneous injection of carrageenin. The effect of naloxone was also studied.

3 Subcutaneous injection of 0.1 ml of carrageenin into the plantar region of the left hindpaw induced a significant increase in the volume of the ipsilateral hindpaw ($P<0.001$), and significant bilateral decreases of the HWL to thermal stimulation (ipsilateral: $P<0.001$; contralateral: $P<0.01$) and HWT to pressure (ipsilateral: $P<0.001$; contralateral: $P<0.01$).

4 Intrathecal administration of 10 nmol of $CGRP_{8-37}$, but not of 1 or 5 nmol, induced a significant bilateral increase in the HWL and HWT in rats with experimentally induced inflammation (thermal test: $P<0.001$; mechanical test: $P<0.001$).

5 The effect of intrathecal administration of 10 nmol $CGRP_{8-37}$ on HWL and HWT was significantly more pronounced in intact rats than in rats with experimentally induced inflammation (ipsilateral: $P<0.001$; contralateral: $P<0.001$).

6 The effect of $CGRP_{8-37}$ on withdrawal responses in the inflamed paw was partly reversed by intrathecal injection of naloxone at a dose of 88 nmol in the thermal (ipsilateral: $P<0.01$; contralateral: $P=0.14$) and mechanical tests (ipsilateral: $P<0.05$; contralateral: $P=0.60$).

7 A significant bilateral increase in the concentration of CGRP-like immunoreactivity in the perfusate of both hindpaws was demonstrated 24 h after unilateral injection of carrageenin (ipsilateral: $P<0.001$; contralateral: $P<0.05$). There was also an increase in the amount of CGRP-like immunoreactivity in the cerebrospinal fluid ($P<0.001$), but not in plasma ($P=0.75$).

8 The present study demonstrates that acute experimentally-induced unilateral hindpaw inflammation, induces bilateral increases in the amount of CGRP-like immunoreactivity in hindpaw perfusates. Intrathecal administration of $CGRP_{8-37}$ increased the HWL to thermal stimulation and HWT to pressure bilaterally.

9 The results indicate that CGRP plays a role in the transmission of presumed nociceptive information in the spinal cord of rats with experimentally induced inflammation. Furthermore, our findings suggest that opioids can modulate CGRP-related effects in the spinal cord.

Keywords: Calcitonin gene-related peptide (CGRP); $CGRP_{8-37}$; naloxone; carrageenan; hindpaw withdrawal latency (HWL); hindpaw withdrawal threshold (HWT); CGRP-like immunoreactivity; unilateral experimental inflammation; bilateral effects; nociceptive test

Introduction

Calcitonin gene-related peptide (CGRP) is known to be present in widespread areas of the peripheral and central nervous system (Tschopp *et al.*, 1985; McNeill *et al.*, 1988; Ishida-Yamamoto & Tohyama, 1989; Hökfelt *et al.*, 1992). It has been reported that CGRP may be involved in the transmission of presumed nociceptive information in the spinal cord. Biella *et al.* (1991) reported that CGRP has a facilitatory role on the excitation of rat spinal dorsal horn neurones induced by substance P and peripheral noxious stimuli. Furthermore, Kuraishi *et al.* (1988), Kawamura *et al.* (1989) and Satoh *et al.* (1992) reported that intrathecal injection of antiserum against CGRP induced prolonged reflex latencies in rats subjected to cold stress or inflammation. On the other hand, Cridland & Henry (1989) found that intrathecal injection of CGRP in the rat attenuated the excitatory effect on the tail flick reflex induced by either substance P or noxious cutaneous stimulation.

The role of the nervous system in inflammation is complex. It has been reported that experimentally induced acute or chronic inflammation results in an enhanced release of CGRP-like immunoreactivity from dorsal horn slices *in vitro* (Nanayama *et al.*, 1989; Donner & Stein, 1992; Garry & Hargreaves, 1992). Recently, it was found that unilateral intra-articular injection of pro-inflammatory substances induces a bilateral increase in substance P-, CGRP-, neurokinin A- and neuropeptide Y-like immunoreactivity in rat synovial fluid (Bileviciute *et al.*, 1993), as well as an increase in the amount of

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the previously mentioned peptides in the cerebrospinal fluid during acute monoarthritis (Bileviciute *et al.*, 1994a). Inflammation has also been shown to induce an up-regulation of the expression of preprodynorphin mRNA and the mRNA of the proto-oncogene c-fos in the dorsal horn of the spinal cord (Draisci *et al.*, 1991).

$CGRP_{8-37}$ is a selective antagonist of CGRP receptors (Chiba *et al.*, 1989; Dennis *et al.*, 1990; Maggi *et al.*, 1992). Recently it has been demonstrated that intrathecal administration of $CGRP_{8-37}$ increased the hindpaw withdrawal latency (HWL) induced by thermal stimulation and hindpaw withdrawal threshold (HWT) to pressure in normal rats (Yu *et al.*, 1994). The effects were more pronounced in intact rats than in rats with mononeuropathy (Yu *et al.*, 1996). These results indicate that CGRP to a different degree is involved in the transmission of presumed nociceptive information in the spinal cord of intact and mononeuropathic rats. The objectives of the present study were to further the understanding of the role of CGRP in spinal transmission of presumed nociceptive information in an inflammation model in rats, and to investigate the effect of the opioid antagonist naloxone in this model. In addition, the effect of acute unilateral inflammation on CGRP-like immunoreactivity in bilateral hindpaw perfusates, cerebrospinal fluid and plasma was investigated.

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±1°C with a 12 h light/dark cycle. All rats were accustomed to the testing conditions for five days (9 times daily) before starting the experiment in order to obtain stable response latencies or thresholds and to decrease the stress induced by handling and measurements. On the experimental day rats were pretreated with 2% lignocaine subcutaneously in the region of intrathecal injection. A stainless steel needle with an outer diameter of 0.5 mm was inserted into the subarachnoid space between L4–L5 or L3–L4 (Lundeberg *et al.*, 1993; Yu *et al.*, 1994). Ten microliters of solution (see below) were thereafter infused intrathecally during 1 min. Rats showing signs of distress during the experimental day were returned to their cages and results obtained were not used for further analyses.

Inflammation model

Inflammation was produced by unilateral subcutaneous injection of 0.1 ml of 2% carrageenin into the plantar region of the rat left hindpaw. One group of rats received injections of 0.1 ml of 0.9% saline as a control. Three h after carrageenin injection, the intrathecal injection of $CGRP_{8-37}$ was given and the hindpaw withdrawal tests started. The hindpaw volume was measured by a Plethysmometer (UGO Basile, type 7150, Italy).

Tests of withdrawal responses

The latency to hindpaw withdrawal during thermal stimulation was measured as well as the pressure force exerted to induce hindpaw withdrawal. The thermal response was assessed by the hot-plate test. The entire ventral surface of the rat's left or right hindpaw was placed on the hot-plate which was maintained at a temperature of 52°C (51.8–52.4°C). The time to hindpaw withdrawal was measured and is referred to as hindpaw withdrawal latency (HWL). The Randall Selitto Test (UGO Basile, Type 7200, Italy) was used to assess hindpaw withdrawal threshold (HWT) to pressure. A wedged-shaped pusher with a loading rate of 48 g s^{-1} was applied to the dorsal surface of the manually handled hindpaw and the pressure required to initiate the struggle response was assessed. The HWT is expressed in grams, i.e., pressure force exerted to induce hindpaw withdrawal. The measurements after plantar injection, but before intrathecal injection, were regarded as the basal HWL to thermal stimulation or basal HWT to pressure. The HWL and HWT recorded during subsequent experiments were expressed as percentage change of the mean basal level for each rat (% change of basal HWL or HWT). Each rat was tested with both thermal stimulation and pressure. Every tested group for withdrawal responses contained 8 rats. Each testing time 4 rats received first thermal and then mechanical stimulation, while the other 4 received first mechanical then thermal stimulation to avoid the influence of stimulation sequence. The tests were performed before intrathecal injection and repeated at 5, 15, 30 and 60 min after the injection as shown in Figures 2 and 5. In Figure 3 the tests were performed before intrathecal injection and 5 and 15 min afterwards; a second intrathecal injection was given and tests were carried out at 20, 30 and 60 min after the first injection.

Radioimmunoassay

Thirty-six rats were anaesthetized with chloral hydrate (0.4 g kg^{-1}) intraperitoneally, and then divided into three groups depending on the timing for examination, i.e., before (control, $n=12$), 3 ($n=12$) or 24 ($n=12$) h after the injection of 0.1 ml of 2% carrageenin subcutaneously into the plantar region of the left hindpaw. Samples of cerebrospinal fluid, plasma and the perfusate from the ipsilateral and contralateral hindpaws were obtained for radioimmunoassay. For collection of cerebrospinal fluid rats were placed in a stereotactic frame. The atlanto-occipital membrane was exposed by retracting the overlaying muscles and samples of 80–150 μl of cerebrospinal fluid were obtained through a 27-gauge needle with a 1 ml syringe via a polyethylene tube. Blood (1.5–4.5 ml) was then collected by puncture of the heart with a vacutainer tube containing heparin 143 iu ml^{-1} and Trasylol 500 iu ml^{-1}. The sample of blood was centrifuged and the plasma was removed (Bileviciute *et al.*, 1993; 1994a,b). The hindpaw perfusion was carried out through two 27-gauge needles inserted into the plantar region of the hindpaws (using a perfusion technique earlier described for the knee joint, Bileviciute *et al.*, 1993). One ml of perfusate was collected. A reverse-phase C18 cartridge (Sep Pak, Waters) was used for sample extraction from the perfusate, plasma or cerebrospinal fluid (CSF). All samples were rapidly cooled and stored at −80°C until analysis.

Radioimmunoassay of CGRP-like immunoreactivity was performed using CGRPR8 (Theodorsson *et al.*, 1990) raised against conjugated rat CGRP according to Bileviciute *et al.* (1993). [^{125}I]-histidyl rat CGRP purified by high-performance liquid chromatography (Waters) was used as radioligand, and rat CGRP as standard. The cross-reactivity of the assay to substance P, neurokinin A, neurokinin B, neuropeptide K, β-endorphin, Leu-enkephalin, Met-enkephalin, dynorphin, gastrin, neurotensin, bombesin, neuropeptide Y and calcitonin was less than 0.01%. Cross-reactivity toward rat α-CGRP and β-CGRP was 100% and 120%, respectively.

Chemicals

Carrageenin (Sigma Chemical Company, St. Louis, MO, U.S.A.) (2%) was diluted in 0.9% saline. Solutions for intrathecal administration were prepared with sterilized saline (0.9%), each with a volume of 10 μl: (1) 1, 5 or 10 nmol of $CGRP_{8-37}$ ($hCGRP_{8-37}$; Peninsula Labs Inc, Europe LIT); (2) 22 nmol (8 μg), 44 nmol (16 μg) or 88 nmol (32 μg) of naloxone (naloxone hydrochloride, Sigma Chemical Company, St. Louis, Mo, U.S.A.). Control groups were given 10 μl of 0.9% saline.

Statistical analysis

All data are presented as mean±s.e.mean. The statistical differences between groups were evaluated by one-way analysis of

variance (ANOVA) or Student's t test (two tailed). $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ were considered as significant differences.

Results

Effects of subcutaneous injection of carrageenin into the left plantar hindpaw on hindpaw volume, hindpaw withdrawal latency to thermal stimulation and withdrawal threshold to pressure

Eight rats received an injection of 0.1 ml of carrageenin into the left plantar hindpaw. The hindpaw volume, the HWL to thermal stimulation and the HWT to pressure were assessed before and after the injection of carrageenin (Figure 1). Three h after carrageenin injection, the ipsilateral hindpaw volume was significantly increased ($P<0.001$) but the volume of the contralateral hindpaw showed no significant change as shown in Figure 1a. The hindpaw volumes between 3 and 4 h after carrageenin injection remained constant.

There was no significant difference between ipsilateral and contralateral HWLs to thermal stimulation before carrageenin injection. Three h after carrageenin injection the HWLs were significantly decreased bilaterally and the HWL obtained on the ipsilateral side was significantly shorter than on the contralateral side as shown in Figure 1b. There was no significant difference in HWLs between 3 and 4 h after the injection of carrageenin.

Further, 3 h after carrageenin injection the HWTs to pressure were significantly decreased bilaterally compared to the HWT before carrageenin injection as shown in Figure 1c. The ipsilateral HWT was significantly lower than that obtained on the contralateral side and similar results were observed at 4 h.

For control, 8 rats received 0.1 ml of 0.9% saline into the left plantar hindpaw and no differences were observed after the injection in either tests (results not shown).

Effects of intrathecal administration of $CGRP_{8-37}$ on hindpaw withdrawal latency to thermal stimulation and withdrawal threshold to pressure in rats with carrageenin induced hindpaw inflammation

Thirty-two rats with left hindpaw inflammation, 3 h after carrageenin injection, were divided into four groups receiving intrathecal injections of: (1) 10 μl of 0.9% saline as a control ($n=8$); (2) 1 nmol of $CGRP_{8-37}$ ($n=8$); (3) 5 nmol of $CGRP_{8-37}$ ($n=8$); (4) 10 nmol of $CGRP_{8-37}$ ($n=8$).

There were no significant changes in either HWL or HWT in the group receiving 1 or 5 nmol of $CGRP_{8-37}$, as shown in Figure 2. The group receiving 10 nmol of $CGRP_{8-37}$ demonstrated significantly increased HWLs and HWTs bilaterally compared with the control group and these effects lasted for more than 60 min after $CGRP_{8-37}$ injection (Figure 2).

Effects of intrathecal administration of naloxone on the $CGRP_{8-37}$-induced increases in hindpaw withdrawal latency to thermal stimulation and withdrawal threshold to pressure in rats with carrageenin induced hindpaw inflammation

Thirty-two rats with carrageenin-induced inflammation received an intrathecal injection of 10 nmol of $CGRP_{8-37}$ followed 15 min later by either 22 ($n=8$), 44 ($n=8$) or 88 nmol of naloxone ($n=8$), or by 10 μl of 0.9% saline ($n=8$) as a control. The results are shown in Figure 3.

The HWL to thermal stimulation and HWT to pressure ipsilateral to the inflammation were significantly increased in all four groups after the injection of $CGRP_{8-37}$. After intrathecal injection of 88 nmol of naloxone, 15 min after $CGRP_{8-37}$ administration, the increased HWL to thermal stimulation and HWT to pressure were significantly reduced only on the ipsilateral side. There were no significantly differences in either HWLs or HWTs in the group receiving 22 or 44 nmol of naloxone. Eight rats with left hindpaw inflammation were given an intrathecal injection of 10 μl of 0.9% saline followed 15 min later by 88 nmol naloxone. No significant change in either HWL or HWT was found, as shown in Figure 3.

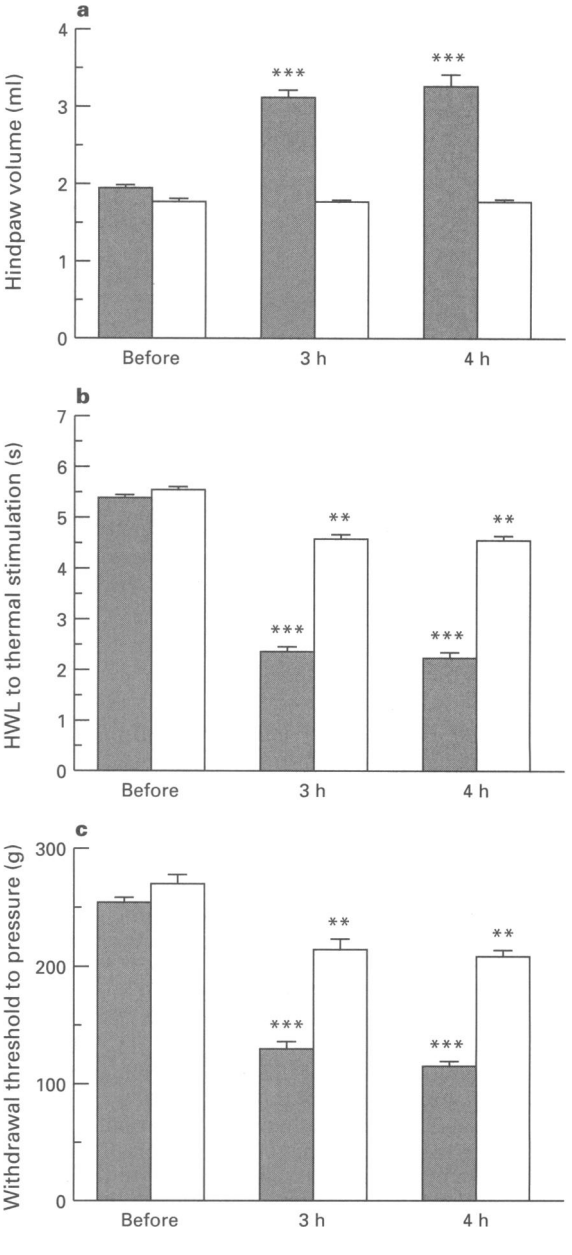

Figure 1 Changes in hindpaw volume (a), HWL to thermal stimulation (b) and HWT to pressure (c) induced by subcutaneous injection of 0.1 ml of 2% carrageenin into the plantar region of the rat left hindpaw ($n=8$). Data show before and at 3 and 4 h after carrageenin injection. HWL: hindpaw withdrawal latency. Volume: (ml); Thermal test: (s); mechanical test: (g). The carrageenin-treated paw; shaded columns; contralateral paw: open columns. Results are presented as mean ± s.e.mean. The statistical difference between groups was evaluated by Student's t test (two tailed): $^{**}P<0.01$ and $^{***}P<0.001$ compared to the data obtained before carrageenin injection.

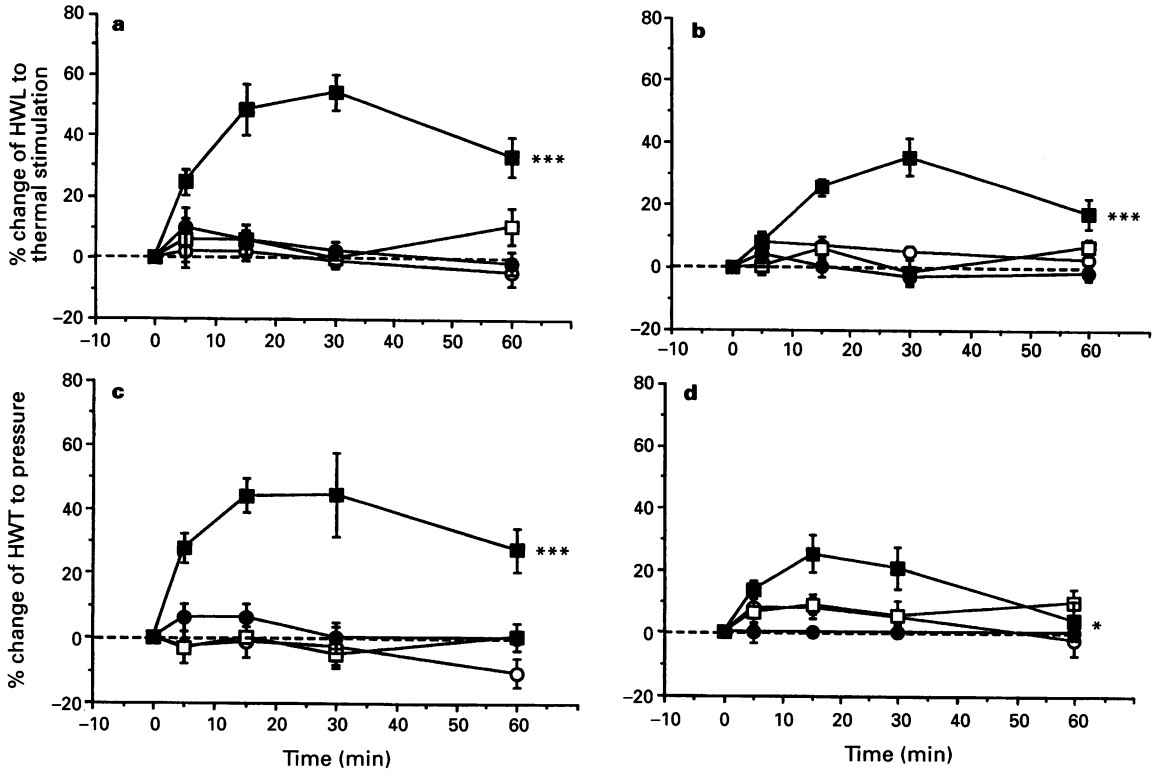

Figure 2 Effects of intrathecal injection of $CGRP_{8-37}$ on the HWL induced by thermal stimulation and HWT to pressure in rats with inflammation induced by 0.1 ml of 2% carrageenin injected into the plantar region of the left hindpaw. One nmol of $CGRP_{8-37}$ (□; $n=8$); 5 nmol of $CGRP_{8-37}$ (●; $n=8$); 10 nmol of $CGRP_{8-37}$ (■; $n=8$), 10 μl of 0.9% saline (○; $n=8$) as control. (a) (Ipsilateral) and (b) (contralateral) show the effect on the HWL to thermal stimulation (hot-plate test); (c) (ipsilateral) and (d) (contralateral) show the effect on the HWT to pressure (Randall Selitto tests). HWL: hindpaw withdrawal latency; HWT: hindpaw withdrawal threshold; $CGRP_{8-37}$: calcitonin gene-related peptide 8–37. Time = 0: intrathecal injection of 10 nmol of $CGRP_{8-37}$. Results are presented as mean ± s.e.mean of % changes in HWL and HWT. The statistical difference between groups was evaluated by one-way analysis of variance (ANOVA): $*P<0.05$, $**P<0.01$ and $***P<0.001$ compared with the control group.

Comparison of the effect of intrathecal injection of $CGRP_{8-37}$ on hindpaw withdrawal responses in intact rats and rats with experimentally induced inflammation

The effect of intrathecal administration of $CGRP_{8-37}$ (10 nmol) in intact rats and rats with experimentally induced inflammation was investigated. Figure 4 depicts the changes in HWL to thermal stimulation and HWT to pressure in intact rats ($n=16$) and in rats with experimentally induced inflammation ($n=16$). In the thermal test (Figure 4a) there was no significant difference in HWLs between left and right sides in intact rats but there was a significant bilateral decrease of HWL in rats with left hindpaw inflammation. In addition, the decrease in HWL was significantly more pronounced in the ipsilateral compared to the contralateral hindpaw (Figure 4a, $P<0.001$) in rats with left hindpaw inflammation.

There was no significant difference in the HWT to pressure in the hindpaw of normal rats but in rats with left hindpaw inflammation the HWT to pressure decreased bilaterally and there was a significantly more pronounced decrease in the ipsilateral hindpaw than in contralateral (Figure 4b; $P<0.001$).

After intrathecal administration of $CGRP_{8-37}$ the HWL to thermal stimulation increased bilaterally in both groups, as shown in Figure 5a, but the increase was less pronounced in the group of rats with inflammation.

As shown in Figure 5b the HWT to pressure increased bilaterally after $CGRP_{8-37}$ injection in rats with inflammation, but the increase was less pronounced than in intact rats. There was also a significant difference in HWTs to pressure between ipsilateral and contralateral sides in rats with left hindpaw inflammation ($P<0.001$).

Changes of CGRP-like immunoreactivity in cerebrospinal fluid, plasma and perfusate of right and left hindpaw after carrageenin injection

In order to determine the effects of carrageenin injected into the plantar area of the left hindpaw on the level of endogenous CGRP, the changes of CGRP-like immunoreactivity in cerebrospinal fluid, plasma and the perfusates of the ipsilateral and contralateral hindpaw in rats with inflammation were measured. The results are shown in Figure 6.

The content of CGRP-like immunoreactivity in the cerebrospinal fluid increased significantly at 3 and 24 h after carrageenin injection, compared with pre-injection data. There was no significant difference in CGRP-like immunoreactivity in the plasma at any time point. There was a significant increase in CGRP-like immunoreactivity in the perfusate of the ipsilateral hindpaw at 3 and 24 h after carrageenin injection. There was also an increased CGRP-like immunoreactivity in the contralateral hindpaw perfusate at 3 h ($P=0.06$) and 24 h ($P<0.05$), although the levels were lower than on the ipsilateral side.

Discussion

Carrageenin-induced inflammation is a commonly used model for the study of oedema formation and/or nociception (Winter *et al.*, 1962; Vinegar *et al.*, 1969; Mayer *et al.*, 1988; Satoh *et al.*, 1992; Lundeberg *et al.*, 1993; Bilevicuiute *et al.*, 1993; 1994a). In the present study injection of carrageenin into the left plantar hindpaw of the rat resulted in an ipsilateral in-

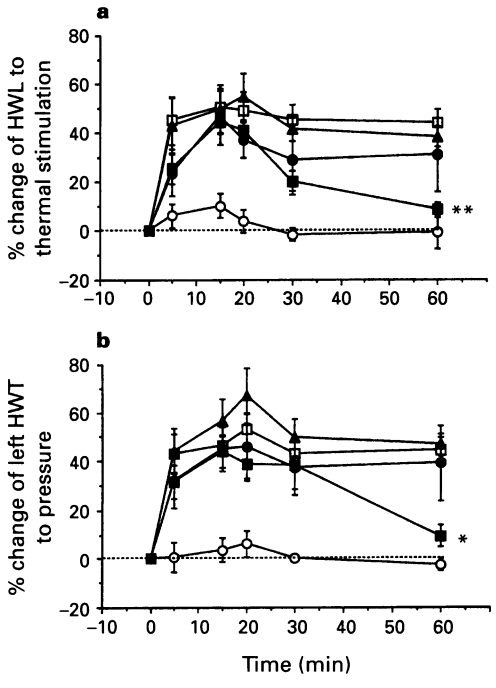

Figure 3 Effects of intrathecal injection of naloxone on $CGRP_{8-37}$-induced increase in ipsilateral HWLs to thermal stimulation (a) and ipsilateral HWTs to pressure (b) in rats with carrageenin-induced inflammation in the left hindpaw. Thirty-two rats with carrageenin-induced inflammation received an intrathecal injection of 10 nmol of $CGRP_{8-37}$ followed 15 min later by 22 (▲; $n=8$), 44 (●; $n=8$) or 88 (■; $n=8$) nmol of naloxone, or 10 μl of 0.9% saline (□; $n=8$) as a control. Another group of rats with carrageenin-induced inflammation received 10 μl of 0.9% saline followed 15 min later by 88 nmol of naloxone (○; $n=8$). HWL: hindpaw withdrawal latency; HWT: hindpaw withdrawal threshold; $CGRP_{8-37}$: calcitonin gene-related peptide 8–37. Time = 0: intrathecal injection of 10 nmol of $CGRP_{8-37}$ or 10 μl of 0.9% saline. Time = 15: intrathecal injection of either 22, 44 or 88 nmol of naloxone or 10 μl of 0.9% saline as a control. Results are presented as mean ± s.e.mean of % changes of HWL and HWT. The statistical difference between groups was evaluated by one-way analysis of variance (ANOVA): $^{*}P<0.05$ and $^{**}P<0.01$ compared with control group.

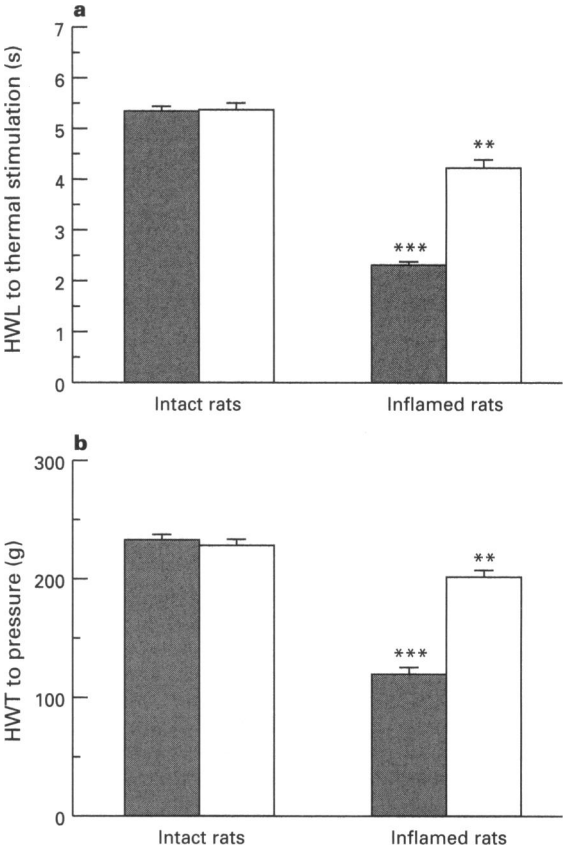

Figure 4 Comparison of the HWL to thermal stimulation (a) and HWT to pressure (b) in intact rats ($n=16$) and in rats with inflammation induced by injection of 0.1 ml of 2% carrageenin into the plantar region of the left hindpaw ($n=16$). HWL: hindpaw withdrawal latency; HWT: hindpaw withdrawal threshold. Contralateral (or right) sides: open columns; ipsilateral (or left) sides: shaded columns. Results are presented as mean ± s.e.mean. The statistical difference between groups was evaluated by Student's t test (two tailed), $^{**}P<0.01$ and $^{***}P<0.001$ compared to the data obtained from intact rats.

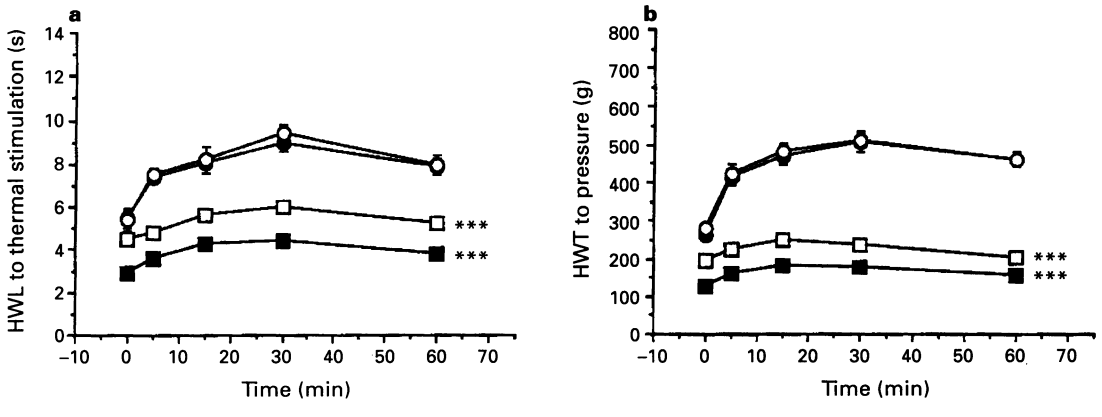

Figure 5 Changes in HWL during thermal stimulation (a) and in HWT to pressure (b) after intrathecal administration of $CGRP_{8-37}$ in intact rats and rats with carrageenin-induced inflammation in the left hindpaw. HWL: hindpaw withdrawal latency; HWT: hindpaw withdrawal threshold; $CGRP_{8-37}$: calcitonin gene-related peptide 8–37. Intact rats: $n=16$, left (●) and right (○) paws. Rats with carrageenin-induced inflammation: $n=16$, ipsilateral (■) and contralateral (□) paws. Time = 0: intrathecal injection of 10 nmol of $CGRP_{8-37}$. Results are presented as mean ± s.e.mean. The statistical difference between groups was evaluated by one-way analysis of variance (ANOVA): $^{***}P<0.001$ compared to the data obtained from intact rats.

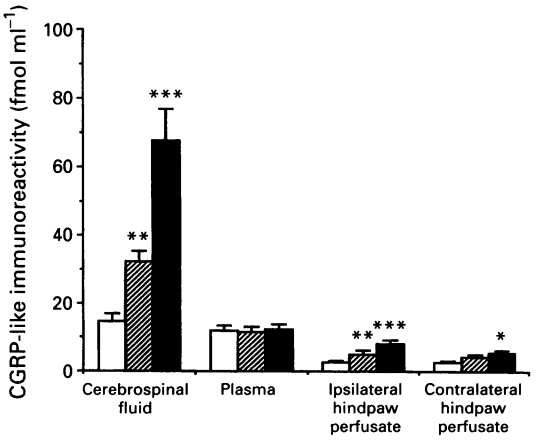

Figure 6 Changes in the content of CGRP-like immunoreactivity in cerebrospinal fluid, plasma and the perfusate of ipsilateral and contralateral hindpaw after 0.1 ml of 2% carrageenin injected into the plantar area of the left hindpaw. Data obtained before carrageenin injection (open columns, $n=12$), 3 h (hatched columns, $n=12$) and 24 h (solid columns, $n=12$) after carrageenin injection. CGRP: calcitonin gene-related peptide. Results are presented as mean ± s.e.mean. The statistical difference between groups was evaluated by Student's t test (two tailed): $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to the data obtained before carrageenin injection.

crease in paw volume, parallelled by an increased content of CGRP-like immunoreactivity in both ipsilateral and contralateral paw perfusates as well as in the CSF. Our results are supported by previous studies demonstrating increased biosynthesis of CGRP in the dorsal root ganglia (Iadarola & Draisci, 1988; Hanesch *et al.*, 1993) and increased release of CGRP-like immunoreactivity in the spinal cord (Nanayama *et al.*, 1989; Bileviciute *et al.*, 1993; 1994a, b), in response to experimentally induced inflammation. Furthermore, Kidd and collaborators have reported that experimentally induced monoarthritis results in changes of vascular reactivity (Cruwys *et al.*, 1992; 1994) and increase in the number of infiltrating cells (Denko & Petricevic, 1978; Levine *et al.*, 1985) in the opposite joint. It is also suggested that the bradykinin-induced plasma extravasation observed in both the injected and contralateral knee joints (Cruwys *et al.*, 1994) may be influenced by a neurogenic mechanism (Levine *et al.*, 1985). However, the contralateral joint swelling was not clinically apparent. As shown in Figure 1, the oedema formation in response to carrageenin injection was seen only in the injected hindpaw whereas there was a bilateral decrease in both the HWL induced by thermal stimulation and in the HWT to pressure which was more pronounced in the carrageenin injected paw. Taken together these findings indicate that there is a link whereby increased activity in sensory neurones on one side of the body can induce reciprocal changes within the dorsal horn and dorsal root ganglia on the opposite side (Levine *et al.*, 1985; Mapp *et al.*, 1992; 1993; Bileviciute *et al.*, 1993; 1994a,b).

Mayer *et al.* (1988) have suggested that primary sensory neurones play a dual role in the response to injury, where the central terminals transmit information set up by the noxious event to the central nervous system, and the peripheral terminals mediate a local inflammatory response via the axon reflex. Several neuropeptides such as CGRP and substance P, as well as other compounds, participate in this response. That CGRP has a role in transmission of presumed nociceptive information is supported by the finding that intrathecal administration of 10 nmol of the CGRP antagonist, $CGRP_{8-37}$, produced an increase in both the HWL induced by thermal stimulation and in the HWT to pressure in rats with unilateral hindpaw inflammation, although significantly less pronounced than in normal rats (see Figure 5). It has been reported that CGRP coexists with substance P in the spinal cord (Wiesenfelt-Hallin *et al.*, 1984; Cameron *et al.*, 1988; Willis & Coggeshall, 1991; Hökfelt *et al.*, 1992) and the two peptides may act synergistically to lower the nociceptive flexion withdrawal reflex threshold in rats (Woolf & Wiesenfelt-Hallin, 1986). In the dorsal horn of the spinal cord it has been demonstrated that CGRP acts at both pre- and post-synaptic sites (Oku *et al.*, 1987; Ryu *et al.*, 1988; Ishida-Yamamoto & Tohyama, 1989; Poyner, 1992). Furthermore CGRP has been shown to inhibit the enzymatic degradation of substance P (Le Greves *et al.*, 1985; Mao *et al.*, 1992) as well as to potentiate the release of substance P from primary afferent fibres (Oku *et al.*, 1987; Ryu *et al.*, 1988). CGRP may therefore enhance the effects of substance P at the spinal cord level. It is possible that endogenous CGRP, released in the dorsal horn of the spinal cord during experimentally induced inflammation (Nanayama *et al.*, 1989; Donner & Stein 1992; Garry & Hargreaves, 1992) and/or noxious stimulation, binds to the available CGRP receptors to transmit the nociceptive information. Intrathecal injection of $CGRP_{8-37}$ may antagonize the postsynaptic CGRP receptor as well as the CGRP receptor located presynaptically, the latter resulting in inhibition of substance P release. Our finding of a reduced effect of $CGRP_{8-37}$ on HWLs in rats with inflammation is in line with an increased release of endogenous CGRP in the dorsal horn in acute inflammation (Draisci *et al.*, 1991) compared to intact rats. However, we do not believe that the effect is due simply to antagonist/agonist dynamics, as 10 nmol of $CGRP_{8-37}$ is shown to produce longer HWLs and HWTs on the ipsilateral side in which there presumably are higher concentrations of CGRP than on the contralateral side. This would suggest that CGRP has a modulatory role which is dependent on the interaction with other neurotransmitters at the spinal cord level.

In the present study, the increase in HWLs and HWTs obtained after intrathecal administration of $CGRP_{8-37}$ in rats with left hindpaw inflammation was partly reversed by intrathecal injection of the opioid receptor antagonist naloxone, indicating that endogenous opioid peptides may be involved in the control of CGRP release and/or the pre- or post-synaptic actions of CGRP. Our results are supported by the finding that opioid peptides may exert a pre-synaptic inhibitory effect on primary nociceptive afferents in the dorsal horn of the spinal cord (Lembeck & Donnerer, 1985; Pohl *et al.*, 1989; Yaksh & Malmberg, 1994), including CGRP-containing primary afferent fibres (Pohl *et al.*, 1989). In addition, spinal neurones which exhibit dynorphin-like immunoreactivity are located in laminae I–VII of the rat spinal cord (Lembeck & Donnerer, 1985; Pohl *et al.*, 1989; Yaksh & Malmberg, 1994). Histological studies have demonstrated a monosynaptic connection between dynorphin-containing neurones and CGRP-containing axon terminals in a rat model of peripheral inflammation (Takahashi *et al.*, 1990). Collin *et al.* (1993) recently demonstrated that endogenous opioid peptides, acting at both μ- and κ-receptors, exert a tonic inhibitory control on the release of CGRP-like material in the spinal cord. However, the results of the present study show that intrathecal naloxone in rats with carrageenin induced inflammation was less potent in reversing the effect of $CGRP_{8-37}$, than in normal and neuropathic rats (Yu *et al.*, 1994; 1995; 1996). The reversal of the effect of $CGRP_{8-37}$ appeared when the dosage of naloxone was 88 nmol, which is 4 times the dose needed in normal rats or in rats with mononeuropathy. It is also possible that the reduced effect of $CGRP_{8-37}$ in the carrageenin model of inflammation is related to an increased biosynthesis and release of endogenous opioid peptides in the dorsal horn of the spinal cord (Draisci *et al.*, 1991). Since naloxone induced a decreased reflex latency in rats previously given an intrathecal injection of $CGRP_{8-37}$ it could be that the excitability of the hindpaw withdrawal reflex is affected by administration of naloxone alone. Available results suggest that this is unlikely. The opioid antagonist, naloxone, has been studied extensively and it clearly has little effect in normal animal or human subjects (Iversen, 1995). In the present study we observed no significant change either in HWL induced by thermal stimulation or in HWT to pressure after intrathecal administration of 10 μl of

0.9% saline followed 15 min later by 88 nmol of naloxone. The above data suggest that spinal opioid neurones may directly influence small diameter primary afferents that are likely to be involved in nociceptive transmission.

In summary, the results of the present study show that unilateral injection of carrageenin into the left plantar hindpaw results in a marked increase in the ipsilateral paw volume only. On the other hand, there was a bilateral decrease of HWLs to thermal stimulation and HWTs to pressure as well as a bilateral increased release of CGRP-like immunoreactivity in hindpaw perfusates. Intrathecal administration of $CGRP_{8-37}$ induced a significant bilateral increase in the HWL induced by thermal stimulation and in the HWT to pressure and these effects were partially reversed by naloxone. Overall, the results indicate that CGRP plays a role in the transmission of presumed nociceptive information in the spinal cord of rats with experimentally induced inflammation, and that its release and/or effects are subjected to opioid modulation.

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