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Effects of calcitonin gene-related peptide-(8-37) on withdrawal responses in rats with inflammation

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Abstract

The present study was performed to explore the effect of subcutaneous injection of carrageenan into the rat plantar region on hindpaw edema formation and the latency of hindpaw withdrawal to presumed nociceptive stimulation. Subcutaneous injection of carrageenan into the left hindpaw induced a significant increase in the volume of the left hindpaw, leaving the right side unaffected. In addition, we found a bilateral decrease in hindpaw withdrawal latency to heat and mechanical, but not to cold stimulation. The decreased bilateral hindpaw withdrawal latency to heat stimulation lasted for 14 days after carrageenan injection. The decreased bilateral hindpaw withdrawal latency to mechanical stimulation lasted for 2 days after the injection, then reversed and increased from day 3 to 14. Intrathecal injection of either 10 nmol of calcitonin gene-related peptide 8-37 or 26.6 nmol of morphine induced significant bilateral increases in hindpaw withdrawal latency, which were more pronounced with the morphine. The results show that experimentally induced unilateral hindpaw inflammation induces a bilateral decrease in hindpaw withdrawal latencies to presumed nociceptive stimulation while the sensory systems for heat and mechanical stimulation were differently affected after carrageenan injection. © 1998 Elsevier Science B.V.

Keywords: Inflammation; Carrageenan; Hindpaw withdrawal latency; Intrathecal injection; (CGRP-8-37) (calcitonin gene-related peptide-8-37); Morphine

1. Introduction

It has been Suggested that primary sensory neurons play a dual role in the response to acute 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 (Mayer et al., 1988). Sluka et al. (1995) have suggested that dorsal root reflexes also play an important role in the local inflammatory response. It has been reported that experimentally induced acute inflammation results in an enhanced release of the neuropeptide calcitonin gene-related peptide (CGRP) into peripheral tissues and cerebrospinal fluid (Bileviciute et al., 1993, 1994). In the periphery CGRP has been shown to potentiate edema formation (Brain and Williams, 1985, 1989) and in the spinal cord CGRP has been shown to be involved in the transmission of presumed nociceptive information (Biella et al., 1991; Kawamura et al., 1989; Satoh et al., 1992; Yu et al., 1994, 1995, 1996a,b). Recently, Neugebauer et al. (1996) reported that CGRP is involved in the spinal processing of mechanosensory input and in the generation and maintenance of hyperexcitability of dorsal horn neurons during the development of acute inflammation. A role for CGRP in nociception is also supported by studies reporting that intrathecal administration of a CGRP receptor antagonist, CGRP-(8-37), induced an increase in hindpaw withdrawal latency in rats with acute inflammation (Yu et al., 1996a).

Carrageenan induced inflammation is a commonly used model for the study of edema formation and nociception (Lundeberg et al., 1993; Mayer et al., 1988; Millan et al., 1988; Satoh et al., 1992; Vinegar et al., 1969; Winter et al., 1962). In the present study we set out to further previous observations in our laboratory by investigating changes in hindpaw withdrawal latencies to nociceptive mechanical, heat and cold stimulation in the carrageenan model. To our knowledge the response to cold stimulation

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has not been evaluated in studies on chronic inflammation induced by carrageenan. Previously, the carrageenan model has generally been used to investigate short term changes. In the present study the temporal pattern of hindpaw withdrawal latencies was investigated for a period of 2 weeks. As the primary sensory neurons may contribute to the local inflammatory process edema formation was also assessed. Both pro- and antinociceptive effects of intrathecal administration of the CGRP receptor antagonist CGRP-(8-37) (Chiba et al., 1989) have been reported (Neugebauer et al., 1996; Xu and Wiesenfeld-Hallin, 1996; Yu et al., 1994), therefore, the effects of GRP-(8-37) were again assessed and compared with morphine.

2. Materials and methods

2.1. 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 ± 2°C with a 12 h light/dark cycle. All experiments were approved by the animal ethical committee of Karolinska Institute and every effort was made to minimize animal suffering. Rats were accustomed to the testing conditions for about five days before starting the experiments in order to decrease the stress caused by handling and measurements and to thus obtain stable responses. Before performing the experiments rats were pre-treated with 2% lidocaine subcutaneously in the region of subsequent intrathecal injection. A stainless steel needle with an outer diameter of 0.5 mm was then inserted directly into the subarachnoid space between the L4–L5 or L3–L4 vertebrae (Lundeberg et al., 1993; Yu et al., 1994, 1995, 1996a,b). Ten microliters of solution (see below) were thereafter infused intrathecally within 1 min.

2.2. Inflammatory model

Inflammation was produced by unilateral subcutaneous injection of 0.1 ml of 2% carrageenan 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 hours after carrageenan injection, the intrathecal injection was given. The hindpaw volume was measured by a plethysmometer (UGO Basile, type 7150, Italy) before testing procedures started. The Plethysmometer was calibrated before each test. A line was drawn just above the hindpaw ankle to allow reproducible measurements.

2.3. Tests of the hindpaw withdrawal responses

The latency to hindpaw withdrawal during heat or cold stimulation was measured as well as the withdrawal latency to mechanical stimulation (Lundeberg et al., 1993; Yu et al., 1994, 1995, 1996a,b). The heat 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 temperature for the cold-plate was 4.0–4.5°C. The time to hindpaw withdrawal latency. The Randall Selitto Test (UGO Basile, Type 7200, Italy) was used to assess hindpaw withdrawal latency to mechanical stimulation. A wedged-shaped pusher with a loading rate of 48 g/s was applied to the dorsal surface of the manually handled hindpaw and the mechanical stimulation required to initiate the struggle response was assessed. The hindpaw withdrawal latency is expressed in seconds, i.e., latency to withdrawal from start of stimulation. The measurement after plantar injection, but before intrathecal injection, were regarded as the basal hindpaw withdrawal latency to thermal or mechanical stimulation. The hindpaw withdrawal latencies recorded during subsequent experiments were expressed as percentage change of the basal level for each rat (% change of hindpaw withdrawal latency). The hindpaw withdrawal latencies were tested before intrathecal injection and repeated at 5, 15, 30 and 60 min after the injection.

2.4. Chemicals

Carrageenan (Sigma, St. Louis, MO) (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) 10 nmol of CGRP-(8-37) (hCGRP-(8-37); Peninsula Labs, Europe LIT); (2) 26.6 nmol (10 μg) of morphine (morphine hydrochloride, Kabi Pharmacia, Sweden). Ten μl of 0.9% saline was injected intrathecally as a control.

2.5. Statistical analysis

Data from hindpaw withdrawal tests are presented as mean $\pm$ S.E. The difference between groups was determined by a two-way analysis of variance (ANOVA) for repeated measures or Student's *t*-test (two tailed) where applicable. $* P < 0.05$, $** P < 0.01$ and $*** P < 0.001$ were considered as significant differences. $*** P < 0.001$ was performed by correlation test (Box M, Statistical software) in Fig. 2.

3. Results

3.1. Effects of subcutaneous injection of carrageenan into the plantar area of the rat left hindpaw on hindpaw volumes and hindpaw withdrawal latencies to heat and mechanical stimulation

Ten rats were tested with heat and mechanical stimulation before, at 3 and 4 h, and on days 1, 3, 5, 8, 10, 12 and

Table 1
Hindpaw withdrawal latencies (s) to heat, cold and mechanical stimulation in rats 3 h after the injection of carrageenan into the left hindpaw

Test	Treatments	*n*	Left side	Right side
Hot-plate	0.9% saline	10	5.49 ± 0.45 s	5.38 ± 0.50 s
	carrageenan	10	2.97 ± 0.09 s	4.59 ± 0.11 s[a]
Cold-plate	0.9% saline	10	2.85 ± 0.12 s	2.95 ± 0.05 s
	carrageenan	10	2.90 ± 0.10 s	2.91 ± 0.04 s
Randall Selitto Test	0.9% saline	10	5.14 ± 0.24 s	5.28 ± 0.19 s
	carrageenan	10	2.75 ± 0.13 s[c]	4.15 ± 0.13 s[a]

[a] $P < 0.05$ and [c] $P < 0.001$ compared with control group; s: second.

14 after carrageenan injection into the plantar area of left hindpaw. Results are shown in Table 1 and Fig. 1. Fig. 1A shows that 3 h after carrageenan injection the left hindpaw volume was significantly increased (before carrageenan injection: 1.89 ± 0.03 ml, 3 h after carrageenan injection: 3.39 ± 0.08 ml; $t = 9.54$, $P < 0.001$) but the volume of the non-injected right hindpaw (before carrageenan injection: 1.86 ± 0.03, 3 h after carrageenan injection: 1.85 ± 0.03 ml; $t = 0.24$, $P = 0.82$) showed no significant change.

As shown in Table 1 3 h after carrageenan injection there were significant decreases in hindpaw withdrawal latency to heat ($t$left/left = 17.14, $P < 0.001$; $t$right/right = 3.14, $P < 0.05$) and mechanical ($t$left/left = 12.87, $P < 0.001$; $t$right/right = 2.88, $P < 0.05$) stimulation. There was no significant difference in the hindpaw withdrawal latency between carrageenan treated and the control groups in the cold-plate test.

As shown in Fig. 1A the increase in left hindpaw volume reached its peak after 3 to 4 h, persisted then recovered slowly during the 14 days of observation. There was no change in right hindpaw volume during these 14 days.

As shown in Fig. 1B, there was a bilateral decrease in hindpaw withdrawal latencies to heat stimulation which persisted for 10 days on the injected side and for 3 days on the contralateral side. The change in hindpaw withdrawal latency was most pronounced on the carrageenan treated side as shown in Fig. 1B.

In the Randall Selitto test the hindpaw withdrawal latency to mechanical stimulation decreased bilaterally after the injection of carrageenan and this decrease lasted for 1 day. As shown in Fig. 1C, a bilateral shift followed leading to an increase in withdrawal latencies from three days after the injection of carrageenan. This effect lasted for about one week whereafter latencies slowly returned to values obtained before the injection of carrageenan.

Ten rats received an injection of 0.1 ml of 0.9% saline into the left plantar hindpaw. Before and after the injection of saline the hindpaw volume, the hindpaw withdrawal latency to heat and mechanical stimulation were assessed. The parameters were unaltered after the injection (not shown), indicating that injection of saline had per se.

3.2. Correlation between the changes in left hindpaw volume and left hindpaw withdrawal latency to heat stimulation

The correlation coefficient of the changes in left hindpaw volume and the changes in left hindpaw withdrawal latency to heat stimulation was highly significant (Correlation test: $r = 0.80$, $P < 0.001$), as shown in Fig. 2.

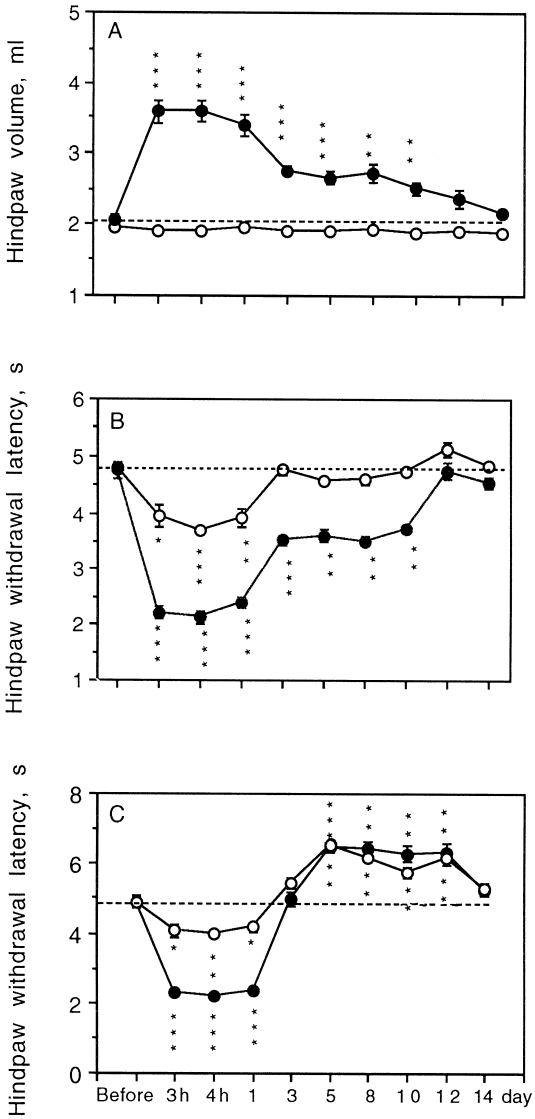

Fig. 1. Changes in hindpaw volume (A), hindpaw withdrawal latency to heat (B) and mechanical (C) stimulation induced by subcutaneous injection of 0.1 ml of 2% carrageenan into the planar region of the rat hindpaw ($n = 10$). Data was collected before (control), 3 and 4 h, 1, 3…, 14 days after carrageenan injection. Hindpaw volume: ml. The carrageenan treated paw: ●; the contralateral paw: ○. Results are presented as mean ± S.E. The statistical difference between groups was evaluated by Student's *t*-test (two tailed), * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

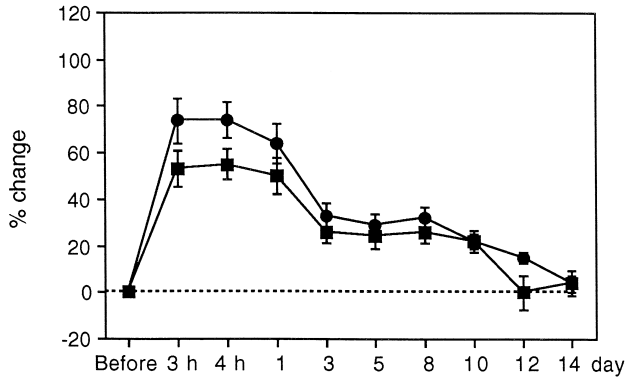

Fig. 2. Comparison of temporal pattern for the % change of left hindpaw volume (●) and the % change of left hindpaw withdrawal latency to heat stimulation (■). Correlation test (Box M, Statistica software): $r = 0.80$, $P < 0.001$.

3.3. Effect of intrathecal injection of CGRP-(8-37) and morphine on the hindpaw withdrawal latency to heat stimulation in rats with left hindpaw inflammation

Thirty rats with left hindpaw inflammation received either 10 nmol of CGRP-(8-37) ($n = 10$), 26.6 nmol of morphine ($n = 10$) or 10 μl of 0.9% saline as a control ($n = 10$). The results from the hot-plate test are shown in Fig. 3.

As shown in Fig. 3A and B, the bilateral hindpaw withdrawal latencies to heat stimulation increased significantly after intrathecal injection of 10 nmol of CGRP-(8-37) (Fleft/left = 41.98, $P < 0.001$; Fright/right = 31.72, $P < 0.001$) or 26.6 nmol of morphine (Fleft/left = 51.45, $P < 0.001$; Fright/right = 45.52, $P < 0.001$) compared with the saline control group.

Fig. 3C shows the % change in hindpaw withdrawal latencies to heat stimulation measured at 30 min after the intrathecal injection of CGRP-(8-37) or morphine. There were significant differences in hindpaw withdrawal latencies during the hot-plate test between rats receiving morphine and CGRP-(8-37) (left: $t = 2.81$, $P < 0.05$; right: $t = 3.54$, $P < 0.01$) as shown in Fig. 3C.

3.4. Effect of intrathecal injection of CGRP-(8-37) or morphine on the hindpaw withdrawal latency to cold stimulation in rats with left hindpaw inflammation

Thirty rats with left hindpaw inflammation received either 10 nmol of CGRP-(8-37) ($n = 10$), 26.6 nmol of morphine ($n = 10$), or 10 μl of 0.9% saline as a control ($n = 10$). The results from the cold-plate test are shown in Fig. 4.

As shown in Fig. 4A and B, the hindpaw withdrawal latencies to cold stimulation increased significantly and bilaterally after intrathecal injection of 10 nmol of CGRP-(8-37) (Fleft/left = 29.67, $P < 0.001$; Fright/right = 20.51, $P < 0.001$) or 26.6 nmol of morphine (Fleft/left = 87.30, $P < 0.001$; Fright/right = 97.27, $P < 0.001$) compared with the saline control group.

Fig. 4C shows the % changes of hindpaw withdrawal latencies to cold stimulation measured at 30 min after the

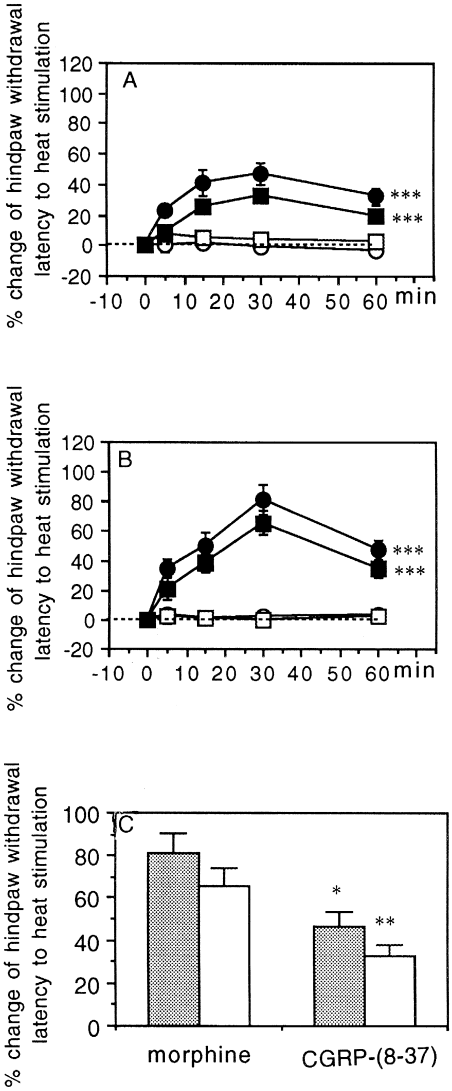

Fig. 3. Effects of intrathecal injection of 10 nmol of CGRP-(8-37) (A; $n = 10$) or 26.6 nmol of morphine (B; $n = 10$) on the hindpaw withdrawal latency to heat stimulation in rats with inflammation induced by 0.1 ml of 2% carrageenan injected into the plantar region of left hindpaw. (C) shows the hindpaw withdrawal latency at 30 min after the injection of either CGRP-(8-37) or morphine. A: CGRP-(8-37) group: left side: ■; right side: ●. Control group: 10 μl of saline, left side: □; right side: ○. Time = 0: intrathecal injection. Two-way ANOVA, $***P < 0.001$ compared with the control group. B: Morphine group: left side: ■; right side: ●. Control group: 10 μl of saline. Left side: □; right side: ○. Time = 0: intrathecal injection. Two-way ANOVA, $***P < 0.001$ compared with the control group. C: Right side: open columns, Left side: closed columns. Results are presented as mean ± S.E. Time: 30 min after intrathecal injection. $*P < 0.05$ and $**P < 0.01$ (Student's t-test, two-tailed for comparison of CGRP-(8-37) with morphine).

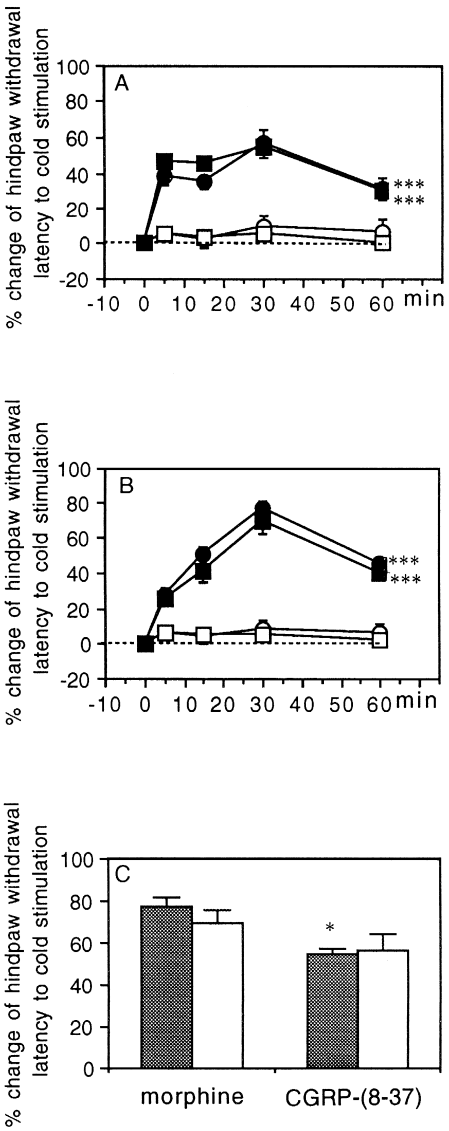

Fig. 4. Effects of intrathecal injection of 10 nmol of CGRP-(8-37) (A; $n = 10$) or 26.6 nmol of morphine (B; $n = 10$) on the hindpaw withdrawal latency to cold stimulation in rats with inflammation induced by 0.1 ml of 2% carrageenan injected into the plantar region of left hindpaw. (C) shows the hindpaw withdrawal latency at 30 min after injection of either CGRP-(8-37) or morphine. A: CGRP-(8-37) group: left side: ■; right side: ●. Control group: 10 μl of saline, left side: □; right side: ○. Time = 0: intrathecal injection. Two-way ANOVA, $***P < 0.001$ compared with the control group. B: Morphine group: left side: ■; right side: ●, Control group: 10 μl of saline, left side: □; right side: ○. Time = 0: intrathecal injection. Two-way ANOVA, $***P < 0.001$ compared with the control group. C: Right side: open columns, Left side: closed columns. Results are presented as mean ± S.E. $*P < 0.05$ (Student's *t*-test, two-tailed for comparison of CGRP-(8-37) with morphine).

intrathecal injection of CGRP-(8-37) or morphine in rats with left hindpaw inflammation. In the cold-plate test there were significant differences in hindpaw withdrawal latencies on the left side (left: $t = 2.65$, $P < 0.05$; right: $t = 1.54$, $P = 0.17$) between rats receiving morphine and CGRP-(8-37) as shown in Fig. 4C.

3.5. Effect of intrathecal injection of CGRP-(8-37) or morphine on the hindpaw withdrawal latency to mechanical stimulation in rats with left hindpaw inflammation

Thirty rats with left hindpaw inflammation received either 10 nmol of CGRP-(8-37) ($n = 10$), 26.6 nmol of morphine ($n = 10$) or 10 μl of 0.9% saline as a control

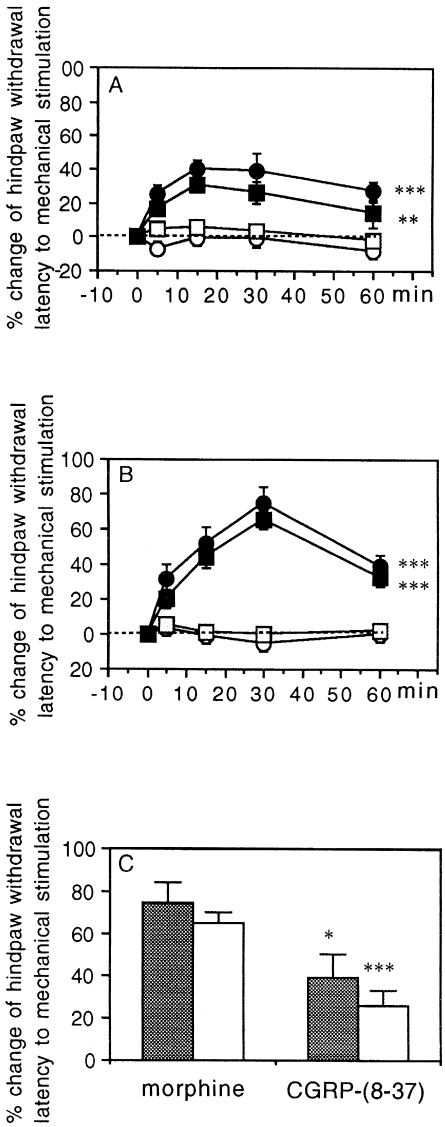

Fig. 5. Effects of intrathecal injection of 10 nmol of CGRP-(8-37) (A; $n = 10$) or 26.6 nmol of morphine (B; $n = 10$) on the hindpaw withdrawal latency to mechanical stimulation in rats with inflammation induced by 0.1 ml of 2% carrageenan injected into the plantar region of left hindpaw. (C) shows the hindpaw withdrawal latency at 30 min after the injections of either CGRP-(8-37) or morphine. A: CGRP-(8-37) group: left side: ■; right side: ●. Control group: 10 μl of saline, left side: □; right side: ○. Time = 0: intrathecal injection. Two-way ANOVA, $**P < 0.01$ and $***P < 0.001$ compared with the control group. B: Morphine group: left side: ■; right side: ●. Control group: 10 μl of saline, left side: □; right side: ○. Time = 0: intrathecal injection. Two-way ANOVA, $***P < 0.001$ compared with the control group. C: Right side: open columns, Left side: closed columns. Results are presented as mean ± S.E. $*P < 0.05$ and $***P < 0.001$ (Student's *t*-test, two-tailed for comparison of CGRP-(8-37) with morphine).

($n = 10$). The results of the Randall Selitto test are shown in Fig. 5.

As shown in Fig. 5A and B, the hindpaw withdrawal latencies to mechanical stimulation increased significantly and bilaterally after intrathecal injection of 10 nmol of CGRP-(8-37) ($F\text{left/left} = 28.45$, $P < 0.001$; $F\text{right/right} = 10.72$, $P < 0.01$) or 26.6 nmol of morphine ($F\text{left/left} = 51.02$, $P < 0.001$, $F\text{right/right} = 85.65$, $P < 0.001$) compared with the control group.

Fig. 5C shows the % changes of hindpaw withdrawal latencies to mechanical stimulation measured at 30 min after the intrathecal injection of CGRP-(8-37) or morphine in rats with left hindpaw inflammation. There are significant differences of hindpaw withdrawal latencies in left ($t = 2.39$, $P < 0.05$) and right ($t = 5.69$, $P < 0.001$) sides to mechanical stimulation in the Randall Selitto test between morphine and CGRP-(8-37) groups (Fig. 5C).

4. Discussion

The results of the present study show that subcutaneous injection of carrageenan into rat's left hindpaw induced a significant increase in the volume of the left hindpaw leaving the right side unaffected. In addition, bilateral decreases were found in withdrawal latencies to heat- and mechanical-, but not to cold stimulation. The decreased hindpaw withdrawal latency to heat stimulation lasted for 14 days after carrageenan injection. The decreased withdrawal latency to mechanical stimulation lasted for 2 days after the injection, was then reversed and increased from day 3 to 14. Intrathecal injection of either 10 nmol of calcitonin gene-related peptide 8-37 or 26.6 nmol of morphine induced significant bilateral increases in hindpaw withdrawal latency.

One of the most interesting findings of the present study was the similarity in temporal profile between the changes in left hindpaw volume and left hindpaw withdrawal latency to heat stimulation. As shown in Fig. 2 the results of the hot-plate test are paralleled by paw volume increase induced by carrageenan injection during the 14 days observation period. Among the neuropeptides with a significant role in edema formation are the tachykinins substance P and neurokinin A (Dray and Bevan, 1993; Dray, 1995). Interestingly, an increased released of neurokinin A and substance P have been shown in the dorsal horn of the spinal cord following heat stimulation (Duggan et al., 1988). It os therefore tempting to suggest that the parallel changes seen in edema formation and response to heat stimulation may be related to the increased release of tachykinins peripherally and their facilitated transmission centrally. Dorsal horn tachykinins, released by noxious but not by nonnoxious stimulator, originate from primary afferent, intrinsic, and descending spinal cord neurons (Dickenson, 1995; Yaksh et al., 1988). The role of substance P in nociception and neuronal hyperexcitability has been extensively studied (Traub, 1996). It has been suggested that substance P facilitates the excitatory amino acid-induced activator of the *N*-methyl D-aspartate receptor via tachykinin NK_1 receptor stimulation. The resulting increased excitability of dorsal horn sensory neurons (wind-up) may be an important mechanism in the long-lasting decrease in hindpaw withdrawal latency to heat stimulation. This proposed role for substance P is supported by the results from behavioural studies using substance P receptor antagonists in animal models of peripheral inflammation (Dickenson, 1995; Traub, 1996). Numerous reports also suggest the involvement of CGRP in the local inflammatory process as well as in spinal cord transmission of presumed nociceptive activity. Studies have shown that CGRP has little or no edema potentiating effect on its own, suggesting that CGRP released peripherally is not directly involved in the edema formation showed (Maggi, 1995; Raud et al., 1991).

In the present study the hindpaw withdrawal latency to mechanical stimulation decreased bilaterally and recovered to basal level 2 days after carrageenan injection. Neugebauer et al. (1996) have reported that CGRP is involved in the spinal processing of mechanosensory input and in the acute generation of hyperexcitability of dorsal horn neurons. It is possible that a decrease in the release of CGRP at the spinal cord level or an increased enzymatic degradation may explain why the hindpaw withdrawal latency to mechanical stimulation increased from the 3rd to the 14th day following carrageenan injection. This would suggest that the release of substance P and CGRP at the spinal cord level is differentially controlled. Such a suggestion is supported by the finding that the $GABA_B$ receptor antagonist was shown to enhance substance P release, whereas CGRP release was unaffected in chronic monoarthritic rats (Malcangio and Bowery, 1996). This suggests an inhibitory effect on spinal substance P turnover mediated via GABAs receptors. It has been suggested that in the chronic inflammatory process this inhibition is out of order, explaining why the release of substance P may increase and in turn result in reduced withdrawal latency to heat. This proposed inhibition of substance P but not CGRP release by GABA is in contrast to the action of endogenous opioids, because naloxone enhanced both substance P and CGRP release in the same model (Malcangio and Bowery, 1996). Pohl et al. (1989) reported that opioids controlled the release of CGRP from primary afferent fibers in rat spinal cord slices. They found that μ and δ agonists inhibited the release of CGRP via presynaptic opioid receptors, thereby possibly contributing to the analgesic action of opioids. Collin et al. (1993) demonstrated that CGRP-like immunoreactive material was spontaneously released from the spinal cord in anaesthetized rats at a rate of about 4 pg/min and that this release was under a tonic inhibitory control by endogenous opioid peptides acting at μ- and κ-opioid receptors. Recently, Yu et al. (1995) reported that μ and δ-opioid receptors are involved in the anti-nociception induced by intrathecal CGRP-(8-37). A

close interaction between μ- and δ-opioids and CGRP have also been demonstrated in studies on opioid tolerance (Menard et al., 1996). Taken together, previous studies indicate that changes in withdrawal latency to mechanical stimulation are associated with changes in CGRP (Neugebauer et al., 1996).

In the present study the cold-plate test was used as well as the hot-plate and the Randall Selitto tests. As shown in Table 1 there was no significant decrease in hindpaw withdrawal latency to cold stimulation in rats with inflammation (Table 1). This contrasts with the outcome of cold hypersensitivity in mononeuropathic rats (Attal et al., 1990) Bennett and Xie, 1988; Carlton et al., 1994).

That CGRP plays a role in the transmission of presumed nociceptive information is supported by the finding that intrathecal administration of 10 nmol of CGRP-(8-37) produced an increase in hindpaw withdrawal latencies to heat, cold and mechanical stimulation in rats with inflammation. Our finding of an antinociceptive effect of CGRP-(8-37) is in contrast to the results of Xu and Wiesenfeld-Hallin (1996), who reported that CGRP-(8-37) facilitates nociceptive responses in decerebrated spinalised rats. However, Neugebauer and collaborators have clearly stated that CGRP-(8-37) inhibited the effect of CGRP in intact rats (Neugebauer et al., 1996). Taken together, this would strongly suggest that the results observed using spinalised rats, elucidating changes in presumed nociceptive responses, should be interpreted with due care. When 10 nmol of intrathecal CGRP-(8-37) was compared with 26.6 nmol of morphine, morphine was shown to induce a more marked prolongation in hindpaw withdrawal responses.

In summary, the present study demonstrated a unilateral edema formation and a bilateral decrease in withdrawal latency to heat and mechanical stimulation after subcutaneous injection of carrageenan into the rat's left hindpaws. The temporal pattern of hindpaw withdrawal latency to heat stimulation was different from what was found to mechanical stimulation during 14 days following carrageenan injection. Hindpaw withdrawal latencies to cold stimulation were unaffected. Intrathecal injection of either CGRP-(8-37) or morphine induced significant increases in hindpaw withdrawal latency.

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