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Spinal cord SP release and hyperalgesia in monoarthritic rats: involvement of the $GABA_B$ receptor system

[1]Marzia Malcangio & Norman G. Bowery

Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX

1 Monoarthritis was induced in Lewis rats by interdermal injection in the left hind paw of a suspension of *Mycobacterium tubercolusis* in mineral oil (500 μg 100 μl^{-1}). Controls were injected with 100 μl mineral oil.

2 Withdrawal latencies to thermal stimuli of the inflamed paw, the contralateral and both paws of control rats were measured at daily intervals after injection by the plantar test.

3 After detection of the pain threshold, rat spinal cords were removed and horizontal dorsal slices were mounted in a 3-compartment bath to measure electrically-evoked release of substance P-like immunoreactivity (SP-LI).

4 The inflamed paw of monoarthritic rats exhibited a lower pain threshold to thermal stimuli than the contralateral paw of the same animals and both paws of control rats. Inflamed paw hyperalgesia was maximal two days after injection, and declined gradually between 7 to 21 days with no evidence of excitability of withdrawal reflexes after 28 days.

5 During the 28 days study, monoarthritic rats gained less weight than control rats.

6 Electrical stimulation of the dorsal roots attached to rat isolated spinal cord slices induced a significant increase (174 ± 18% of basal outflow which was 30.3 fmol 8 ml^{-1}, $n = 5$) in SP-LI release.

7 One-week after induction of inflammation no differences in the amount of SP-LI released from the spinal cord of incomplete Freund's adjuvant-treated rats (IFA) and Freund's adjuvant-treated rats (CFA) were detected. Two weeks after, CFA spinal cord tended to release more SP-LI than IFA cords and, 21 days after injection, the spinal cord of CFA rats released significantly more peptide than IFA rats (17.8 ± 2.8 fmol ml^{-1}, $n = 12$ and 6.9 ± 3.2 fmol ml^{-1}, $n = 9$, respectively).

8 Twenty-one days after treatment, the evoked release from monoarthritic rat spinal cords was increased by 263 ± 42% ($n = 3$) in the presence of the $GABA_B$ receptor antagonist, CGP 36742 (100 μM) which also significantly potentiated monoarthritis-induced hyperalgesia up to 45 min after injection (100 mg kg^{-1}, i.p.).

9 These findings may provide a basis for a novel approach to chronic pain therapy but also an explanation for the lack of analgesia produced by the $GABA_B$ agonist, baclofen, in chronic as compared to acute pain.

Keywords: Monoarthritis; chronic pain; $GABA_B$ receptor; SP-LI release

Introduction

Freund's adjuvant-induced arthritis in rats is associated with chronic pain (Colpaert, 1987). Inflammation of the paw induces a quantitative increase in the afferent input to the spinal cord (Levine *et al.*, 1984). High threshold peripheral afferent fibres respond even to gentle stimulation of the inflamed area (Schaible & Schmidt, 1988). It seems possible that changes in intraspinal mechanisms, which enhance the sensitivity of the spinal neurones, may be implicated in the increased nociception.

The transmission of afferent impulses within the spinal cord is possibly mediated, in part, via peptidergic mechanisms (see Sluka *et al.*, 1992) and among the peptide candidates proposed is the undecapeptide, substance P (SP). This is thought to be released from the central end of sensory fibres as well as from the peripheral terminals where it has been implicated in the genesis of neurogenic inflammation. Increased biosynthesis of SP in the dorsal root ganglia of adjuvant arthritic rats is reflected in an increase in the mRNA levels of preprotachykinin A, the precursor of SP (Minami *et al.*, 1989) and the number of SP-immunoreactive fibres increases in the dorsal horn of the lumbar spinal cord (Kar *et al.*, 1991). In polyarthritic rats, spontaneous release of SP from the spinal dorsal horn exceeds that in control rats (Oku *et al.*, 1987). Movement of the ankle joint which is innocuous and does not affect the release of SP in non-inflamed control rats, is converted into a noxious stimulus producing a significant increase in SP release in polyarthritic rats (Oku *et al.*, 1987).

It has been reported that adjuvant arthritic rats show an increase in the number of γ-aminobutyric acid (GABA)-immunoreactive cells due to an enhanced concentration of intracellular GABA in lamina I–III of the dorsal horn (L3–L5) (Castro-Lopes *et al.*, 1992). This increase in the amount of GABA occurs within the zone of projection of afferent nerves emanating from the inflamed foot, supporting the possibility that GABA modulates the nociceptive input at the segmental level (Castro-Lopes *et al.*, 1992).

GABA activates two receptor types, $GABA_A$ and $GABA_B$ and the latter may be involved in nociception within the spinal cord (see Sawynok, 1987). The $GABA_B$ agonist, baclofen, exhibits antinociceptive activity which stems, at least in part from an activaton of receptors within the spinal cord (Wilson & Yaksh, 1978).

We have recently demonstrated that baclofen can inhibit the electrically-evoked release of SP from the rat spinal cord and that this effect is mimicked by GABA and prevented by $GABA_B$ antagonists (Malcangio & Bowery, 1993). The aim of the present study was to evaluate the potential role of the GABAergic system within the spinal cord in the physiology or pathology of chronic pain associated with monoarthritis. Electrically-evoked release of SP from isolated spinal cords of

[1] Author for correspondence.

monoarthritic and control rats was evaluated and the effect of GABAergic drugs examined. The pain thresholds were determined in the same rats.

Methods

The present study was carried out on male Lewis rats (Perlik & Zidek, 1973) weighing 180–200 g at the time experiments were started. They were housed four to a cage and given food and water *ad libitum*. All behavioural tests were conducted in the morning.

Induction of monoarthritis

Monoarthritis was induced by intradermal injections into the left hind paw of 500 μg heat-killed and dried *Mycobacterium tubercolusis* (H37 Ra, Difco laboratories, U.K.) in 0.1 ml mineral oil (complete Freund's adjuvant) under halothane anaesthesia. Control animals were injected with 0.1 ml mineral oil (incomplete Freund's adjuvant) (Difco laboratories, U.K.). Five normal rats were injected with phosphate buffered saline (PBS) (into the left hind paw) and killed three weeks later to serve as naive controls. Arthritic rats were bedded on soft paper shavings. Rat body weight and pain threshold of both treated and non-treated paws were monitored for four weeks after inoculation, at two-day intervals.

Paw withdrawal test

Thermal hyperalgesia to radiant heat was assessed by using the paw-withdrawal test where the paw withdrawal latency is considered to be an index of the thermal nociceptive index (Hargreaves *et al.*, 1988). The choice of this test was based on its sensitivity for detecting hyperalgesia and we have used the Ugo Basile (Comerio, Italy) plantar test. It consists of a movable infrared (I.R.) generator placed below a glass pane upon which the operator places the rat. A perspex enclosure defines the space within which the animal is unrestrained. It is divided into three compartments so that up to three rats can be tested simultaneously. The radiant heat source is placed directly under the plantar surface of the rat hind paw. The paw-withdrawal latency to radiant heat stimulation is defined as the time from onset of radiant heat to withdrawal of the rat hind paw. The radiant heat source was adjusted to result in baseline latencies of 10–12 s. Three test trials (with an intertrial interval of 3 min) were made and scores from them were averaged to yield a mean withdrawal latency.

Rat spinal cord slice preparation and superfusion

Horizontal spinal cord slices were obtained from adult rats as previously described (Malcangio & Bowery, 1993; 1994). Briefly, hemisected dorsal lumbosacral slices with attached dorsal roots were cut with a Vibratome (Campden Instruments, London) and mounted in a three-compartment chamber. The tissue was positioned in the central compartment where it was continuously perfused with Krebs solution at 1 ml min^{-1} and the dorsal roots were placed across two pairs of bipolar electrodes and immersed in mineral oil in the lateral compartments. Leak-proof partition barriers of perspex and paraffin grease ensured electrical isolation. After 1 h wash, normal Krebs solution (composition in millimolar: NaCl 118, KCl 4, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11) was substituted with Krebs solution containing 0.1% bovine serum albumin, 100 μM captopril, 1 μM phosphoramidon, 20 μg ml^{-1} bacitracin and dithiothreitol (6 μM) to prevent metabolic breakdown of SP. Eight-minute fractions were collected in the following order: 3 fractions to measure basal outflow, one fraction to evaluate release following electrical stimulation at 20 V, 0.5 ms, 1 Hz either in the absence or presence of drugs and 3 fractions to evaluate recovery to basal levels. At the completion of each experiment, the slice was blotted, weighed and extracted. Samples (8 ml volume) were desalted by using 100 mg SEP-PAK C_{18} reverse phase silica gel cartridges (Millipore) as already described (Malcangio & Bowery, 1993; 1994). The eluates were dried by evaporation at 55°C under nitrogen and stored at −80°C until they could be assayed for SP-LI content by radioimmunoassay using the scintillation proximity assay bead technique (Amersham International, U.K.).

SP radioimmunoassay (RIA)

In each experiment, RIA standard curves were constructed for SP by using known amounts of synthetic peptides ranging between 1 and 100 fmol per tube. The dried samples were resuspended in 300 μl of 50 mM phosphate buffer (pH 7.4) containing bovine serum albumin and 10 mM ethylenediaminetetracetic acid (EDTA) and assayed in amounts of SP immunoreactivity sufficient to place them within the range of the standard curve. SP content was measured by using a polyclonal rabbit antiserum specific for the whole SP undecapeptide (Amersham International, U.K.). The incubation mixtures of each assay contained sample or standard (100 μl), tracer containing ~6000 c.p.m. of the isotope (100 μl), diluted antiserum (100 μl) and 50 μl of scintillation proximity assay protein A reagent. All samples were assayed in duplicate. The reaction mixture was vortexed and incubated at room temperature on a shaker for 48 h before being placed in the scintillation-counter.

Drugs

(−)-Baclofen (β-*p*-chlorophenyl-GABA) and CGP 36742 (3-aminopropyl-*n*-butyl-phosphinic acid) were a gift from Ciba-Geigy, Switzerland. Naloxone hydrochloride, bicuculline methiodide and SP were purchased from Sigma, U.K. Antiserum raised against SP and [^{125}]-Bolton-Hunter SP were obtained from Amersham, U.K. Drugs were dissolved in distilled water or saline immediately before use.

Statistical analysis

Student's *t* test was used throughout. *P* values <0.05 were considered to be statistically significant.

Results

Monoarthritis-induced hyperalgesia and loss of body weight in rats

In most rats, moderate inflammatory signs occurred 2–4 h after injection and rose quickly to reach the maximum score of 4 after 2–3 weeks. In every animal the inflammatory signs were restricted to the injected paw. The inflamed paw of Freund's adjuvant-treated (CFA) rats exhibited a lower withdrawal latency to thermal stimuli than the contralateral paw of the same animals and both paws of incomplete Freund's adjuvant-treated (IFA) rats (Figure 1). Inflamed paw hyperalgesia was maximal two days after injection, and declined gradually between 7 and 21 days with no evidence of any excitability of withdrawal reflexes after 28 days (Figure 1). It is worth noting that 21 days after injection, the contralateral paw of CFA rats also showed a significant hyperalgesia. Of course, when the degree of inflammation was so high to cause loss of use of the limb, paw withdrawal latency was not measured.

As expected, CFA rats lost body weight immediately after induction of inflammation and then gained less weight than control rats (IFA) (Figure 1 insert).

Electrically-evoked SP-LI release from the spinal cord of monoarthritic rats

After rats were tested for their threshold to thermal stimuli, their spinal cords were isolated to measured SP-LI release. Electrical stimulation (20 V, 0.5 ms, 1 Hz) of the dorsal roots for 8 min induced a significant release of substance P-like immunoreactivity (SP-LI), which was approximately twice the basal level, from rat spinal cord slices. This evoked release was Ca^{2+}-dependent and inhibited by tetrodotoxin (1 μM) as previously described (Malcangio & Bowery, 1993; 1994) (data not shown).

Electrical stimulation of the dorsal roots attached to control rat isolated spinal cord slices induced a significant increase (174 ± 18% of basal outflow which was 30.4 ± 3.1 fmol per fraction 8 ml^{-1}) in SP-LI release (Figure 2, open column).

One week after injection of CFA no differences in the amount of SP-LI released from the spinal cord of IFA and CFA rats were detected (Figure 2) whereas after two weeks the spinal cord from CFA-treated rats tended to release more SP-LI than IFA cords. Twenty-one days after injection, the spinal cord of CFA rats released significantly more peptide compared to IFA rats (Figure 2). By contrast, four weeks after injection, the release of SP-LI from the spinal cord of CFA rats was almost absent (Figure 2). Basal outflow of SP-LI, total content of SP-LI and SP-LI release induced by capsaicin were unaffected in CFA and IFA rats throughout the 28 days period (Table 1).

Effect of CGP 37642, naloxone and bicuculline on electrically-evoked SP-LI release from monoarthritic rat spinal cord slices

In view of the higher release of SP-LI from CFA spinal cords and the contralateral hyperalgesia which were both detectable 21 days after treatment, all subsequent experiments were performed at this time after injection.

Evoked release of SP-LI from the spinal cord of CFA rats was greatly increased by perfusion with the GABA$_B$ antagonist, CGP 36742 (10 and 100 μM) which did not alter the release from IFA spinal cord. The effect of this drug was dose-dependent, achieving statistical significance at 100 μM (Figure 3). The GABA$_A$ antagonist, bicuculline (100 μM),

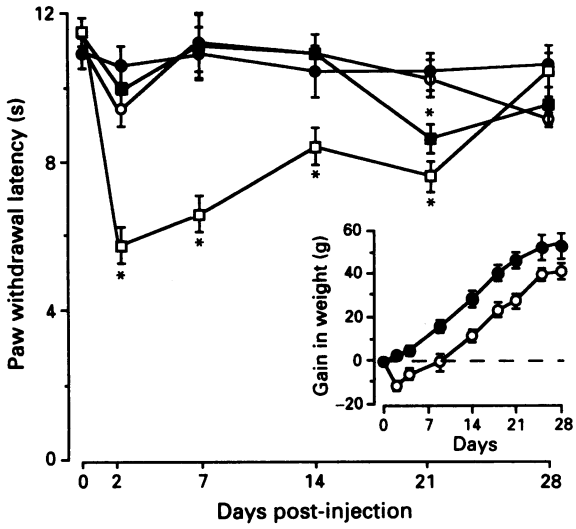

Figure 1 Freund's adjuvant-induced hyperalgesia in the rat paw detected by the paw withdrawal test, and cumulative weight gained by CFA rats and IFA rats (insert). Paw withdrawal latency to radiant heat was measured in the ipsilateral (open symbols) and contralateral (closed symbols) paws of IFA (circles) and CFA (squares) rats, respectively. Insert panel shows the cumulative weight gain in CFA (○) and IFA (●) rats from day 0 to day 28. Values are mean ± s.e.mean of 6–30 rats. *$P<0.05$ CFA ipsilateral vs. IFA ipsilateral (Student's t test).

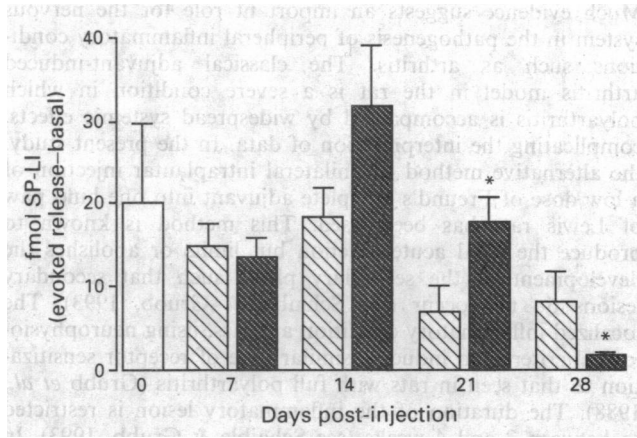

Figure 2 Effect of monoarthritis on substance P-like immunoreactivity (SP-LI) from Lewis rat spinal cord slices. Basal outflow of SP-LI from the spinal cord of saline-injected (open column) IFA (widely hatched column) and CFA (closely hatched column) rats was 36.7 ± 3.0 fmol 8 ml^{-1} per fraction, $n = 61$ (pooled means). SP-LI was released by electrical stimulation of the dorsal roots at 20 V, 0.5 ms, 1 Hz for 8 min. Values are mean ± s.e.mean. $n = 5$ spinal cords for open column, 9 spinal cords for IFA except at 28 days when they were 3; 8 spinal cords for CFA at 7 days; 12 spinal cords for CFA at 14 and 21 days; 3 spinal cords for CFA at 28 days. *$P<0.05$ CFA vs. IFA (Student's t test).

Table 1 Substance P-like immunoreactivity (SP-LI) basal outflow, SP-LI released by capsaicin and SP-LI total content from spinal cord slices of monoarthritic rats

Days after injection	*Injection*	n	*SP-LI basal outflow* (fmol 8 ml^{-1})	*SP-LI released by capsaicin* (1 μM) (fmol 8 ml^{-1})	*SP-LI total content* (fmol mg^{-1} tissue)
21	PBS	5	30.3 ± 3.1	110.8 ± 29.7	64.0 ± 11.0
7	IFA	6	34.7 ± 5.7	216.7 ± 82.5	103.0 ± 40.8
7	CFA	6	38.0 ± 7.3	214.0 ± 57.0	76.4 ± 5.4
14	IFA	9	42.2 ± 5.6	156.5 ± 40.8	84.6 ± 5.8
14	CFA	8	36.4 ± 4.4	146.6 ± 28.9	83.1 ± 13.8
21	IFA	9	38.2 ± 3.9	166.9 ± 72.0	56.4 ± 9.7
21	CFA	12	37.1 ± 3.5	156.7 ± 44.0	57.2 ± 3.8
28	IFA	3	30.9 ± 2.3	144.7 ± 53.1	78.4 ± 5.1
28	CFA	3	33.6 ± 0.3	100.3 ± 24.1	78.1 ± 9.0

Lewis rats were injected intradermally with 100 μl phosphate-buffered saline (PBS), complete or incomplete Freund's adjuvant (CFA or IFA) in the left hind paw. Their spinal cords were excised after the period indicated. SP-LI basal outflow was calculated as the mean of the first three 8 ml fraction collected before stimulation. Capsaicin (1 μM) was perfused over the spinal cord slices at the end of each experiment for 2 min out of 8 min collection time. Data are mean ± s.e.mean.

failed to enhance the peptide release from either the CFA or IFA rat spinal cord removed 21 days after injection (Figure 3). Naxolone (1 μM) increased the peptide release from both IFA and CFA spinal cord to the same extent (Figure 3) as well as from naive rat spinal cord (330 ± 157% of control evoked release, $n = 3$, $P < 0.05$).

Effect of CGP 37642 on monoarthritis-induced hyperalgesia

On the 21st day after injection, intraperitoneal injection of the $GABA_B$ antagonist, significantly decreased the withdrawal latency of both inflamed and contralateral paw of CFA rats up to 45 min after treatment (Figure 4a). CGP 36742 was not hyperalgesic in IFA rats (Figure 4a). The $GABA_B$ agonist, (−)-baclofen, could reverse the hyperalgesia in the inflamed paw but a high dose of 3 mg kg^{-1} was necessary to raise the paw withdrawal latency from 5.2 ± 0.8 s to 8.2 ± 0.5 s ($n = 6$, $P < 0.05$, Student's t test). Naloxone (3 mg kg^{-1}) did not modify monoarthritis-induced hyperalgesia (Figure 4b).

Discussion

Much evidence suggests an important role for the nervous system in the pathogenesis of peripheral inflammatory conditions such as arthritis. The classical adjuvant-induced arthritis model in the rat is a severe condition in which polyarthritis is accompanied by widespread systemic effects, complicating the interpretation of data. In the present study, the alternative method of unilateral intraplantar injection of a low dose of Freund's complete adjuvant into one hind paw of Lewis rats has been used. This method is known to produce the local acute reaction but limits or abolishes the development of the secondary phase such that secondary lesions do not occur (see Schaible & Grubb, 1993). The localized inflammatory condition appears, using neurophysiological criteria, to induce a similar type of receptor sensitization to that seen in rats with full polyarthritis (Grubb *et al.*, 1988). The duration of the inflammatory lesion is restricted to between 2 and 4 weeks (see Schaible & Grubb, 1993). In the present work, the injection of Freund's adjuvant in one paw led to a unilateral, localized inflammation which reached its peak between 2 and 3 weeks, as already reported (Stein *et al.*, 1988; Donaldson *et al.*, 1993).

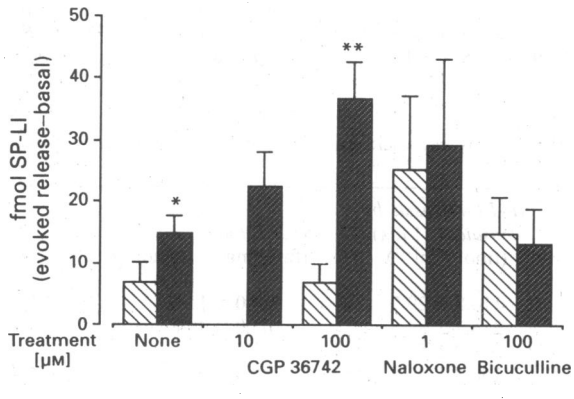

Figure 3 Effect of CGP 36742, naloxone and bicuculline on electrically-evoked substance P-like immunoreativity (SP-LI) release from the spinal cord of IFA and CFA rats. Basal outflow of SP-LI from IFA (widely hatched column) and CFA (closely hatched column) rat spinal cord slices was 6.5 ± 1.5 fmol 8 ml^{-1} per fraction, $n = 26$. Drugs were perfused 5 min prior to and during stimulation (20 V, 0.5 ms, 1 Hz for 8 min). Values are mean ± s.e.mean of 3–4 slices for each group. *$P < 0.01$ CFA in absence vs presence of drug (Student's t test).

Onset of monoarthritis-induced hyperalgesia in rats detected by the plantar test

Animals inoculated with Freund's adjuvant showed the decrease in threshold for nociceptive reflexes already observed in polyarthritic rats (Millan *et al.*, 1987) and rats bearing a carrageenin-induced hindlimb inflammation (Hargreaves *et al.*, 1988). Initial inflammation-induced elevation of paw threshold to heat, declined in the course of 4 weeks; thus it followed neither the time course nor the peak of severity of inflammation. This finding is consistent with data obtained in monoarthritic rats followed for only 1 week (Millan *et al.*, 1988). Interestingly, a significant hyperalgesia was also detected at the level of the contralateral paw, 3 weeks after treatment. This might suggest a spread of the inflammation even though no other major signs, like redness or swelling of the paw, were observed. It has been previously shown that the inflammatory process could, even within one week, significantly influence vascular responses and blood flow in the knee joint (Lam & Ferrell, 1993) involving alteration in sympathetic and neuropeptidergic actions (McDougall *et al.*, 1994). The inflammation induced by a low dose of Freund's adjuvant in the present study, would have very likely affected the blood flow in the inflamed paw and changed the skin temperature. However, in carrageenin-induced inflammation it has been demonstrated that the treated paw local erythema

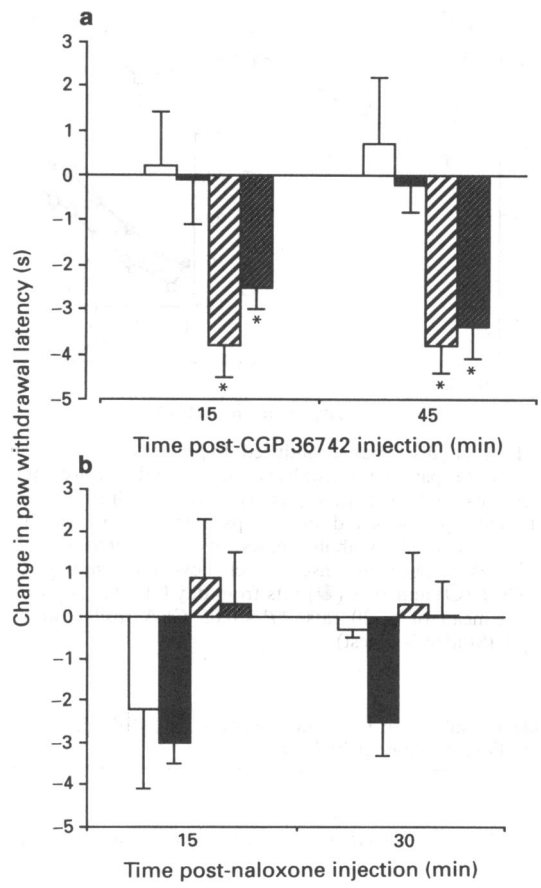

Figure 4 Effect of CGP 36742 (a) and naloxone (b) on monoarthritis-induced hyperalgesia. CGP 36742 (100 mg kg^{-1}, i.p.) and naloxone (3 mg kg^{-1}, i.p.) were injected to all rats immediately after determination of pre-injection paw withdrawal latency. Paw withdrawal latency was subsequently measured at 15 and 45 min after injection of CGP 36742 and at 15 and 30 min after injection of naloxone. Pre-injection values were subtracted from the 15, 30 and 45 min values and data are mean ± s.e.mean of 6 rats per group. Key to histograms: Contralateral paw IFA (open column); ipsilateral paw IFA (solid column); contralateral paw CFA (widely hatched column); ipsilateral paw CFA (closely hatched column). *$P < 0.05$ CFA vs. IFA (Student's t test).

and hypothermia, do not contribute to the decrease in paw withdrawal latency observed after carrageenin (Hargreaves *et al.*, 1988).

Monoarthritic rats displayed a diminished rate of body weight gain which is also normally observed in polyarthritic rats and could be due to factors released during stress like β-endorphin and corticotropin-releasing factor (Morley *et al.*, 1985) or simply to the fact that the motor activity which is required to obtain food causes discomfort and thus, the animals avoid such activities.

SP-LI release from the spinal cord of monoarthritic rats in vitro

Spinal cord slices from CFA rats released more SP-LI than those from IFA rats after electrical stimulation of the dorsal roots whereas the basal outflow of the peptide remained constant irrespective of treatment. However, the increase in SP-LI release was significant only 21 days after the treatment. By contrast, four weeks after injection, the release of SP-LI from the spinal cord of CFA rats was almost absent. This latter observation is in line with a previously reported decrease in the density of SP immunoreactive nerves in rat dorsal horn four weeks after induction of inflammation (Mapp *et al.*, 1993). The failure to detect SP-LI release earlier than 2–3 weeks after induction of monoarthritis, is in agreement with the delayed release of SP from the spinal cord of anaesthetized rats with an acute inflammation of the joint (Schaible *et al.*, 1990). This delay was suggested to be due to the period that fibres, which release the peptide, require to be sensitized by inflammatory mediators before they can be excited by joint stimuli (Schaible *et al.*, 1990).

On the basis of recent reports (Oku *et al.*, 1987; Schaible *et al.*, 1990; Kar *et al.*, 1991; Smith *et al.*, 1992), we were expecting a more pronounced release of SP-LI from the spinal cord of CFA rats. For example, it has been reported that in polyarthritic rats even spontaneous, basal release of SP from the dorsal horn exceeded that of control rats (Oku *et al.*, 1987). In our study, only one in 141 rats showed clear signs of polyarthritis, 21 days after injection. The spinal cord slice excised from this rat released the largest amount of SP-LI after electrical stimulation of the dorsal roots (759% of IFA evoked release) of any of the CFA rats, however, the basal outflow of the peptide was in the same range as controls. The lower increase in the amount of the peptide released by CFA cords might be attributed to the lesser severity of the monoarthritis model compared to polyarthritis. However, in view of the rapid reduction from the initial hyperalgesia developed in the chronically inflamed paw, we considered whether inhibitory influences develop rapidly within the spinal cord in response to chronic inflammation to reduce the release of the nociceptive peptide. The rapid rise in the threshold to heat does not appear to be opioid-mediated (Millan *et al.*, 1987; 1988).

Effect of GABAergic antagonists and naloxone on SP-LI release and monoarthritis-induced hyperalgesia at 21 days after treatment

The inhibitory neurotransmitter GABA is highly concentrated in interneurones of the superficial dorsal horn (Magoul *et al.*, 1987; Todd & McKenzie, 1989) which is also the main site of termination of the unmyelinated (C) fibres, containing SP (Hokfelt *et al.*, 1975), and of small myelinated (Aδ) fibres. Both of these systems convey nociceptive inputs arising in the periphery (see Perl, 1984). GABA levels increase 2–3 weeks after induction of monoarthritis and this has been attributed either to an enhancement of its synthesis or to a reduced release of the neurotransmitter (Castro-Lopes *et al.*, 1992). GABA can inhibit SP-LI release from rat spinal cord (Malcangio & Bowery, 1993), thus antagonists of both $GABA_A$ and $GABA_B$ receptors were tested in the paw withdrawal test and on SP-LI release from the spinal cord 21 days after injection. Evoked release of SP-LI from the spinal cord of CFA rats was greatly increased by perfusion with the $GABA_B$ antagonst, CGP 36742 (Olpe *et al.*, 1993). The range of CGP 36742 concentrations used in the present study has already been shown to antagonize the inhibition of electrically-evoked SP-LI release induced by either GABA or the $GABA_B$ agonist (−)-baclofen and not to modify the basal outflow of peptide (Malcangio & Bowery, 1993). Thus, the effect of CGP 36742 could be assumed to be due to blockade of $GABA_B$ receptors. The $GABA_B$ antagonist-induced release of SP-LI from monoarthritic rat spinal cord correlated with the potentiation of the paw hyperalgesia. CGP 36742 was not hyperalgesic in IFA rats, whereas it significantly decreased the withdrawal latency of both the inflamed and contralateral paw of CFA rats. The lack of any behavioural effect of the $GABA_B$ antagonist in control rats is not surprising (Olpe *et al.*, 1993) and augurs well for possible therapeutic use since adverse effects may be limited (Bowery, 1993). The $GABA_B$ agonist, (−)-baclofen, could reverse the hyperalgesia in the inflamed paw but a high dose of 3 mg kg^{-1} was necessary to raise the paw withdrawal latency. Chronically inflamed rats were less sensitive to the antinociceptive effect of baclofen than animals subjected to acute pain.

The $GABA_A$ antagonist, bicuculline, failed to enhance the peptide release from either the CFA or IFA rat spinal cord, thus supporting the concept that $GABA_A$ receptors are unlikely to mediate the GABA control of SP-LI release from primary afferent terminals either in normal rats (Malcangio & Bowery, 1993) or in rats with chronic inflammatory pain.

It has been reported that rats with unilateral chronic inflammation of the paw developed a supersentivity to morphine in the paw pressure withdrawal test (Kayser & Guilbaud, 1983; Millan *et al.*, 1988) even though naloxone is inactive in polyarthritic rats (Millan *et al.*, 1987) and unilaterally inflamed rats at 1 week (Millan *et al.*, 1988). As a consequence we tested the effect of naloxone on electrically-evoked SP-LI release. This drug was not specific in its action, increasing the peptide release from both IFA and CFA spinal cord to the same extent as well as from naive rat spinal cord. Furthermore, in contrast to the $GABA_B$ antagonists but in line with previous studies (Millan *et al.*, 1987; 1988), naloxone did not modify monoarthritis-induced hyperalgesia. These observations, support the concept of a role for opioids in the control of SP release from primary afferent fibres (Mudge *et al.*, 1979; Cesselin *et al.*, 1984; Go & Yaksh, 1987), and also support the idea that this system is not specifically activated when chronic pain develops (Przewlocka *et al.*, 1992; Millan *et al.*, 1987; 1988). On the contrary, it would seem that the GABAergic system within the spinal cord, during chronic pain, might be activated to counteract pain sensation. In our model it seems very likely that a tonic GABAergic control is activated in CFA rats since the selective block of $GABA_B$ receptors caused a significant increase in the release of SP from primary afferent fibres of monoarthritic rats coupled with an intense hyperalgesia. This could explain the low efficacy of baclofen as a therapeutic analgesic in chronic pain despite its effectiveness in acute pain models (Malcangio *et al.*, 1991) and in trigeminal neuralgia in man (Fromm & Terence, 1987). Thus activation of the GABAergic system which is strongly activated as a consequence of chronic pain would be expected to be little affected by the addition of a GABA agonist.

Conclusions

In conclusion, the induction of chronic inflammation appears to activate the GABAergic system within the spinal cord to counteract pain sensation. This increases presynaptic inhibition of the primary afferent input to the dorsal spinal cord via $GABA_B$ receptors reducing the sensation of painful stimuli. This endogenous pathological activation of $GABA_B$

receptors could explain the weak analgesic activity of baclofen in human chronic pain, and further information about this mechanism may provide a basis for a novel approach to alternative pain therapy.

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