ELSEVIER

European Journal of Pharmacology 299 (1996) 113–118

ejp

Effect of interleukin-1β on the release of substance P from rat isolated spinal cord

Marzia Malcangio [a,1], Norman G. Bowery [a,2], Roderick J. Flower [b], Mauro Perretti [b,*]

[a] *Department of Pharmacology, The School of Pharmacy, 29 / 39 Brunswick Square, London WC1N 1AX, UK*

[b] *Department of Biochemical Pharmacology, The William Harvey Research Institute, The Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ, UK*

Received 16 October 1995; revised 4 December 1995; accepted 8 December 1995

Abstract

Superfusion of rat spinal cord slices with rat interleukin-1β resulted in a significant enhancement of electrically evoked substance P-like immunoreactivity with a maximal effect (> 2-fold increase) at 0.1 ng/ml, whereas higher concentration (10–50 ng/ml) of the cytokine inhibited (≈ 50%) the release of the neuropeptide. Interleukin-1β (0.1 ng/ml) potentiation of substance P-like immunoreactivity release was abrogated by co-perfusion with interleukin-1 receptor antagonist (10–100 ng/ml) or with indomethacin (1 μM). Superfusion of spinal cord with interleukin-1β inhibited electrically evoked calcitonin gene-related peptide-like immunoreactivity release. Modulation of substance P-like immunoreactivity release from the spinal cord by interleukin-1β may represent a mechanism responsible for the hyperalgesic action of the cytokine characteristic of the inflammatory response.

Keywords: CGRP (calcitonin gene-related peptide); Indomethacin; Interleukin-1 receptor antagonist; Hyperalgesia

1. Introduction

Interleukin-1 is a polypeptide cytokine that possesses a wide variety of immunogenic and inflammatory activities such that it is thought to play an important role in the host body's response in several pathological conditions (Dinarello, 1991). Two isoforms of the protein have been identified, termed interleukin-1α and interleukin-1β, which are both pro-inflammatory and share virtually all biological activities (Dinarello, 1991). In more recent years another form of the protein has been discovered and termed interleukin-1 receptor antagonist (Eisenberg et al., 1990). Interleukin-1 receptor antagonist was found to be devoid of any agonistic effect but able to prevent interleukin-1α and interleukin-1β interaction with their receptors and the consequent biological effects (McIntyre et al., 1991; McMahan et al., 1991). In view of the high number of interleukin-1 spare receptors on target cells (Dinarello, 1991), it was soon clear that a high ratio of antagonist/agonist concentrations (≥ 10-fold in vitro and ≥ 100-fold in vivo) was required to effectively inhibit the action of the cytokine.

Besides the effect on the immune response, interleukin-1 is a pro-inflammatory cytokine actively involved in the initiation and consolidation of the inflammatory response. Interleukin-1 induces mechanical hyperalgesia after injection into the rat hindpaw (Ferreira et al., 1988) or knee-joint (Davis and Perkins, 1994), and after intraperitoneal or intracerebroventricular administration (Watkins et al., 1994), thus indicating that this protein is involved in the hyperalgesia which characterizes acute inflammation. Although interleukin-1-induced hyperalgesia has been confirmed by several groups (Follenfant et al., 1991; Oka et al., 1994) in some studies an analgesic effect has been observed following intracerebroventricular injection (Nakamura et al., 1988), and some groups reported no effect on pain threshold after intrathecal administration (Watkins et al., 1994). Nevertheless, intratechal co-administration of interleukin-1 and γ-interferon has been reported to produce a synergistic hyperalgesic effect (Meller et al., 1994).

* Corresponding author. Tel.: +44-171-982.6073; fax: +44-171-982.6076; e-mail: m.perretti@qmw.ac.uk.

[1] Present address: Department of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK.

[2] Present address: Department of Pharmacology, The Medical School, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

SSDI 0014-2999(95)00845-4

The transmission of noxious stimuli from the periphery to the spinal cord is mediated by primary afferent fibres which also contain the undecapeptide substance P. This neuropeptide can be released from primary afferent terminals following electrical stimulation of the dorsal roots attached to isolated spinal cord slices (Malcangio and Bowery, 1993). The evoked release of substance P-like immunoreactivity can be prevented by antinociceptive drugs such as baclofen (Malcangio and Bowery, 1993) and morphine (Malcangio and Bowery, unpublished data) and enhanced by substance P antagonists (Malcangio and Bowery, 1994).

Recent findings suggest that substance P and other tachykinins are involved in the acute inflammatory response caused by interleukin-1 administration into specific tissue site (Perretti et al., 1993) and, an increase in synovial substance P-like immunoreactivity content has been observed following intra-articular injection of interleukin-1 in the rabbit (O'Byrne et al., 1990). Co-culturing of bovine adrenal chromaffin cells (Eskay and Eiden, 1992) or rat myenteric plexus (Hurst et al., 1993) with interleukin-1 augments the expression and/or the synthesis of substance P. This latter action of the cytokine was attributed to an interaction with its receptor since interleukin-1 receptor antagonist was an effective antagonist (Hurst et al., 1993).

Blood-borne interleukin-1 can be actively transported into certain regions of the brain through a specific and saturable transport system (Banks et al., 1991) which has more recently been shown to occur also within the spinal cord (Banks et al., 1994). In the present study we have investigated whether superfusion of rat spinal cord slices with homologous interleukin-1β could influence the basal outflow or electrically evoked substance P-like immunoreactivity release, with the aim of relating the effects of interleukin-1 on pain threshold to an action on substance P-like immunoreactivity release within the spinal cord.

2. Materials and methods

2.1. Neuropeptide release from rat spinal cord slices

Horizontal spinal cord slices were obtained from adult male Wistar rats (~250 g) as previously described (Malcangio and Bowery, 1993, 1994). Briefly, hemisected dorsal lumbosacral slices with attached dorsal roots were cut 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 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 (in mM: 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 bacitracin and 6 μM dithiothreitol to prevent metabolic breakdown of substance P. Eight-minute fractions were collected in the following order: 2 fractions to measure basal outflow, 2 fractions in the presence or absence of drugs, one fraction to evaluate release following electrical stimulation at 20 V, 0.5 ms, using a frequence of 1 Hz (for substance P-like immunoreactivity release) or of 10 Hz (for calcitonin gene-related peptide-like immunoreactivity release) either in the absence or presence of drugs and 3 fractions to evaluate recovery to basal levels. Samples (8 ml volume) were desalted by using 100 mg SEP-PAK C_{18} reverse phase silica gel cartridges (Millipore) as already described for substance P (Malcangio and Bowery, 1993) and calcitonin-gene related peptide-like immunoreactivity (CGRP-like immunoreactivity) (Malcangio and Bowery, 1994). The eluates were dried by evaporation at 55°C under nitrogen and stored at −80°C until they could be assayed for substance P-like immunoreactivity or CGRP-like immunoreactivity content by radioimmunoassay using the scintillation proximity assay bead technique already described (Malcangio and Bowery, 1993, 1994). Rat interleukin-1β did not directly interfere with the radioimmunoassay determinations in the concentration range tested of 1–100 ng/ml.

2.2. Materials

Rat recombinant interleukin-1β (10^7 units/mg) and rat recombinant interleukin-1 receptor antagonist were generously supplied by Dr R.C. Newton (Du Pont-Merck, Wilmington, DE, USA). Both cytokines were stored at −80°C and fresh aliquots were used in each experiment. Indomethacin (Sigma Chem., Poole, UK) was dissolved in $NaHCO_3$ to prepare a stock solution of 1 mM. Dilutions were made in the superfusion buffer. Specific antisera for substance P-like immunoreactivity and CGRP-like immunoreactivity were obtained from Amersham and Peninsula laboratories, respectively.

2.3. Data and statistics

Data are reported as means ± S.E.M. of n separate determinations. Statistical analysis was performed using one-way analysis of variance followed by Bonferroni test for intergroup differences. A threshold P value < 0.05 was taken as significant.

3. Results

3.1. Effect of interleukin-1β on substance P-like immunoreactivity release from rat isolated spinal cord slices

Electrical stimulation of the dorsal roots induced a significant release (50% increase of basal outflow) of

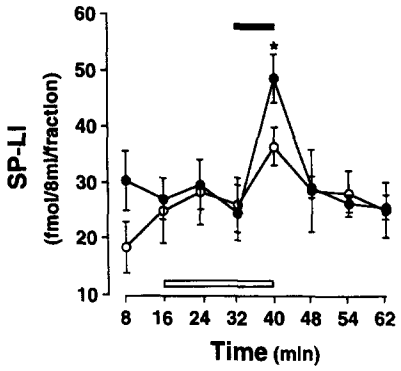

Fig. 1. Effect of superfusion with interleukin-1β on electrically evoked substance P-like immunoreactivity (SP-LI) release from rat isolated spinal cord. Interleukin-1β (0.1 ng/ml, $n = 6$; closed circles) or vehicle ($n = 4$; open circles) was present (white horizontal bar) 2 fractions prior to and during stimulation (black horizontal bar 20 V, 0.5 ms, 1 Hz for 8 min). Values are mean ± S.E.M. * $P < 0.05$.

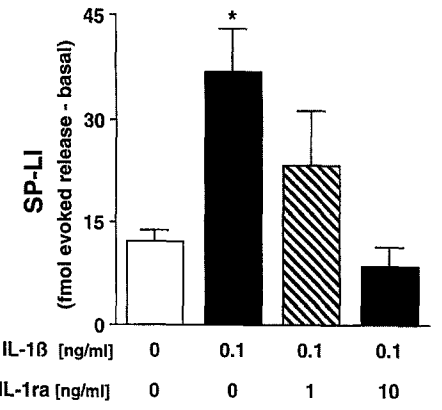

Fig. 3. Effect of interleukin-1 receptor antagonist on interleukin-1β-induced increase in electrically evoked substance P-like immunoreactivity (SP-LI) release from rat spinal cord slices. Interleukin-1 receptor antagonist (IL-1ra) and interleukin-1β (IL-1β) were concomitantly superfused 2 fractions prior to and during 8-min stimulation (20 V, 0.5 ms, 1 Hz). Values (fmol/8 ml/fraction) are means ± S.E.M. * $P < 0.05$, $n = 4$ in each group. The basal outflow was 10.6 ± 2.3 fmol/8 ml/fraction ($n = 18$).

substance P-like immunoreactivity from spinal cord slices (Fig. 1). Superfusion of slices with interleukin-1β prior to stimulation did not alter the basal outflow of substance P-like immunoreactivity (Fig. 1). However superfusion with interleukin-1β (0.1–50 ng/ml) induced a dual effect on electrically evoked release of the neuropeptide. Fig. 1 shows the cumulative data of the electrically evoked substance P-like immunoreactivity release in the presence or absence of 0.1 ng/ml interleukin-1β. Fig. 2 shows the concentration dependency of the effect of the cytokine, with an enhancement of substance P-like immunoreactivity release at 0.1–1 ng/ml (up to a 2-fold increase in substance P-like immunoreactivity) and an inhibition of the release at the higher concentrations of 10 and 50 ng/ml (80 ± 9 and 78 ± 8% inhibition, respectively).

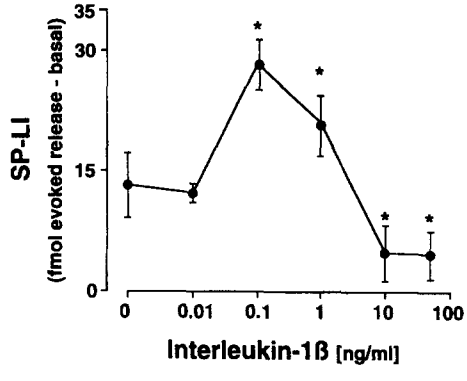

Fig. 2. Interleukin-1β concentration-response curve on electrically evoked substance P-like immunoreactivity (SP-LI) release from rat spinal cord slices. Interleukin-1β was present 2 fractions prior to and during stimulation (20 V, 0.5 ms, 1 Hz for 8 min). Values (fmol/8 ml/fraction) are means ± S.E.M. ($n = 4$–6) and were obtained subtracting basal outflow from release values both in the presence of the drug. The basal outflow in absence of drugs was 14.5 ± 1.5 fmol/8 ml/fraction ($n = 26$). * $P <$ 0.05.

3.2. *Antagonism of interleukin-1β-induced increase in electrically evoked substance P-like immunoreactivity release by interleukin-1-receptor antagonist or indomethacin.*

Superfusion of spinal cord slices with interleukin-1 receptor antagonist (1–10 ng/ml) did not modify basal and electrically stimulated substance P-like immunoreactivity release to any extent (data not shown). However, when interleukin-1 receptor antagonist was superfused together with interleukin-1β (0.1 ng/ml) the stimulatory action of the cytokine was significantly attenuated: from a 2.5-fold potentiation of substance P-like immunoreactivity release with interleukin-1β alone to a 1.7-fold increase in the presence of interleukin-1 receptor antagonist 1 ng/ml (10:1 ratio antagonist/agonist), which was no longer significant. In the presence of 10 ng/ml interleukin-1 receptor antagonist (100:1 ratio antagonist/agonist) the enhancement in neuropeptide release was abolished (Fig. 3).

Concomitant superfusion of interleukin-1β (0.1 ng/ml) with indomethacin (1 μM) reduced the effect of the cytokine (data expressed as percentage of basal outflow which was 13 ± 2 fmol/fraction): control 180 ± 26 ($n = 3$), interleukin-1β 263 ± 21 ($n = 3$; $P < 0.05$), interleukin-1β + indomethacin 170 ± 36 ($n = 4$, not significant). Similarly to interleukin-1 receptor antagonist, indomethacin alone did not exert any effect on substance P-like immunoreactivity basal outflow (10 ± 1 fmol/fraction in control slices, $n = 3$, and 12 ± 3 fmol/fraction in the slices superfused with 1 μM indomethacin, $n = 3$) or on electrically evoked release (215 ± 36% basal outflow, $n = 3$).

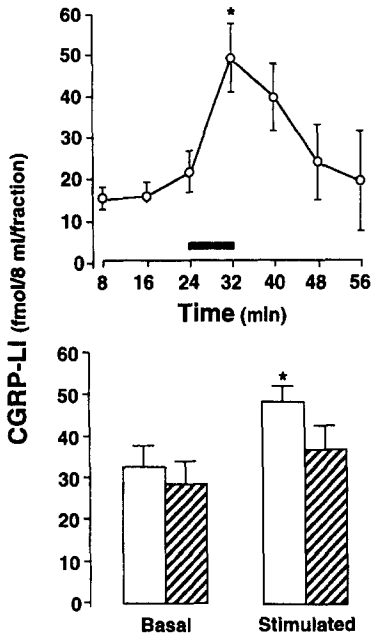

Fig. 4. Electrically evoked calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) release from rat spinal cord slices and effect of interleukin-1β. Panel A: CGRP-LI in 8-min fractions collected prior to and after electrical stimulation (20 V, 0.5 ms, 10 Hz; black horizontal bar); panel B: effect of vehicle ($n = 3$; white columns) or interleukin-1β (0.1 ng/ml, $n = 6$, hatched columns) on basal outflow (fraction 2) or 8-min electrically stimulated fraction. Values are means ± S.E.M. * $P < 0.05$.

3.3. Effect of interleukin-1β on CGRP-like immunoreactivity release from the spinal cord

Basal outflow of CGRP-like immunoreactivity was variable between different preparations, this possibly being a consequence of the partial (60%) recovery of peptide after loading samples through SEP-PAK cartridges (Malcangio and Bowery, 1995). Electrical stimulation of the dorsal roots evoked a significant release of CGRP-like immunoreactivity over basal outflow (Fig. 4A). Electrically evoked CGRP-like immunoreactivity release was no longer significantly higher than basal outflow when rat spinal cord slices were superfused with 0.1 ng/ml interleukin-1β (Fig. 4B).

4. Discussion

Superfusion of rat isolated spinal cord slices with low concentrations of interleukin-1β failed to modify the basal outflow of substance P-like immunoreactivity but strongly potentiated the release of the neuropeptide consequent to electrical stimulation of the dorsal roots. This is the first demonstration of interleukin-1-induced substance P-like immunoreactivity release after a short period of incubation. Previous studies in vivo have shown an increase of substance P-like immunoreactivity in the rabbit synovial fluid at 2 h post-interleukin-1 administration (O'Byrne et al., 1990) or have postulated a role for this neuropeptide in the chemotactic action of interleukin-1 after 4 h (Perretti et al., 1993). In vitro studies have reported an effect of the cytokine upon the synthesis of substance P by rat cells or tissues within 6–24 h (Eskay and Eiden, 1992; Hurst et al., 1993).

The release of substance P is one of the mechanisms responsible for the transmission of noxious stimuli from the periphery to higher centres (Levine et al., 1993) with particular reference to inflammatory pain (Konttinen et al., 1994). Peripheral production of interleukin-1 during acute and chronic inflammation is also important for the hyperalgesia characteristic of acute inflammation. Injection of interleukin-1 in the rat hind paw or articular space produces a hyperalgesic effect (Ferreira et al., 1988; Davis and Perkins, 1994). On the basis of our data we propose that these two mechanisms are related and that an enhanced release of substance P by interleukin-1β is at least one of the mechanisms responsible for the hyperalgesic property of the cytokine. The possibility that blood-borne interleukin-1 can be actively taken up by the spinal cord (Banks et al., 1994) supports this hypothesis. During inflammation interleukin-1 produced in the periphery could accumulate in the spinal cord to potentiate the release of substance P thus lowering the threshold to pain perception. However, it is also true that the extent of cytokine accumulation within the spinal cord is minimal (Banks et al., 1994; Watkins et al., 1995), and other possible mechanism(s) must be taken into account. For instance, local injection of interleukin-1 can activate peripheral nerves that, in turn, stimulate several phenomena within the central system, neurones and/or glia (Meller et al., 1994; Watkins et al., 1995).

The observed bell-shaped effect of interleukin-1 on substance P-like immunoreactivity release might also explain the analgesic effect detected in the mouse writhing test after intravenous administration of interleukin-1 (Nakamura et al., 1988). Thus, high doses of the cytokine, which may lead to high concentrations within the spinal cord, cause analgesia possibly as a consequence of an inhibition of the release of substance P, thus resulting in suppression of one of the mediators of the sensation of pain. However, the relevance of interleukin-1β effects observed at high doses/concentrations may be merely of pharmacological nature.

The specificity of interleukin-1 effect was confirmed by the experiments using its natural antagonist, interleukin-1 receptor antagonist. As expected, high ratios interleukin-1 receptor antagonist: interleukin-1β were required to suppress the potentiating effect of the cytokine on substance P-like immunoreactivity release, however the total inhibition achieved suggested that interleukin-1β was acting through interaction with its specific receptors. Since interleukin-1 receptor antagonist blocks interleukin-1 binding both to interleukin-1 receptor type I and type II (McMahan et al., 1991) our data do not allow the identification of the

receptor type involved in the potentiation of substance P-like immunoreactivity release by the cytokine.

Besides interleukin-1 receptor antagonist, the effect of interleukin-1β was inhibited by indomethacin. This suggests that endogenous prostanoids may be generated following interaction of the cytokine with its specific receptors, and this would potentiate electrically evoked substance P-like immunoreactivity release. Prostaglandins have been shown to increase the release of neuropeptides from rat spinal cord in vitro (Andreeva and Rang, 1993) and also to induce hyperalgesia in the rat after intrathecal injection (Taiwo and Levine, 1986). Short-time incubations of interleukin-1β with isolated organs, such as the stomach strip, induces the release of prostaglandins following activation of phospholipase A_2 (Mugridge et al., 1989). This mechanism may also occur under the present experimental conditions.

The effectiveness of indomethacin provides further support to the hypothesis of interleukin-1-induced hyperalgesia via an action upon substance P-like immunoreactivity release from the spinal cord, since treatment of animals with indomethacin prevents interleukin-1-induced hyperalgesia (Ferreira et al., 1988) and inhibition of spinal cyclooxygenase blocks substance P-mediated hyperalgesia (Malmberg and Yaksh, 1992).

In the experimental conditions used, interleukin-1β failed to potentiate electrically evoked CGRP-like immunoreactivity release. The explanation for the different effect of the cytokine upon substance P-like immunoreactivity and CGRP-like immunoreactivity is at the moment unclear and requires further investigations. Nevertheless, it points to a preferential, or selective, functional interaction between interleukin-1 and substance P.

This study demonstrates the ability of rat interleukin-1β to enhance the electrically evoked substance P-like immunoreactivity release from rat spinal cord slices. It is suggested that an effect on the release of this nociceptive neuropeptide can be responsible for the hyperalgesic action of peripherally injected interleukin-1.

Acknowledgements

Personal support to M.P. was by ONO Pharmaceutical Co. (Osaka, Japan) whereas R.J.F. is a Principal Research Fellow of the Wellcome Trust. M.M. and N.G.B. thank the Arthritis and Rheumatism Council for financial support (grant B 0196).

References

Andreeva, L. and H.P. Rang, 1993, Effect of bradykinin and prostaglandins on the release of calcitonin gene-related peptide-like immunoreactivity from the rat spinal cord in vitro, Br. J. Pharmacol. 108, 185.

Banks, W.A., L. Ortiz, S.R. Plotkin and A.J. Kastin, 1991, Human interleukin (IL) 1α, murine IL-1α and murine IL-1β are transported from blood to brain in the mouse by a shared saturable mechanism, J. Pharmacol. Exp. Ther. 259, 988.

Banks, W.A., A.J. Kastin and C.A. Ehrensing, 1994, Blood-borne interleukin-1α is transported across the endothelial blood-spinal cord barrier of mice, J. Physiol. (London) 479, 257.

Davis, A.J. and M.N. Perkins, 1994, The involvement of bradikinin B_1 and B_2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat, Br. J. Pharmacol. 113, 63.

Dinarello, C.A., 1991, Interleukin-1 and interleukin-1 antagonism, Blood 77, 1627.

Eisenberg, S.P., R.J. Evans, W.P. Arend, E. Verderber, M.T. Brewer, C.H. Hannum and R.C. Thompson, 1990, Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist, Nature 343, 341.

Eskay, R.L. and L.E. Eiden, 1992, Interleukin-1α and tumor necrosis factor-α differentially regulate enkephalin, vasoactive intestinal polypeptide, neurotensin, and substance P biosynthesis in chromaffin cells, Endocrinology 130, 2252.

Ferreira, S.H., B.B. Lorenzetti, A.F. Bristow and S. Poole, 1988, Interleukin-1β as a potent hyperalgesic agent antagonized by tripeptide analogue, Nature 334, 698.

Follenfant, R.L., M. Nakamura-Craig, B. Henderson and G.A. Higgs, 1991, Inhibition by neuropeptides of interleukin-1β-induced, prostaglandin-independent hyperalgesia, Br. J. Pharmacol. 98, 41.

Hurst, S.M., A.M. Stanisz, K.A. Sharkey and S.M. Collins, 1993, Interleukin-1β-induced increase in substance P in rat myenteric plexus, Gastroenterology 105, 1754.

Konttinen, Y.T., P. Kemppinen, M. Segerberg, M. Hukkanen, R.Rees, S. Santavirta, T. Sorsa, A. Pertovaara and J.M. Polak, 1994, Peripheral and spinal neural mechanisms in arthritis, with particular reference to treatment of inflammation and pain, Arthritis Rheum. 37, 965.

Levine, J.D., H.L. Fields and A.I. Basbaum, 1993, Peptides and primary afferent nociceptor, Neuroscience 13, 2273.

Malcangio, M. and N.G. Bowery, 1993, γ-Aminobutyric $acid_B$, but not γ-aminobutyric $acid_A$, receptor activation, inhibits electrically-evoked substance P-like immunoreactivity from rat spinal cord in vitro, J. Pharmacol. Exp. Ther. 266, 1490.

Malcangio, M. and N.G. Bowery, 1994, Effect of tachykinin NK_1 receptor antagonists, RP 67580 and SR 140333, on electrically-evoked substance P release from rat spinal cord, Br. J. Pharmacol. 113, 635.

Malcangio, M. and N.G. Bowery, 1995, Calcitonin gene-related peptide content, basal outflow and electrically-evoked release from monoarthritic rat spinal cord in vitro, Pain (in press).

Malmberg. A.B. and T.L. Yaksh, 1992, Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition, Science 257, 1276.

McIntyre, K.W., G.J. Stepan, K.D. Kolisnky, W.R. Benjamin, J.M. Plocinski, K.L. Kaffka, C.A. Campen, R.A. Chizzonite and P.L. Kilian, 1991, Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody, J. Exp. Med. 173, 931.

McMahan, C.J., J.L. Slack, B. Mosley, D. Cosman, S.D. Lupton, L.L. Brunton, C.E. Grubin, J.M. Wignall, N.A. Jenskins, C.I. Brannan, N.G. Copeland, K. Huebner, C.M. Croce, L.A. Cannizzaro, D. Benjamin, S.K. Dower, M.K. Spriggs and J.E. Sims, 1991, A novel IL-1 receptor, cloned from B cells by mammalin expression, is expressed in many cell types, EMBO J. 10, 2821.

Meller, S.T., C. Dykstra, D. Grzybycki, S. Murphy and G.F. Gebhart, 1994, The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat, Neuropharmacology 33, 1471.

Mugridge, K.G., D. Donati, S. Silvestri and L. Parente, 1989, Arachidonic acid lipoxygenation may be involved in interleukin-1 induction of prostaglandin biosynthesis, J. Pharmacol. Exp. Ther. 250, 714.

Nakamura, H., K. Nakanishi, A. Kita and T. Kadokawa, 1988, Interleukin-1 induces analgesia in mice by a central action, Eur. J. Pharmacol. 149, 49.

O'Byrne, E.M., V. Blancuzzi, D.E. Wilson, M. Wong and A.Y. Jeng, 1990, Elevated substance P and accelerated cartilage degradation in rabbit knees injected with interleukin-1 and tumor necrosis factor, Arthritis Rheum. 33, 1023.

Oka, T., S. Aou and T. Hori, 1994, Intracerebroventricular injection of interleukin-1β enhances nociceptive neuronal responses of the trigeminal nucleus caudalis in rats, Brain Res. 656, 236.

Perretti, M., A. Ahluwalia, R.J. Flower and S. Manzini, 1993, Endogenous tachykinins play a role in IL-1-induced neutrophil accumulation: involvement of NK-1 receptors, Immunology 80, 73.

Taiwo, Y.O. and J.D. Levine, 1986, Indomethacin blocks central nociceptive effects of $PGF_{2\alpha}$, Brain Res. 373, 81.

Watkins, L.R., E.P. Wiertelak, L.E. Goehler, K.P. Smith, D. Martin and S.F. Maier, 1994, Characterization of cytokine-induced hyperalgesia, Brain Res. 654, 15.

Watkins, L.R., S.F. Maier and L.E. Goehler, 1995, Cytokine-to-brain communication: a review and analysis of alternative mechanisms, Life Sci. 57, 1011.