J. Pharm. Pharmacol. 2000, 52: 431–436
Received July 19, 1999
Accepted December 6, 1999

© 2000 J. Pharm. Pharmacol.

The Role of Nitric Oxide and Prostaglandin E_2 on the Hyperalgesia Induced by Excitatory Amino Acids in Rats

YANG HAE PARK, CHANG YELL SHIN, TAI SANG LEE, IN HOI HUH AND UY DONG SOHN

Department of Pharmacology, School of Pharmacy, Chung Ang University, Seoul 156-756, Republic of Korea

Abstract

The present study was designed to investigate the role of nitric oxide (NO), *N*-methyl-D-aspartate (NMDA) receptor and prostaglandins on hyperalgesia induced in rats by excitatory amino acids and the possibility that prostaglandins may act as the retrograde messenger in the spinal cord like NO.

*N*ω-nitro-L-arginine methyl ester (L-NAME; 500 μg/paw, intraplantarly (i.pl.)), MK-801 (10 μg/paw, i.pl.) or indomethacin (300 μg/paw, i.pl.) reduced the duration of phase 2 of the biting/licking and scratching (B/L + S) response induced by formalin injection from 255·6 ± 16·7 s to 155·6 ± 16·9, 172·2 ± 33·3 or 205·6 ± 16·7 s, respectively. L-NAME (0·3 mg, i.th.), MK-801 (8 μg, i.th.) or indomethacin (20 μg, i.th) reduced the duration of phase 2 of the B/L + S response induced by saline injection from 288·5 ± 7·7 s to 207·7 ± 19·2, 184·6 ± 7·7 or 192·3 ± 38·5 s, respectively.

L-NAME or indomethacin injected into the spinal cord of the rat significantly reduced the hyperalgesia induced by NMDA (1 μg, i.th.) from 43·8 ± 4·6% to 12·3 ± 3·1 and 19·2 ± 2·3%, respectively. It is assumed that NO produced by excitatory amino acids may increase prostaglandin production by cyclooxygenase activation. L-NAME, MK-801 or indomethacin injected into the rat spinal cord significantly reduced the hyperalgesia induced by prostaglandin E_2 (PGE_2, 25 ng, i.th.) in the tail-flick test from 40·6 ± 3·5% to 18·2 ± 3·2, 18·8 ± 1·8 or 17·6 ± 4·1%, respectively, but had little effect on hyperalgesia in the paw pressure test (except for indomethacin).

In conclusion, NO and PGE_2 affect the hyperalgesia induced by excitatory amino acids. It is suggested that PGE_2, like NO, may act as a retrograde messenger in the spinal cord.

Hyperalgesia is an enhanced sensitivity to pain (Kitto et al 1992). Noxious stimuli of an intensity sufficient to cause tissue damage are associated with the release of numerous inflammatory mediators including 5-hydroxytryptamine, bradykinin, cytokines (interleukin, tumour necrosis factor-α), substance P and calcitonin gene-related peptide (Meller et al 1992; Roche et al 1996). These inflammatory mediators are transmitted to the spinal cord through the primary afferent nerve. C and A fibres are believed to participate in nociception (Urban et al 1994).

N-Methyl-D-aspartate (NMDA) receptors have been shown to be involved in hyperalgesia, since intrathecal administration of NMDA, but not quisqualate or kainate, produced a marked hyperalgesia in thermal nociceptive testing (Ren et al 1992). Coderre & Empel (1994) have shown that the non-competitive NMDA receptor antagonist MK-801 reduced nociceptive behaviour in the formalin test.

Nitric oxide (NO) has a role as a retrograde messenger in the spinal cord. NO may diffuse from its site of production to either the presynaptic neuron or adjacent neuron where it activates soluble guanylate cyclase or facilitates the release of excitatory amino acids. Hyperalgesia can be produced by NO (Barinaga 1991; Bohme et al 1991).

NO may be important in the regulation of the activity of cyclooxygenase (McCormack 1994). Cyclooxygenase is rate limiting in the synthesis of prostaglandins, thromboxane A_2 and prostacyclin from arachidonic acid (Shimizu & Wolfe 1990). Salvemini et al (1993) have evaluated the role of NO in the activity of constitutive and induced forms of cyclooxygenase. Their results suggest that NO may activate cyclooxygenase, which results in

Correspondence: U. D. Sohn, Department of Pharmacology, School of Pharmacy, Chung Ang University 221, Huksuk-dong, Dongjak-Ku, Seoul, 156-756, Republic of Korea.
E-Mail: udsohn@cau.ac.kr

the increase of prostaglandin synthesis (Cashman 1996).

In this study, we investigated the role of NO, the NMDA receptor and prostaglandins on hyperalgesia induced by excitatory amino acids and the possibility that prostaglandins may act as a retrograde messenger in the spinal cord, like NO.

Methods

Animals and drugs

Male Sprague–Dawley rats, 200–250 g, were used. All rats were housed in a room maintained at constant temperature and humidity ($21 \pm 2^{\circ}$C, 50%; MJ-800 ECS, Myung-jin, Seoul, Korea) on a 12-h light–dark cycle with food and water freely available. Indomethacin, methylene blue, Nω-nitro-L-arginine methyl ester (L-NAME), prostaglandin E_2 (PGE_2) and other reagents were purchased from Sigma Chemical Co. (St Louis, MO). MK-801 was purchased from Tocris Cookson Ltd (Bristol, UK) and atropine sulphate crystals and pentobarbital sodium were purchased from Merck (Rahway, NJ).

Surgical preparation

Administration at the spinal level in the rat is usually performed via a catheter introduced along the spinal cord according to the technique described by Yaksh & Rudy (1976).

Hylden & Wilcox (1981) have introduced a method for a direct intrathecal injection in the mouse. In this study, the method improved by Mestre et al (1994) was used. Anaesthesia was induced in rats by intraperitoneal injection of sodium pentobarbital ($40\,mg\,kg^{-1}$). To reduce the volume of salivary
and bronchial secretions that might block the airways, atropine sulphate ($1\,mg\,kg^{-1}$) was injected intraperitoneally 15 min before pentobarbital.

A 26·5-G needle connected to a polyethylene tube (i.d., 0·58 mm; o.d., 0·96 mm; Natume, Tokyo, Japan) was inserted into the tissues between the dorsal aspects of L5 and L6, perpendicular to the vertebral column and fixed with the dental cement. The L5–L6 site was selected so that the injection was restricted to the region where the spinal cord ends and the cauda equina begins to reduce the possibility of spinal damage and increase the intervertebral accessibility. When the needle entered the subarachnoidal space, a sudden lateral movement of the tail was observed. The reflex was used as an indicator of successful puncture. No other specific behaviour or sign of distress or pain was observed at this time. After the operation, the rats recovered from the anaesthesia and were kept in individual cages. After 24 h, the experiment was performed.

After the experiment, rats were injected intrathecally with 10 μL of 1% methylene blue and were killed to verify the site of injection. If the site of injection was wrong, the data was excluded from the results.

Drugs and administration

All drugs were dissolved in 0·9% saline shortly before administration, except for PGE_2 and indomethacin, which were dissolved in 10% ethanol and then diluted with 0·9% saline.

To investigate the central mechanism, the drug was injected by microsyringe (Hamilton Co., Reno, NV) over 20–30 s. A drug-containing solution (5 μL) was always followed by 0·9% saline (10 μL) to ensure that the drug was cleared from the catheter.

Formalin test

Formalin solution 2·5% (50 μL) was injected intraplantarly (i.pl.) into the dorsal surface of the right hind-paw. The rat was then individually placed in a cage and observed at 3-min intervals for 60 min. Two phases of spontaneous behaviour were observed. Phase 1 started immediately after formalin injection and lasted through the second or third interval (0–10 min) followed by phase 2 with maximum response observed about 20–30 min after the formalin injection. Pain behaviour was quantified by the duration of spontaneous biting/licking and scratching (B/L + S).

Tail-flick test

A rat was gently restrained by hand and radiant heat was directed onto the tail (7350, UGO Basile, Comerio, Italy). The time between stimulus presentation and the rapid removal of the tail was assigned as the response latency. The cut-off time was 10 s.

$$\text{Tail-flick latency (\%)} = \left[\frac{(\text{maximal latency} - \text{baseline latency})}{\text{baseline latency}}\right] \times 100 \quad (1)$$

Paw pressure (Randall-Selitto) test

A rat was gently restrained by hand and constant pressure ($66\,g\,s^{-1}$, 7200, UGO Basile, Comerio, Italy) was applied onto the dorsal surface of the hind-paw.

$$\text{Paw-withdrawal latency (\%)} = \left[\frac{(\text{maximal latency} - \text{baseline latency})}{\text{baseline latency}}\right] \times 100 \quad (2)$$

Statistical analysis

All values are expressed as means ± s.e.m. Statistical differences between groups were established using Student's *t*-test or analysis of variance. $P < 0{\cdot}05$ was considered significant.

Results

Effect of NO and prostaglandin E_2 on hyperalgesia induced by formalin

General behaviour. Formalin 2·5% injected i.pl. into the dorsal surface of the right hind-paw resulted in a B/L + S response with two distinct phases (Figure 1A). An immediate increase (during the first 6 min) occurred in B/L + S response, which was followed by a drop at 6–12 min. Phase 2 was characterized by a gradual increase in

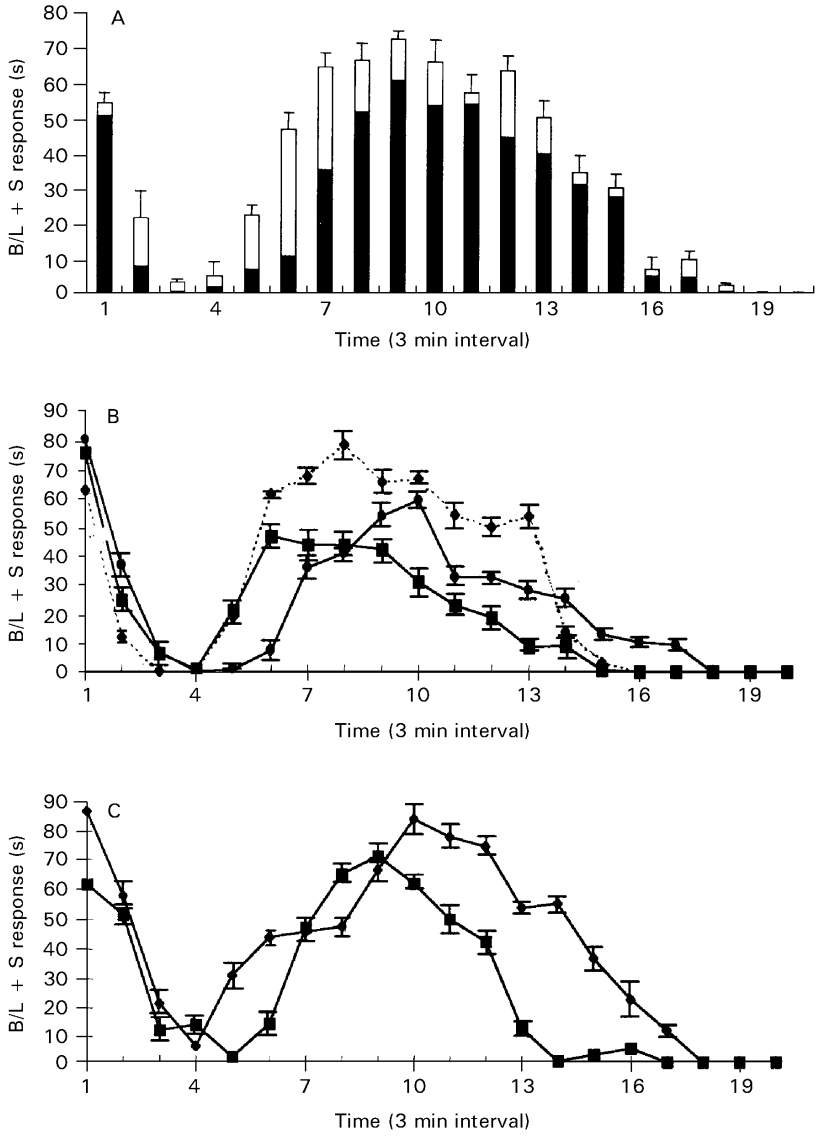

Figure 1. Time-dependent responses of mean biting/licking and scratching (B/L + S response) in rats. A. After 2·5% formalin (50 μL/paw) injection, an immediate increase occurred in B/L + S response (□: scratching response; ■: biting/licking response) which was followed by a drop from 6 to 12 min. A gradual increase in B/L + S response was observed from 15 to 30 min. Each column represents a 3 min interval. B. L-NAME (■; 500 μg/paw) and MK-801 (●; 10 μg/paw) were injected 30 min before 2·5% formalin injection (50 μL/paw). They had little effect on phase 1 of the B/L + S response (◆, saline), however, they significantly suppressed phase 2. C. Indomethacin (■; 300 μg/paw) was injected 30 min before 2·5% formalin injection (50 μL/paw) (◆, ethanol). Indomethacin significantly suppressed phase 2 but had no effect on phase 1 of the B/L + S response. All values are mean ± s.e.m., n = 6.

B/L + S response (15–30 min) that was followed by a decrease over the next 30 min. Phase 1 reflects acute, transient chemical stimulus and phase 2 involves peripheral inflammation and central sensitization (Goettl & Larson 1996).

Peripheral effect of L-NAME, MK-801 and indomethacin on B/L + S response. L-NAME (500 μg/paw), MK-801 (10 μg/paw) and indomethacin (300 μg/paw) were injected i.pl. into the right hindpaw 30 min before formalin injection. L-NAME, MK-801 and indomethacin had little effect on phase 1 of the B/L + S response but produced a significant suppression of phase 2 (Figures 1B and 1C; Table 1).

Central effect of L-NAME, MK-801 and indomethacin on B/L + S response. To examine the effects of NO- and prostaglandin-induced hyperalgesia in the spinal cord, L-NAME (0·3 mg), MK-801 (8 μg) and indomethacin (20 μg) were injected intrathecally. L-NAME, MK-801 and indomethacin significantly suppressed phase 2 of the B/L + S response (Table 1). The results were consistent with those of Malmberg & Yaksh (1992a), and suggested that NO and prostaglandins mediated hyperalgesia through the release of excitatory amino acids.

Effects of L-NAME and indomethacin on NMDA-induced hyperalgesia

To clarify the involvement of the NO or prostaglandin systems in hyperalgesia induced by NMDA (NMDA receptor agonist), the effects of L-NAME and indomethacin on hyperalgesia were examined. L-NAME (0·3 mg, i.th.) and indomethacin (20 μg, i.th.) were injected 10 min before NMDA (1 μg, i.th.). The effects of these agents on hyperalgesia were evaluated by tail-flick test. The maximal effect of NMDA on tail-flick latency was observed at 3 min after administration (Table 2). L-NAME and indomethacin reduced the hyperalgesia induced by NMDA (Table 3). These results demonstrate that NMDA-induced hyperalgesia may be produced by interaction with prostaglandin and the NO system.

Effects of L-NAME, MK-801, and indomethacin on prostaglandin E_2-induced hyperalgesia

Before assessment of the involvement of the NO system on PGE_2-induced hyperalgesia, PGE_2-induced hyperalgesia was examined. Using microdialysis, Malmberg & Yaksh (1992b) reported that the PGE_2 released in the spinal cord by formalin injection was changed. Therefore, PGE_2 (25 ng) was intrathecally injected, and resulted in hyperalgesia as observed in NMDA-induced hyperalgesia. The maximal effect of PGE_2 on tail-flick latency was observed at 30 min after administration (Table 2).

L-NAME (0·3 mg, i.th.), MK-801 (8 μg, i.th.) or indomethacin (20 ng, i.th.) were injected, together with PGE_2 (25 ng, i.th.) simultaneously. All three significantly suppressed hyperalgesia induced by PGE_2 in the tail-flick test (thermal stimuli), but had little effect in the paw pressure test (mechanical stimuli) (Table 3). From these results, the effect of PGE_2 on hyperalgesia induced by mechanical stimulus was not deduced.

These results suggest that PGE_2 administered intrathecally produces hyperalgesia by an action on the primary sensory neuron. The fact that L-NAME and MK-801 inhibited hyperalgesia induced by

Table 1. Effects of L-NAME, MK-801 and indomethacin on biting, licking and scratching (B/L + S) responses in rats.

	Duration of Phase 2 of B/L + S response (s)
Intraplantar	
Control	255·6 ± 16·7
L-NAME (500 μg/paw)	155·6 ± 16·9**
MK-801 (10 μg/paw)	172·2 ± 33·3**
Indomethacin (300 μg/paw)	205·6 ± 16·7**
Intrathecal	
Control	288·5 ± 7·7
L-NAME (0·3 mg)	207·7 ± 19·2*
MK-801 (8 μg)	184·6 ± 7·7**
Indomethacin (20 μg)	192·3 ± 38·5*

L-NAME, MK-801 and indomethacin reduced phase 2 (20–30 min) of the B/L + S response. Values are expressed as means ± s.e.m., n = 6. *$P < 0·05$, **$P < 0·01$ vs control (analysis of variance).

Table 2. Time-dependent effects of NMDA (1 μg, i.th.) and PGE_2 (25 ng, i.th.) on Δ tail-flick latency (%) in rats.

Time (min)	NMDA (1 μg/2 μL, i.th.)
0	32·3 ± 2·6
3	46·6 ± 4·9
6	42·5 ± 5·5
9	32·6 ± 4·9
12	22·1 ± 3·2
30	10·5 ± 2·8
	PGE_2 (25 ng, i.th.)
0	6·9 ± 3·3
30	38·8 ± 3·4
60	27·8 ± 3·7
90	31·1 ± 3·8
120	26·0 ± 3·7

The maximal effect of PGE_2 on tail-flick latency was observed at 30 min after administration. Values are expressed as mean ± s.e.m., n = 6.

Table 3. Effects of L-NAME, MK-801 and indomethacin on PGE_2- or NMDA-induced hyperalgesia in rats.

	NMDA-induced hyperalgesia	PGE_2-induced hyperalgesia	
Drug	Δ Tail-flick latency (%)	Δ Tail-flick latency (%)	Δ Paw-pressure latency (%)
Control	43·8 ± 4·6	40·6 ± 3·5	37·3 ± 2·6
L-NAME	12·3 ± 3·1**	18·2 ± 3·2**	30·0 ± 8·9
Indomethacin	19·2 ± 2·3*	17·6 ± 4·1**	23·2 ± 7·4*
MK-801	–	18·8 ± 1·8**	26·3 ± 4·7

NMDA (1 μg, i.th.) was injected 10 min after L-NAME (0·3 mg, i.th.) and indomethacin (20 μg, i.th.). PGE_2 (25 ng) was injected simultaneously with L-NAME (0·3 mg, i.th.), MK-801 (8 μg, i.th.) or indomethacin (20 μg, i.th.). L-NAME, MK-801 or indomethacin significantly suppressed hyperalgesia induced by PGE_2 in the tail-flick test. Indomethacin significantly suppressed hyperalgesia but L-NAME and MK-801 had little effect in the paw-pressure test. Values are expressed as mean ± s.e.m., n = 6. *$P < 0·05$, **$P < 0·01$ vs control (analysis of variance).

PGE_2 suggests that NO system may be involved in PGE_2-induced hyperalgesia. These results are consistent with hyperalgesia induced by a single intrathecal injection of PGE_2 being the result of a continuous release of glutamate (an excitatory amino acid) in the spinal cord.

Discussion

It is known that noxious stimuli, transmitted to the spinal cord, may increase the excitability of nociceptive-specific neurons in the spinal dorsal horn (Urban et al 1994). Increased spike discharge and sustained depolarization has been described after peripheral stimulation in acute complete Freund's adjuvant-induced arthritis or skin inflammation and in chemically elicited inflammation (Abbott et al 1995). A-fibre activation produces a brief excitatory postsynaptic potential in the dorsal horn cells, with no cumulative response upon low-frequency stimulation.

In the same cell, however, C-fibre activation produces a long-lasting excitatory postsynaptic potential that, upon repetitive stimulations, gives rise to a cumulative depolarization and firing of an action potential. In response to the stimulus, excitatory amino acids are released in the spinal cord; a significant increase (50–100%) in spinal levels of excitatory amino acids has been reported (Malmberg & Yaksh 1995). The NMDA receptor is a voltage-dependent ion channel that, once activated by excitatory amino acids, allows Ca^{2+} to enter the neuron. Therefore, it has been proposed that high-frequency barrages of C-fibre discharges result in the release of excitatory amino acids, fast synaptic potentials produced by actions at non-NMDA excitatory amino-acid receptors (AMPA/KA) and slow synaptic potentials produced by continual depolarization and peptides (substance-P, calcitonin gene-related peptide) (Raigorodsky & Urca 1987; Mao et al 1995).

In our study, we have shown that MK801 significantly reduced the hyperalgesia induced by PGE_2 in the tail-flick test and this reduction by MK801 may be explained by it being a non-competitive NMDA receptor antagonist which inhibits inward Ca^{2+} flux through the calcium channel, thereby diminishing intracellular calcium concentration (del Pilar Fernandez et al 1998). As a result, the slow synaptic potentials and high-frequency prolonged discharges are able to overcome the voltage-dependent Mg^{2+} block on the NMDA receptor, and the expulsion of Mg^{2+} from the ion channel allows an influx of Ca^{2+} through the NMDA receptor ion channel.

L-NAME significantly reduced licking behaviour associated with injection of formalin into the left hind-paw of the rat. L-NAME or methylene blue (soluble guanylate cyclase inhibitor) blocked the thermal hyperalgesia in rats with chronic gut ligatures for a period of 2 and 4 h, respectively. In this study we show that L-NAME, a nonselective NO synthase inhibitor, significantly prevented the hyperalgesia, suggesting that NO plays a significant role in hyperalgesia in the spinal cord (Meller & Gebhart 1993).

Cyclooxygenase is the potential target for NO because it contains an iron-haem centre at their active site, and indeed, most of the effects mediated by NO are a consequence of its interaction with iron or iron-containing enzymes (Garthwaite & Boulton 1995). Recent studies have linked NMDA receptor activation with the subsequent production of NO. The increase in intracellular Ca^{2+} triggers

a cascade of events that include stimulation of phospholipases to produce the diacylglycerol of inositol-1,4,5-triphosphate, activation of protein kinase C and, importantly, activation of the constitutive form of NOS synthase. There is evidence to suggest that NO may be produced by dorsal root ganglion neurons maintained in culture (Bauer et al 1992). Morris et al (1992) have suggested that NO produced in neurons in dorsal root ganglion may act as a signal between neurons and satellite cells in sensory ganglia.

Minami et al (1994) reported that intrathecal administration of PGE_2 increased the release of excitatory amino acids in the spinal cord. This would suggest that prostaglandins may be involved in hyperalgesia mediated by excitatory amino acids (Malmberg & Yaksh 1992b).

In summary, NO and PGE_2 affects the hyperalgesia induced by excitatory amino acids, suggesting that PGE_2, like NO, may act as a retrograde messenger in the spinal cord.

Acknowledgements

Uy Dong Sohn was supported by the Brain Korea 21 Hacsim Project.

References

Abbott, F. V., Franklin, K. B. J., Westbrook, R. F. (1995) The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain 60: 91–102

Barinaga, M. (1991) Is nitric oxide the retrograde messenger. Science 254: 1296–1297

Bauer, M. B., Murphy, S., Gebhart, G. F. (1992) Capscicin stimulates cGMP via nitric oxide in dorsal root ganglion cells. Soc. Neurosci. Abstr. 18: 689

Bohme, G. A., Bon, C. H., Stutzmann, J. M., Doble, A., Blanchard, J. C. (1991) Possible involvement of nitric oxide in long-term potentiation. Eur. J. Pharmacol. 199: 379–381

Cashman, J. N. (1996) The mechanisms of action of NSAIDs in analgesia. Drugs 52 (Suppl. 5): 13–23

Coderre, T. J., Empel, I. V. (1994) The utility of excitatory amino acid (EAA) antagonists as analgesic agents. I. Comparison of the antinociceptive activity of various classes of EAA antagonists in mechanical, thermal and chemical nociceptive tests. Pain 59: 345–352

del Pilar Fernandez, M., Meizoso, M. J., Lodeiro, M. J., Belmonte, A. (1998) Effect of desmethyl tirilazad, dizocipine maleate and nimodipine on brain nitric oxide synthase acitivity and cyclic guanosine monophosphate during cerebral ischemia in rats. Pharmacology 57: 174–179

Garthwaite, J., Boulton, C. L. (1995) Nitric oxide signaling in the central nervous system. Annu. Rev. Physiol. 57: 683–706

Goettl, V. M., Larson, A. A. (1996) Nitric oxide mediates long-term hyperalgesic and antinociceptive effects of the N-terminus of substance P in the formalin assay in mice. Pain 67: 435–441

Hylden, J. L. K., Wilcox G. L. (1981) Intrathecal morphine in mice. A new technique. Eur. J. Pharmacol. 67: 313–316

Kitto, K. F., Haley, J. E., Wilcox, G. L. (1992) Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. Neurosci. Lett. 148: 1–5

Malmberg A. B., Yaksh, T. L. (1992a) Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. J. Pharmacol. Exp. Ther. 263: 136–146

Malmberg, A. B., Yaksh, T. L. (1992b) Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. Science 257: 1276–1279

Malmberg, A. B., Yaksh, T. L. (1995) The effect of morphine on formalin-evoked behavior and spinal release of excitatory amino acids and prostaglandin E2 using microdialysis in conscious rats. Br. J. Pharmacol. 114: 1069–1075

Mao, J., Price, D. D., Mayer, D. J. (1995) Mechanisms of hyperalgesia and morphine tolerance: a current view of their possible interactions. Pain 62: 259–274

McCormack, K. (1994) Non-steroidal anti-inflammatory drugs and spinal nociceptive processing. Pain 59: 9–43

Meller, S. T., Gebhart, G. F. (1993) Nitric oxide (NO) and nociceptive processing in the spinal cord. Pain 52: 127–136

Meller, S. T., Pechman, P. S., Gebhart, G. F., Maves, T. J. (1992) Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. Neuroscience 50: 7–10

Mestre, C. M., Pelissier, T., Fialip, J., Wilcox, G., Eschalier, A. (1994) A method to perform direct transcutaneous intrathecal injection in rats. J. Pharmacol. Toxicol. Meth. 32: 197–200

Minami, T., Uda, R., Horiguch, S., Ito, S., Hyodo, M., Hayishi, O. (1994) Allodynia evoked by intrathecal administraion of prostaglandin E2 to conscious mice. Pain 57: 217–223

Morris, R., Southam, E., Braid, D. J., Garthwaite, J. (1992) Nitric oxide may act as a messenger between dorsal root ganglion neurons and their satellite cells. Neurosci. Lett. 137: 29–32

Raigorodsky, G., Urca, G. (1987) Intrathecal N-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems. Brain. Res. 422: 158–162

Ren, K. Hylden, J. L. K., Williams, G. M., Ruda, M. A., Dubner, R. (1992) The effect of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal acitivity in rats with unilateral inflammation. Pain 50: 331–334

Roche, A. K., Cook, G. L., Wilcox, G. L., Kajander, K. C. (1996) A nitric oxide synthesis inhibitor (L-NAME) reduces licking behavior and Fos-labeling in the spinal cord of rats during formalin-induced inflammation. Pain 66: 331–341

Salvemini, D., Misko, T. P., Masferrer, J. L., Seibert, K., Currie, M. G., Needleman, P. (1993) Nitric oxide activates cyclooxygenase enzyme. Proc. Natl Acad. Sci. USA 90: 7240–7244

Shimizu, T., Wolfe, L. S. (1990) Arachidonic acid cascade and signal transduction. J. Neurochem. 55: 1–15

Urban, L., Thompso, S. W. N., Dray, A. (1994) Modulation of spinal excitability: co-operation between neurokinin and excitatory amino acid neurotransmitters. Trends Neurosci. 17: 432–438

Yaksh, T. L., Rudy, T. A. (1976) Chronic catheterization of the spinal subarachnoid space. Physiol. Behavior 17: 1031–1036