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Pharmacological modulation of secondary mediator systems – cyclic AMP and cyclic GMP – on inflammatory hyperalgesia

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1 The objective of the present paper was to evaluate the relevance of neuronal balance of cyclic AMP and cyclic GMP concentration for functional regulation of nociceptor sensitivity during inflammation.

2 Injection of PGE_2 (10–100 ng paw^{-1}) evoked a dose-dependent hyperalgesic effect which was mediated *via* a cyclic AMP-activated protein kinase (PKA) inasmuch as hyperalgesia was blocked by the PKA inhibitor H89.

3 The PDE4 inhibitor rolipram and RP73401, but not PDE3 and PDE5 inhibitors potentiated the hyperalgesic effects of PGE_2. The hyperalgesic effect of dopamine was also enhanced by rolipram. Moreover, rolipram significantly potentiated hyperalgesia induced by carrageenan, bradykinin, TNFα, IL-1β, IL-6 and IL-8. This suggests that neuronal cyclic AMP mediates the prostanoid and sympathetic components of mechanical hyperalgesia. Moreover, in the neuron cyclic AMP is mainly metabolized by PDE4.

4 To examine the role of the NO/cyclic GMP pathway in modulating mechanical hyperalgesia, we tested the effects of the soluble guanylate cyclase inhibitor, ODQ. This substance counteracts the inhibitory effects of the NO donor, SNAP, on the hyperalgesia induced by PGE_2.

5 The ODQ potentiated hyperalgesia induced by carrageenan, bradykinin, TNFα, IL-1β, IL-6 and IL-8. In contrast, ODQ had no significant effect on the hyperalgesia induced by PGE_2 and dopamine. This indicates that the hyperalgesic cytokines may activate soluble guanylate cyclase, which down-regulate the ability of these substances to cause hyperalgesia. This event appears not to be mediated by prostaglandin or dopamine.

6 In conclusion, the results presented in this paper confirm an association between (i) hyperalgesia and elevated levels of cyclic AMP as well as (ii) antinociception and elevated levels of cyclic GMP. The intracellular levels of cyclic AMP that enhance hyperalgesia are controlled by the PDE4 isoform and appear to result in activation of protein kinase A whereas the intracellular levels of cyclic GMP results from activation of a soluble guanylate cyclase.

Keywords: Inflammatory hyperalgesia; cyclic AMP; cyclic GMP; PDEs; rolipram; ODQ; protein kinase A; SNAP; cytokines; dopamine; prostaglandin E_2

Abbreviations: cyclic AMP, adenosine 3′,5′-cyclic monophosphate; cyclic GMP, guanosine 3′,5′-cyclic monophosphate; H89, N-2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulphonamide; IL-1β, Interleukin 1 beta; NO, nitric oxide; ODQ, (1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one); PDEs phosphodiesterases; PGE_2, prostaglandin E_2; PKA, protein kinase A; PKC, protein kinase C; SNAP, S-nitroso-N-acetyl-D,L-penicillamine; TNF-α, tumour necrosis factor alpha

Introduction

Sensitization of the pain receptor is the common denominator of all types of 'inflammatory pain'. Following such sensitization, previously ineffective stimuli cause 'overt pain' in humans, or a characteristic behaviour in laboratory animals that may be used as an end point in nociceptive tests. C-polymodal, high-threshold receptors or receptors connected by fine myelinated fibres have long been associated with inflammatory hyperalgesia (Handwerker, 1976; Perl, 1976). More recently, a 'sleeping' pain receptor (nociceptor) associated with a small afferent fibre has been described in deep visceral enervation (colon and bladder) and in joints (McMahon & Koltzenburg, 1990; Messlinger, 1997). Sleeping nociceptors cannot be activated in normal (healthy) tissues but are 'switched on' during inflammation. Distinct clinical symptoms such as hyperalgesia and allodynia may be due to a functional up-regulation of pain receptors.

Hyperalgesic agents that satisfy clinical and experimental criteria for directly acting nociceptor sensitizers are the products of arachidonic acid/cyclo-oxygenase, e.g., prostaglandins (PGE_2, PGI_2), and also the sympathomimetic amines. The capacity of the prostaglandins to sensitize primary sensory neurons has been studied extensively in man and in experimental animals using both behavioural and electrophysiological techniques (Ferreira, 1990; Moncada *et al*., 1975; Ferreira *et al*., 1978). Sympathomimetic amines (noradrenaline and dopamine) have also been shown to functionally up-regulate nociceptors in man and in animals (Nakamura & Ferreira, 1987; Duarte *et al*., 1988; Coderre *et al*., 1984; Wall & Gutnick, 1974). In some experimental models of inflammatory pain both prostaglandins and sympathomimetic amines may be involved and their relative

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contribution may well depend on the characteristics of the pathological stimulus.

It is now becoming apparent that the release of these final hyperalgesic mediators are events secondary to the release of a cascade of cytokines (Ferreira *et al*., 1988; Cunha *et al*., 1991). For example, we have previously demonstrated that carrageenan or LPS induced tissue formation of bradykinin, which stimulated the release of TNFα. The TNFα produced subsequently induced the release of IL-1β and IL-6, which stimulated the production of cyclo-oxygenase products, and IL-8, which stimulated production of sympathomimetic mediators (Cunha *et al*., 1992). Similarly, using the tail-flick method, it was recently demonstrated that the ability of LPS to induce hyperalgesia also appeared to be mediated by TNFα and IL-1β (Watkins *et al*., 1994, 1995).

The molecular events associated with hyperalgesia triggered by the final hyperalgesic mediators (i.e., prostanoids and sympathomimetic amines) are not yet fully understood. We have provided experimental evidence to suggest that up or down functional regulation of the primary sensory neuron sensitivity to mechanical stimulation may result from a neuronal balance of Ca^{2+}/cyclic AMP and cyclic GMP concentrations, respectively (Ferreira & Nakamura, 1979; Taiwo *et al*., 1989; Duarte *et al*., 1990; Kress *et al*., 1996). The pivotal role of cyclic AMP in the sensitization of the primary sensory neuron is particularly convincing when mechanical stimulation was used as the hyperalgesic stimulus (Ferreira & Nakamura, 1979; Taiwo *et al*., 1989; Taiwo & Levine, 1991). This idea is in line with the general observation that prostanoid receptors and adrenoceptors are coupled with adenylate cyclase (Namba *et al*., 1994; Coleman *et al*., 1994; Aantaa *et al*., 1995; Hingtgen *et al*., 1995; Smith *et al*., 1998), and supported by the fact that the intraplantar administration of stable cyclic AMP analogues or the adenylate cyclase activator, forskolin, or inhibitors of the phosphodiesterases (PDEs) enhanced the mechanical hyperalgesia induced by PGE_2 (Ferreira & Nakamura, 1979; Taiwo *et al*., 1989; Taiwo & Levine, 1991; Ouseph *et al*., 1995). During hyperalgesia to mechanical stimulation, adenylate cyclase activation seems to occur also in the spinal cord (Sluka, 1997). In contrast, at this site, nociception evoked by thermal stimulation apparently involves cyclic GMP rather than cyclic AMP (Meller *et al*., 1992; Garry *et al*., 1994; Inoue *et al*., 1998).

The levels of cytosolic cyclic AMP are controlled by the rate of cyclic AMP production by receptor-coupled adenylate cyclase and by the rate of cyclic AMP degradation by 3′,5′-cyclic nucleotide phosphodiesterases (PDEs, Teixeira *et al*., 1997). Specific and non-specific inhibitors of PDEs have been used to support the participation of cyclic AMP in mechanical hyperalgesia (Ferreira & Nakamura, 1979; Taiwo & Levine, 1991; Ouseph *et al*., 1995). The PDE4 isoenzyme seems to be the most relevant enzyme in cyclic AMP inactivation in cells involved in the inflammatory process (Teixeira *et al*., 1997). In order to provide further understanding of the role of PDEs in mechanical inflammatory hyperalgesia we tested the possibility that specific inhibitors of PDE4, (rolipram and RP-73401), PDE3 (Org-9935) and PDE5 (zaprinast) potentiated hyperalgesia induced by carrageenan, bradykinin, inflammatory cytokines and the final hyperalgesic mediators, PGE_2 and dopamine.

In addition to a role for cyclic AMP in modulating hyperalgesia, direct blockade of ongoing mechanical hyperalgesia has been observed after local administration of dibutyryl cyclic GMP or substances that stimulate neuronal soluble guanylate cyclase (acetylcholine or the NO donors, SNAP and sodium nitroprusside) (Wang, 1996; Duarte *et al*., 1990; Ferreira *et al*., 1991, 1992). In order to provide further support for the suggestion that inflammatory hyperalgesia depends upon the balance between neuronal cyclic AMP and cyclic GMP levels, we investigated the effect of ODQ, a specific inhibitor of soluble guanylate cyclase (Moro *et al*., 1996), on the antinociceptive effect of SNAP, a NO donor. Subsequently ODQ was used to investigate the role of guanylate cyclase activation in the inflammatory hyperalgesia triggered by the same range of stimuli described above.

Methods

Nociceptive test

A constant pressure of 20 mmHg (measured using a sphygmomanometer), was applied (*via* a syringe piston moved by compressed air) to an area of 15 mm^2 of the dorsal surface of the hind paws of rats, and discontinued when they presented a typical 'freezing reaction'. The freezing reaction was signalled by a brief apnoea, concomitant with a retraction of the head and forepaws and a reduction in the escape movements that animals frequently make to escape from the position imposed by the experimental situation. Usually, the apnoea was associated with successive waves of muscular tremor. For each animal, the latency to the onset of the freezing reaction (from the time of first application of the pressure) was measured before administration (zero time) and again, 3 h after administration of a hyperalgesic agent. The intensity of hyperalgesia was quantified as the reduction in reaction time, calculated by subtracting the value of the second measurement from that of the first (Ferreira *et al*., 1978). Reaction times were typically 32–34 s (with standard errors of the mean [s.e.m.] of 0.5–1.0 s) before injection and 2–4 s after stimulation with hyperalgesic agents. Multiple paw treatments did not alter basal reaction times. Different individuals prepared the solutions to be injected, made the injections, and measured the reaction times. The experimenter who measured the reaction times did not have access to the drugs protocols.

Experimental protocols

Effect of rolipram (racemate mixture of 4-[3′-cyclopentyloxy-4′-methoxyphenyl]-2-pyrolidone), ODQ (1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one), zaprinast, Org-9935, RP-73401 and H89 (N-p-bromocinnamylaminoethyl-5-isoquinolinesulphonamide) on inflammatory hyperalgesia Hyperalgesia was measured 3 h after injections of carrageenan (50 μg), bradykinin (100 ng), TNFα (0.25 pg), IL-1β (0.05 pg), IL-6 (0.1 ng), IL-8 (0.05 ng), PGE_2 (10 ng), or dopamine (3 μg), each injected in 100 μl, into the hind paws (intraplantar, i.pl.) of rats. Rolipram, a type 4 phosphodiesterase (PDE) inhibitor (1–9 μg in 50 μl, i.pl.), ODQ, a NO-sensitive guanylyl cyclase inhibitor (2–8 μg in 50 μl, i.pl.) or saline (control, 50 μl) were injected in the same hind paws, 30 min before all hyperalgesic substances. In other sets of experiments, zaprinast, type 5 PDE inhibitor (9 μg in 50 μl), Org-9935, a type 3 PDE inhibitor (9 μg in 50 μl), RP-73401, a type 4 PDE inhibitor (9 μg in 50 μl) or H89, a kinase A protein inhibitor (1–27 μg in 50 μl) were administered i.pl. 30 min before prostaglandin administration (10 ng/paw in zaprinast and Org-9935 pretreated animals and 100 ng paw^{-1} in H89 pretreated animals). The hyperalgesia was measured 3 h after prostaglandin injection. The time of injection of the inhibitors (30 min before the

hyperalgesic stimuli) were selected on the basis of preliminary experiments.

Effect of ODQ on anti-hyperalgesic activity of the NO donor, SNAP (S-nitroso-N-acetyl-D,L-penicillamine) Rats were injected with PGE_2 (100 ng in 100 μl, i.pl.) into the hind paws. ODQ (8 μg in 50 μl, i.pl.) or saline (control, 50 μl. i.pl.) were injected in the same hind paws, 90 min after PGE_2 injection. After a further 30 min, the NO donor, SNAP (50, 100 or 200 μg in 50 μl, i.pl.) was also injected in the same hind paws. The hyperalgesia was determined 3 h after the PGE_2 administration.

Drugs

Recombinant human IL-1β, IL-6, IL-8 and TNFβ were a gift from Dr Steve Poole from NIBSC (National Institute for Biological Standards and Control) preparations. The specific activities of these materials are IL-1β: 100,000 international units (IU μg^{-1} ampoule^{-1}, IL-6: 100,000 IU μg^{-1} ampoule^{-1}, IL-8: 1,000 IU μg^{-1} ampoule^{-1} and TNFα: 40,000 IU μg^{-1} ampoule^{-1}. Carrageenan was a gift from the FMC Corporation (Philadelphia, U.S.A.). Prostaglandin E_2, bradykinin and dopamine were purchased from Sigma (St. Louis, U.S.A.). ODQ and SNAP were purchased from Trocris Cookson (St. Louis, U.S.A.) and H89 from Calbiochem (U.S.A.). Rolipram, zaprinast, RP73401 and Org-9935 were a kind gift from Chiroscience, (Cambridge, U.K.).

Statistical analysis

Results are presented as means and standard errors of the means of groups of at least five animals in each group. Differences between responses were evaluated by ANOVA, followed by the Bonferroni *t*-test. Results with $P<0.05$ were considered significant.

Animals

Male Wistar rats, weighing 150–180 g, housed in temperature controlled-rooms (22–25°C) with water and food *ad libitum* until use.

Results

Effect of phosphodiesterase inhibitors on the hyperalgesic response to PGE_2 and dopamine

The injection of PGE_2 (10 ng) or dopamine (3 μg) into the hind paw of rats evoked a small hyperalgesic effect, measured 3 h later. Rolipram, a PDE4 inhibitor (1, 3 and 9 μg) injected i.pl. into the same paw 30 min before potentiated in a dose-dependent manner the PGE_2 or dopamine-evoked hyperalgesia (Figure 1a and b, respectively). The i.pl. injection of another PDE4 inhibitor, RP-73401, also significantly enhanced the PGE_2-evoked hyperalgesia (RP, 9 μg, Figure 1c). In contrast,

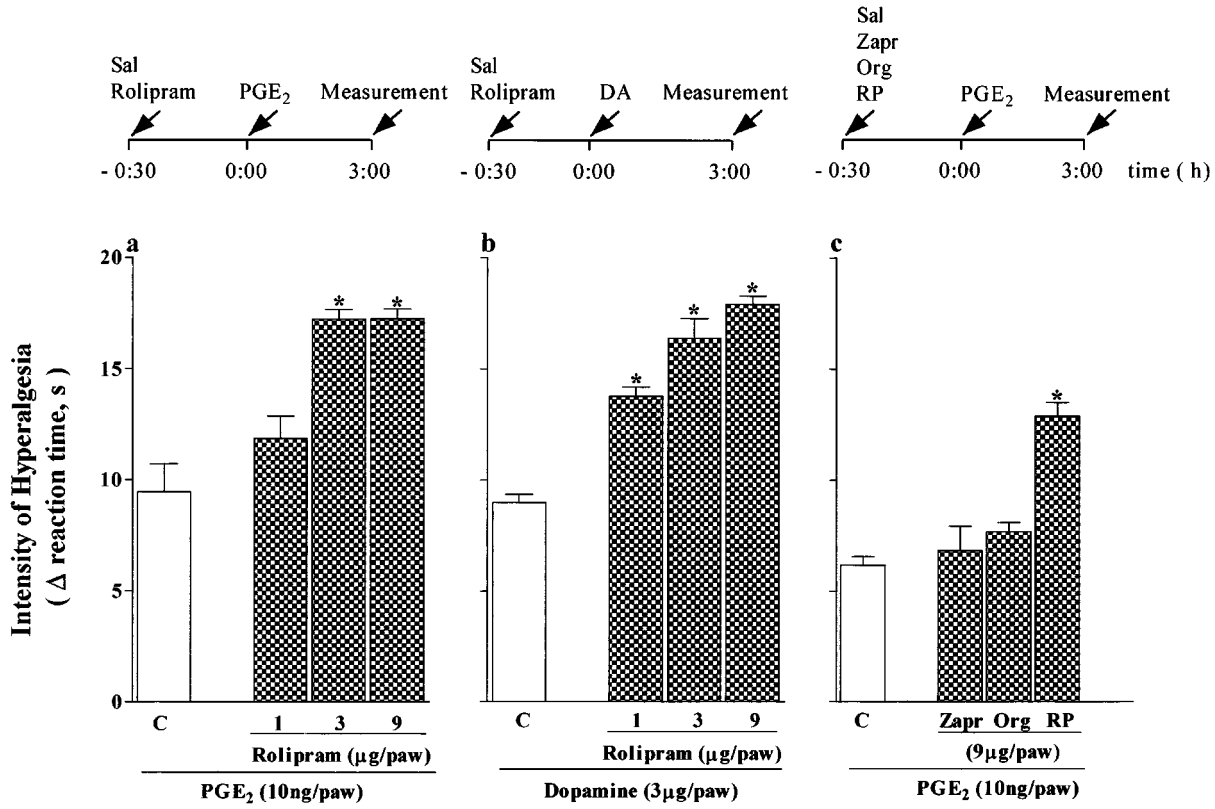

Figure 1 Effect of local administration of rolipram on the hyperalgesic responses to PGE_2 (a), and dopamine (b) and of zaprinast, Org-9935 or RP-73401 on the +hyperalgesic responses to PGE_2 (c). Hyperalgesic responses were measured 3 h after injection (i.pl.) of PGE_2 (10 ng in 100 μl, i.pl., a and c) or dopamine (3 μg in 100 μl, i.pl., b). Rolipram (1, 3 and 9 μg in 50 μl, i.pl., filled bars) or saline (50 μL, i.pl., C, open bars) were given 30 min before PGE_2 or dopamine. Zaprinast (9 μg in 50 μl, i.pl., filled bar), Org-9935 (9 μg in 50 μL, i.pl., filled bar) or RP-73401 (9 μg in 50 μL. i.pl., filled bar) were also given 30 min before PGE_2. The schemes of drugs administrations were present in the top of the panels. Results are expressed as means$\pm$s.e.mean in groups of five rats. *$P<0.005$, compared with the respective control (ANOVA, followed by Bonferroni *t*-test).

the i.pl. administration of zaprinast (a PDE5 inhibitor, 9 μg) or Org-9935 (a PDE3 inhibitor, 9 μg), 30 min before the i.pl. injection of PGE_2, did not affect the eicosanoid-evoked hyperalgesia.

Effect of rolipram on the hyperalgesic response to carrageenan, bradykinin, TNFα, IL-1β, IL-6 and IL-8

Next, we investigated whether the potentiating effects of rolipram occurred in response to stimuli other than the final mediators, PGE_2 and dopamine. The hyperalgesia induced by i.pl. injection of carrageenan, at a sub-maximal dose (50 μg), was augmented in a dose-dependent manner by the pretreatment of the paw with rolipram (1–9 μg, Figure 2a). Similarly, the hyperalgesia induced by bradykinin (100 ng paw^{-1}), TNFα (0.25 pg paw^{-1}), IL-1β (0.05 pg paw^{-1}), IL-6 (0.1 ng paw^{-1}) or IL-8 (0.05 ng paw^{-1}) was also significantly enhanced by the i.pl. pretreatment with rolipram (3 μg) administered 30 min before the hyperalgesic stimuli (Figure 2b).

Effect of compound H89 on the hyperalgesic response to PGE_2

To investigate whether the hyperalgesic effect of PGE_2 was mediated by activation of protein kinase A (PKA), we used the PKA inhibitor H89. Intraplantar injection of PGE_2 (100 ng) evoked hyperalgesia determined 3 h later. PGE_2-evoked hyperalgesia was inhibited in a dose-dependent manner by the i.pl. administration (30 min before) of H89 (1, 3, 9 and 27 µg, Figure 3).

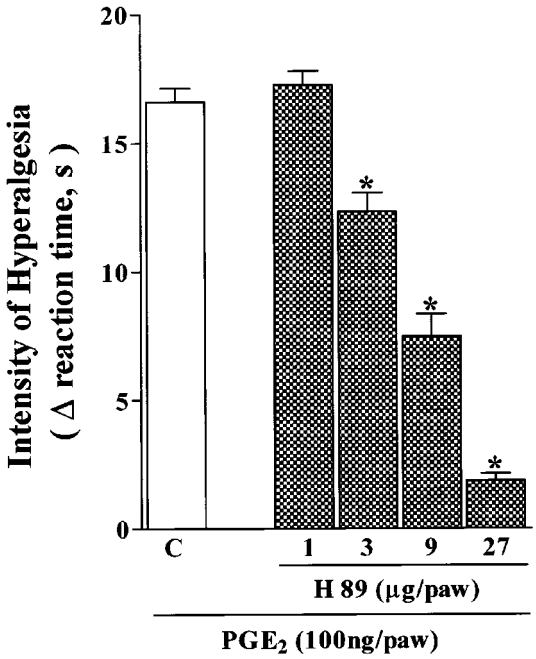

Figure 3 Effect of local administration of the compound H89 on the hyperalgesic responses to PGE_2. Responses were measured 3 h after injection (i.pl.) of PGE_2 (100 ng/100 μl, i.pl.). Compound H89 (1, 3, 9, 27 μg in 50 μl, i.pl.) or saline (control, C, 50 μl, i.pl.) were given 30 min before PGE_2. Results are expressed as means (s.e.mean in groups of five rats. $^*P<0.005$, compared with the control (C, ANOVA, followed by Bonferroni *t*-test).

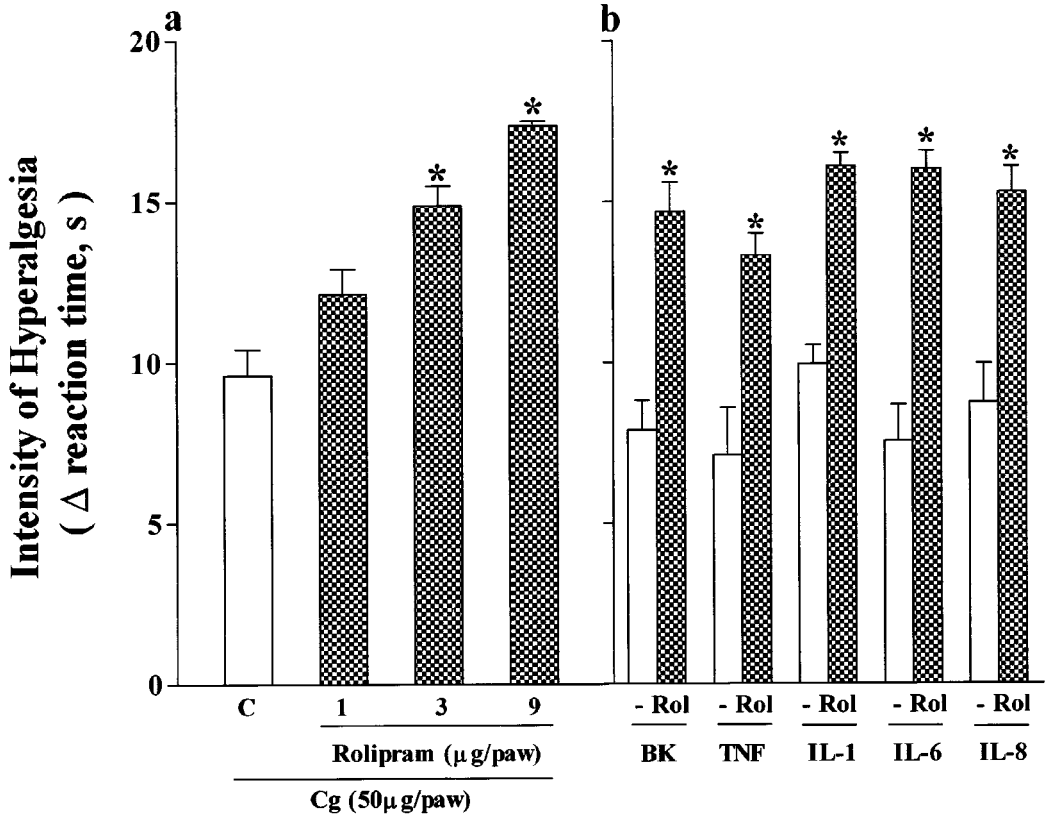

Figure 2 Effect of local administration of rolipram on the hyperalgesic responses to carrageenan (a) or to bradykinin, TNFα, IL-1β, IL-6 and IL-8 (b). Hyperalgesic responses were measured 3 h after injection of carrageenan (50 μg in 100 μl, i.pl.) or bradykinin (BK, 100 ng in 100 μl, i.pl.), TNFα (0.25 pg in 100 μl, i.pl.), IL-1β (0.05 pg in 100 μl, i.pl.), IL-6 (0.1 ng in 100 μl, i.pl.) or IL-8 (0.05 ng in 100 μl, i.pl.). Rolipram (1, 3 and 9 μg in 50 μl, i.pl.) or saline (control, C, 50 μl, i.pl.) were given 30 min before carrageenan. The dose of rolipram administered i.pl. 30 min before bradykinin, TNFα, IL-1β, IL-6 and IL-8 was 9 μg paw^{-1} diluted in 50 μl of saline. Results are expressed as means ± s.e.mean in groups of five rats. $^*P<0.005$, compared with the respective control (ANOVA, followed by Bonferroni *t*-test).

Effect of ODQ on the anti-hyperalgesic effect of the NO donor, SNAP

For these experiments hyperalgesia was evoked by the intraplantar injection of PGE_2 (100 ng) and determined 3 h later. SNAP (50, 100 and 200 μg) injected into the same paw 60 min after PGE_2 reduced, in a dose-dependent manner, the hyperalgesic response. In order to investigate whether the inhibitory effects of the NO donor were mediated by cyclic GMP, we used the soluble guanylate cyclase inhibitor ODQ (8 μg paw^{-1}). As shown in Figure 4, the anti-hyperalgesic effects of the SNAP were significantly inhibited by the pre-treatment of the paw (30 min before SNAP injection) with ODQ.

Effect of the ODQ on the hyperalgesic response to carrageenan, bradykinin, TNFα, IL-1β, IL-6, IL-8, PGE_2 and dopamine

In order to investigate whether a cyclic GMP pathway is triggered during hyperalgesia, we examined the effect of ODQ on the hyperalgesia evoked by a range of stimuli, including the final hyperalgesic mediators. Hyperalgesia induced by a small dose of carrageenan (50 μg paw^{-1}) was potentiated in a dose-dependent manner by ODQ (2, 4 and 8 μg), injected into the paw (i.p.) 90 min after carrageenan (Figure 5a). Similarly, the hyperalgesia induced by small doses of bradykinin (100 ng paw^{-1}), TNFα (0.25 pg paw^{-1}), IL-1β (0.05 pg paw^{-1}), IL-6 (0.1 ng paw^{-1}) or IL-8 (0.05 ng paw^{-1}) was also potentiated by ODQ (8 μg paw^{-1}). On the other hand, the hyperalgesia induced by the final mediator PGE_2 and dopamine was not affected by the treatment of the paw with ODQ (8 μg, Figure 5b).

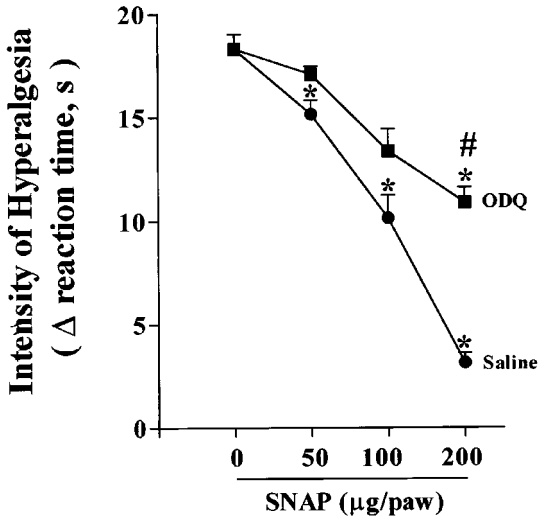

Figure 4 Effect of ODQ on the anti-hyperalgesic effect of the NO donor, SNAP. Rats were injected with PGE_2 (100 ng in 100 μl, i.pl.), and 90 min later received in the same paw saline (50 μl, i.pl.) or ODQ (8 μg in 50 μl, i.pl.). After a further 30 min, SNAP (50, 100 or 200 μg in 50 μl, i.pl.) was injected in the same paw. The hyperalgesia was determined 3 h after the PGE_2 injection. Results are expressed as means$\pm$s.e.mean in groups of five rats; $^*P<0.005$, compared with animals that received only PGE_2, $^*P<0.05$, compared with animals that received PGE_2, saline and SNAP (ANOVA, followed by Bonferroni *t*-test).

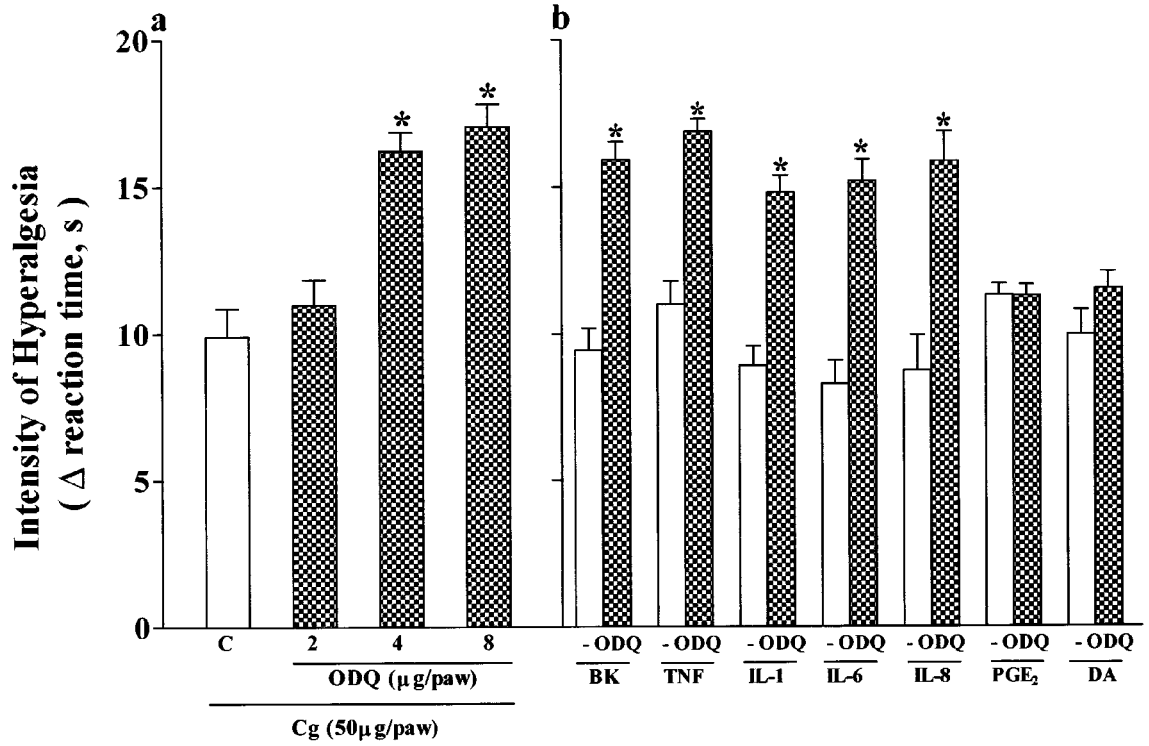

Figure 5 Effect of local administration of ODQ on the hyperalgesic responses to carrageenan (a) or to bradykinin, TNFα, IL-1β, IL-6, IL-8, PGE_2 and dopamine. Responses were measured 3 h after injection (i.pl.) of carrageenan (50 μg in 100 μl, i.pl.), bradykinin (BK, 100 ng in 100 μl, i.pl.), TNFα (0.25 pg in 100 μl, i.pl.) IL-1β (0.05 pg in 100 μl, i.pl.), IL-6 (0.1 ng in 100 μl, i.pl.), IL-8 (0.05 ng in 100 μl, i.pl.), PGE_2 (10 ng in 100 μl, i.pl.) or dopamine (DA, 3 μg in 100 μl, i.pl.). (a) ODQ (2, 4 and 8 μg in 50 μl, i.pl., filled bars) or saline (control, C, 50 μl, i.pl., open bar) were given 90 min after carrageenan. (b) ODQ (8 μg in 50 μl, i.pl., filled bars) or saline (50 ml, i.pl., open bar) were given 90 min after BK, TNFα, IL-1β, IL-6 or IL-8, PGE_2 and dopamine. Results are expressed as means$\pm$s.e.mean in groups of five rats. $^*P<0.005$, compared with the respective control (ANOVA, followed by Bonferroni *t*-test).

Discussion

There is experimental evidence to suggest that modification of the intracellular levels of cyclic AMP or cyclic GMP in peripheral sensitive neurons modulates hyperalgesia. Overall, elevated levels of cyclic AMP are associated with enhanced hyperalgesia, whereas elevated levels of cyclic GMP are associated with inhibition of hyperalgesia, at least in mechanical hyperalgesia. Most of these studies focus on the exogenous addition of stimuli that modulate the levels of these cyclic nucleotides. However, there are studies that have investigated the importance of the balance of these cyclic nucleotides in the genesis of inflammatory hyperalgesia (Ferreira & Nakamura, 1979; Taiwo *et al*., 1989; Duarte *et al*., 1990; Ferreira *et al*., 1991; Taiwo & Levine, 1991; Kress *et al*., 1996; Wang, 1996).

In this study, we have demonstrated that the mechanical hyperalgesia induced by PGE_2 and dopamine were significantly enhanced by rolipram, a specific inhibitor of PDE4 (Teixeira *et al*., 1997). This was confirmed by using another PDE4 inhibitor, RP-73401, which also augmented hyperalgesia induced by PGE_2. In contrast, zaprinast and Org-9935, inhibitors of PDE5 and PDE3, respectively, had no effect on the PGE_2-induced hyperalgesia. These results confirm the involvement of cyclic AMP in the primary sensory neuron in hyperalgesia induced by mechanical stimulation. Moreover, these results suggest that in these cells, cyclic AMP is mostly metabolized by PDE4 isoenzymes.

In addition to the hyperalgesia induced by the final mediators, PGE_2 and dopamine, we also tested the effects of rolipram on the hyperalgesia induced by carrageenan, bradykinin and the cytokines TNFα, IL-1β, IL-6 and IL-8. These mediators have previously been shown to induce hyperalgesia *via* the release of prostaglandins and/or sympathomimetic mediators (Ferreira *et al*., 1988; Cunha *et al*., 1991; 1992) in our model. As expected, our results clearly demonstrate that rolipram effectively enhanced the hyperalgesic effects of all the stimuli tested. Thus, our results suggest that cyclic AMP is produced during inflammatory reactions and its enhancement, *via* inhibition of its degradation, is associated with worsening of inflammatory hyperalgesia. This may be relevant in the development of PDE4 inhibitors for the treatment of clinical situations associated with inflammatory pain (e.g. rheumatoid arthritis). In these situations, it is possible that treatment with PDE4 inhibitors may be associated with worsening of clinical symptoms (pain), at least until the inflammation in the joint is controlled by the anti-inflammatory effects of the drugs. We are presently addressing this situation in animal models of rheumatoid arthritis.

There is evidence to suggest that at least some of the inhibitory effects of cyclic AMP elevation in leukocytes are mediated *via* the production of IL-10 and/or inhibition of the production of pro-inflammatory cytokines, such TNFα (Eigler *et al*., 1998). In our model, the anti-hyperalgesic effect of rolipram does not appear to be due to increased IL-10 production or decreased TNFα release, inasmuch as neither IL-10 nor TNF (interfere with the hyperalgesia induced by PGE_2 or dopamine (Cunha *et al*., 1992; Poole *et al*., 1995). The final biochemical events responsible for the functional up-regulation of nociceptors following an increase in cytosolic cyclic AMP are not clear. The mechanisms are likely to involve the activation of protein kinase A, with subsequent phosphorylation of an ion channel or the modulation of cytosolic structures that control intracellular calcium levels (Ouseph *et al*., 1995; Taiwo *et al*., 1992; Wang *et al*., 1996; Sluka, 1997; Lynn & O'Shea, 1998). To test this possibility in our model, we investigated the effect of H89, a specific inhibitor of protein kinase A (Lynn & O'Shea, 1998) on the hyperalgesia induced by PGE_2. It was demonstrated that H89 inhibits in a dose-dependent manner the hyperalgesic effects of PGE_2 injected i.pl. (Figure 3). Overall our data suggest that PGE_2 and the sympathetic mediators cause mechanical hyperalgesia due to the increase in cyclic AMP, which activated PKA, of the primary sensitive neuron. Although intraplantar injection of PMA causes hyperalgesia (Taniguchi *et al*., 1997), activation of the protein kinase C (PKC) in the primary sensory neurons during inflammation was not found (Taiwo & Levine, 1991). However, PKC has been shown to be involved in nociceptive transmission in the spinal cord (Coderre, 1992; Coderre & Yashpal, 1994; Lin *et al*., 1996; Sluka *et al*., 1997). Thus, it is plausible that PKA and PKC contributes to the inflammatory hyperalgesia *via* sensitization of the primary and secondary sensory neurons respectively.

In order to demonstrate the contribution of cyclic GMP for the function down-regulation of the primary sensory neuron to mechanical stimulation, first we tested the effect of ODQ, a specific inhibitor of soluble guanylate cyclase (Moro *et al*., 1996), on the analgesic effect of a NO donor, SNAP (Duarte *et al*., 1990; Ferreira *et al*., 1991). It was observed that the anti-hyperalgesic effect of SNAP on the hyperalgesia induced by PGE_2 was significantly inhibited by ODQ (Figure 4). This result is in line with previous experiments in which we demonstrated that compounds capable of releasing NO had an anti-hyperalgesic effect in mechanical (Duarte *et al*., 1990; Ferreira *et al*., 1991; Lorenzetti & Ferreira, 1996) and in other tests (Tonussi & Ferreira, 1994; Granados-Soto *et al*., 1997). Moreover, the anti-hyperalgesic effects of NO donors were inhibited by the non-specific inhibitor of soluble guanylate cyclase, methylene blue. Overall these results are in good agreement with our hypothesis that NO can modulate hyperalgesia *via* its action on soluble guanylate cyclase (Duarte *et al*., 1990; Ferreira *et al*., 1991). In addition, our results suggest that ODQ is a useful tool to investigate the role of soluble guanylate cyclase in modulating inflammatory hyperalgesia.

Pretreatment of paws with ODQ potentiated, in a dose-dependent manner, the hyperalgesia induced by a sub-maximal dose of carrageenan (Figure 5a). Furthermore, ODQ also enhanced the hyperalgesia induced by sub-maximal doses of bradykinin, TNFα, IL-1β, IL-6 and IL-8. On the other hand, the hyperalgesia induced by prostaglandin and dopamine, the final mediators of hyperalgesia, was not potentiated by ODQ (Figure 5b). These results indicate that bradykinin, or the hyperalgesic cytokines may also directly or indirectly (*via* NO production) activate soluble guanylate cyclase. The activation of this enzyme will then down-regulate the ability of the substances to cause hyperalgesia. This event appears not to be mediated by prostaglandin or dopamine released by those cytokines, inasmuch as their hyperalgesic effect was not enhanced by ODQ.

Several observations indicate that the L-arginine/NO/cyclic GMP pathway has a peripheral hyperalgesic rather than analgesic effect. Thus, the intraplantar or systemic administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, another NOS inhibitor), but not D-NAME, has been reported to produce dose-dependent antinociception in the second phase of the formalin test in rats (Haley *et al*., 1992). A nociceptive role for the L-arginine/NO/cyclic GMP pathway has also been demonstrated using other tests, such as the tail-flick and hot-plate tests, acetic acid- or phenyl-ρ-quinone-induced writhing and formalin-induced paw licking in mice (Morgan *et al*, 1992; Malmberg & Yaksh, 1993; Kawabata *et al*., 1994; Mustafa,

1992; Moore *et al*., 1991; Meller *et al*., 1994). The simplest explanation for these conflicting observations may be that the role and importance of this pathway varies among the groups of primary sensory neurones mobilized by different types of nociceptive stimuli.

In conclusion, the results presented in this paper show an association between hyperalgesia and activation of adenylate cyclase as well as antinociception with activation of a soluble guanylate cyclase. The intracellular levels of cyclic AMP that enhance hyperalgesia are controlled by the PDE4 isoform and appear to result in the activation of protein kinase A. The balance between the intracellular levels of cyclic AMP and cyclic GMP may be fundamental in the control of pain in response to inflammatory mediators. In this sense, the discovery of substances that act by tilting the cyclic AMP/cyclic GMP balance and/or function in primary sensory neurons may constitute a new class of peripheral analgesics.

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