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Evidence for the participation of the nitric oxide–cyclic GMP pathway in the antinociceptive action of meloxicam in the formalin test

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Abstract

The involvement of the nitric oxide–cyclic GMP pathway in the antinociceptive action of the cyclooxygenase-2 preferential inhibitor meloxicam was assessed in the rat formalin test. Rats received local pretreatment with saline or meloxicam and then 50 μl of dilute formalin (1%). Local administration of meloxicam produced a dose-dependent antinociception in the second phase of the formalin test. The antinociception produced by meloxicam was due to a local action as its administration in the contralateral paw was ineffective. Local pretreatment of the paws with saline or N^G-D-nitro-arginine methyl ester (D-NAME) did not affect the antinociception produced by meloxicam. However, N^G-L-nitro-arginine methyl ester (L-NAME, a NO synthesis inhibitor) or 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ, a soluble guanylyl cyclase inhibitor) blocked in a dose-dependent way the effect of meloxicam. It is concluded that the peripheral antinociceptive effect of meloxicam involves a local NO–cyclic GMP pathway.

Keywords: Meloxicam; Nitric oxide (NO); cGMP; N^G-L-nitro-arginine methyl ester (L-NAME); ODQ (1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one)

1. Introduction

The peripheral initiation of the nociceptive process has been a topic of intense scientific and medical study for years (Hill, 1999). The discovery of drugs, opiates and non-opiates, which exert their antinociceptive action solely in the periphery is perhaps the only way to avoid central side effects. Therefore, the peripheral administration of non-steroidal anti-inflammatory drugs (NSAIDs) is a possible strategy to avoid gastrointestinal and other side effects.

There is evidence to support a critical role of Ca^{2+} and cyclic AMP in the sensitization of the primary sensory neuron (Ferreira and Nakamura, 1979; Taiwo and Levine, 1991). The evidence agrees with the observation that prostanoid receptors are coupled with adenylyl cyclase (Namba et al., 1994; Smith et al., 1998) to produce cyclic AMP. In addition, it is supported by the demonstration that intraplantar administration of stable cyclic AMP analogues or inhibitors of phosphodiesterase 4 enhanced prostaglandin E_2-induced mechanical hyperalgesia (Ferreira and Nakamura, 1979; Taiwo and Levine, 1991; Ouseph et al., 1995). The final biochemical events responsible for the sensitization following an increase in cyclic AMP are not clear. The activation of protein kinase A and C, with subsequent phosphorylation of ion channels or modulation of cytosolic structures that control intracellular Ca^{2+} levels, has been reported to participate (Sluka et al., 1997; Gold et al., 1998; Aley and Levine, 1999). In contrast, it has been proposed that cyclic GMP is involved in antinociception. This proposal was based on the observation that local administration of L-arginine produces antinociception in rats with carrageenin-induced hyperalgesia, the effect being blocked by nitric oxide (NO) inhibitors and methylene blue (a soluble guanylyl cyclase inhibitor) (Duarte et al., 1990). In prostaglandin- and carrageenin-induced hyperalgesia, the local administration of opiates or non-enzymatic NO donors also produces antinociception. While

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pretreatment of the paws with methylene blue inhibited the action of morphine and the NO donor, the NO synthase inhibitor only inhibited opiate analgesia (Ferreira et al., 1991). The effect of morphine was potentiated by specific inhibitors of phosphodiesterase 5, whereas intraplantar injection of dibutyryl-cyclic GMP caused antinociception (Ferreira and Nakamura, 1979). Therefore, the neuronal balance of cyclic AMP and cyclic GMP concentrations seems to be very important for the up- or down-regulation of the nociceptor (Ferreira and Nakamura, 1979; Duarte et al., 1990).

More recently, there have been reports that the antinociceptive effect of certain NSAIDs, such as dipyrone, diclofenac and ketorolac, involves the activation of the L-arginine–NO–cyclic GMP pathway in addition to inhibition of prostaglandin synthesis (Tonussi and Ferreira, 1994; Granados-Soto et al., 1995). In contrast, we have evidence that not all NSAIDs act through this pathway (López-Muñoz et al., 1996b). Therefore, the purpose of the present work was to examine if a local NO–cyclic GMP pathway is involved in the peripheral antinociceptive effect of meloxicam, assayed in the rat formalin test.

2. Materials and methods

2.1. Animals

Female Wistar rats (weight range, 120–160 g) from our own breeding facilities were used in this study. Rats had free access to food and drinking water before the experiment. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (IASP, 1983). Additionally, the study was approved by the local Animal Care Committee.

2.2. Measurement of antinociceptive activity

Antinociception was assessed with the formalin test. Rats were placed in an open Plexiglas observation chamber for 30 min to allow them to accommodate to their surroundings, then they were removed for formalin administration. The right hind paw of the rat was injected with 50 μl of dilute formalin (1%), using a 30-gauge needle. The animal was then returned to the chamber for observation. A mirror was placed behind the chamber to enable unhindered observation of the formalin-injected paw. The rats were observed for nociceptive behavior immediately after formalin injection. Nociceptive behavior was quantified as the number of flinches of the injected paw during 1-min periods each 5 min until 60 min after injection (Malmberg and Yaksh, 1992). Flinching was readily identified and was characterized as rapid and brief withdrawal or flexing the injected paw. Formalin-induced flinching behavior is biphasic (Fig. 1). The initial acute phase (0–10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15–60 min). At the end of the experiment, rats were killed in a CO_2 chamber.

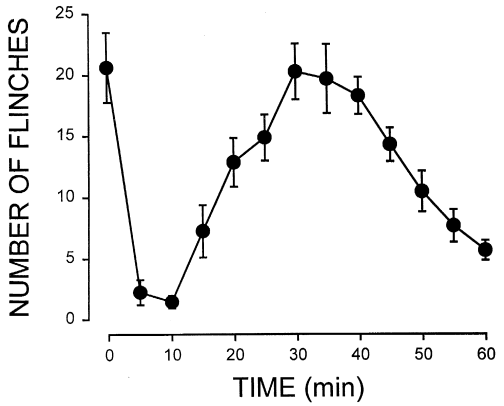

Fig. 1. Time course of the nociceptive behavior observed after local administration of 1% formalin in the rat. Rats were injected with formalin and immediately observed for flinching behavior for the next 60 min. Data are expressed as the number of flinches by minute (mean ± S.E.M. for six to eight animals).

2.3. Drugs

Meloxicam was a gift of Laboratorios Promeco (Mexico City). N^G-L-nitro-arginine methyl ester (L-NAME), N^G-D-nitro-arginine methyl ester (D-NAME), 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ) were purchased from Research Biochemical International (Natick, MA, USA).

2.4. Study design

Rats received appropriate vehicle or increasing doses of meloxicam (50, 100 and 200 μg) 20 min before formalin injection. To determine if the meloxicam-induced antinociception was mediated by the NO–cyclic GMP pathway, the effect of L-NAME and ODQ on the antinociceptive effect induced by meloxicam was assessed. Rats in all groups were tested for possible side effects such as reduction in righting, stepping, corneal and pinna reflexes and catalepsy.

2.5. Data analysis and statistics

All results are presented as means ± S.E.M. for six to eight animals per group. Curves were made for number of flinches against time. The area under the number of flinches against time curves (AUC) for the second phase was calculated by the trapezoidal rule. Analysis of variance followed by Dunnett's test was used to test differences between treatments. A $P < 0.05$ was considered significant.

3. Results

3.1. Peripheral antinociceptive effect of meloxicam

Formalin (1%) administration (50 μl) produced a typical pattern of flinching behavior. The first phase started immediately after administration of formalin and then diminished gradually in about 10 min. The second phase started at 15 min and lasted until 1 h (Fig. 1). Ipsilateral (IL), but not contralateral, local administration of meloxicam produced a dose-dependent reduction in the flinching behavior otherwise observed after injection of 1% formalin (Fig. 2). Meloxicam significantly reduced in a dose-dependent manner the number of flinches during phase 2 ($P < 0.05$). No side effects were observed in either group, control or treated.

3.2. Effect of L-NAME and ODQ on the peripheral antinociceptive effect of meloxicam

Local pretreatment with the NO synthesis inhibitor L-NAME (100 μg, IL) did not produce any antinociceptive effect by itself. However, it reversed in a dose-dependent manner the antinociception ($P < 0.05$) produced by meloxicam (Fig. 3). Administration of the inactive isomer of L-NAME, D-NAME was not able to produce antinociception or reverse that produced by meloxicam (Fig. 3).

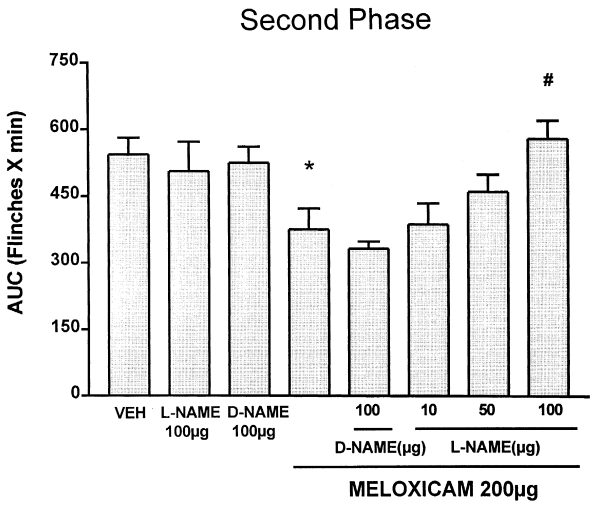

Fig. 3. Effect of L-NAME on the peripheral antinociception produced by meloxicam. Rats received intraplantar L-NAME (30 min) and meloxicam (20 min) pretreatment on the right paw and then an injection of 1% formalin (50 μl). Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the means ± S.E.M. for six to eight animals. * Significantly different from the saline group ($P < 0.05$) and #significantly different from the meloxicam group ($P < 0.05$), as determined by analysis of variance followed by Dunnett's test.

Local pretreatment with the NO-sensitive guanylyl cyclase inhibitor ODQ (50 μg, IL) did not produce any antinociceptive effect by itself. However, pretreatment with ODQ, but not saline, significantly reversed in a dose-de-

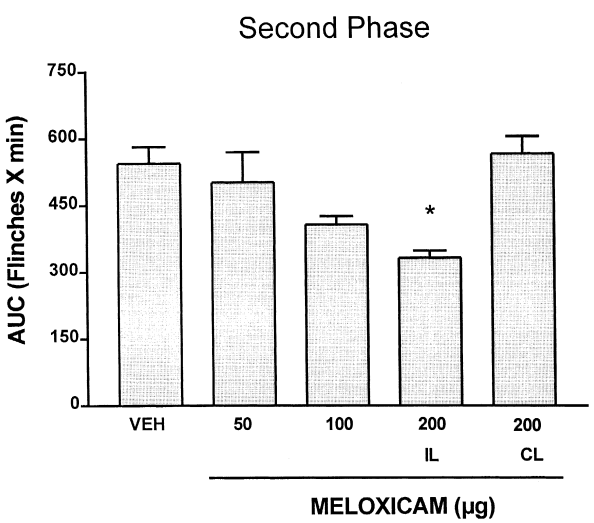

Fig. 2. Local antinociceptive effect of meloxicam in the formalin test. Rats received saline (30 min) and meloxicam pretreatment (20 min) on either the right (ipsilateral, IL) or left paw (contralateral, CL) and then an injection of 1% formalin (50 μl). Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the means ± S.E.M. for six to eight animals. * Significantly different from saline ($P < 0.05$), as determined by analysis of variance followed by Dunnett's test.

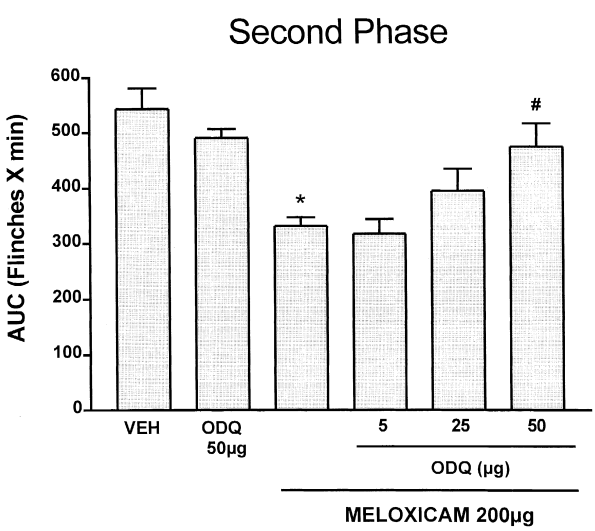

Fig. 4. Effect of ODQ on the peripheral antinociception produced by meloxicam. Rats received intraplantar ODQ (30 min) and meloxicam (20 min) pretreatment on the right paw and then an injection of 1% formalin (50 μl). Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the means ± S.E.M. for six to eight animals. * Significantly different from the saline group ($P < 0.05$) and #significantly different from the meloxicam group ($P < 0.05$), as determined by analysis of variance followed by Dunnett's test.

pendent manner ($P < 0.05$) the antinociception produced by meloxicam (Fig. 4).

4. Discussion

In the present investigation, we were able to observe peripheral antinociception with meloxicam. The antinociceptive effect was not due to a systemic or central action since the administration of meloxicam in the contralateral paw did not produce any effect. The effect of meloxicam was blocked in a dose-dependent manner by the NO synthesis inhibitor, L-NAME, but not by saline or the inactive isomer of L-NAME, D-NAME. These results suggest that NO is involved in the peripheral antinociception induced by meloxicam. Moreover, the antinociceptive effect was also blocked by the NO-sensitive soluble guanylyl cyclase inhibitor ODQ (Moro et al., 1996), suggesting that the NO–cyclic GMP pathway plays an important role in the meloxicam-induced antinociception in the formalin test. The results confirm the participation of the NO–cyclic GMP pathway in the antinociception produced by several NSAIDs (Duarte et al., 1990, 1992; Tonussi and Ferreira, 1994; Granados-Soto et al., 1995; Lorenzetti and Ferreira, 1996) and morphine (Duarte et al., 1992; Granados-Soto et al., 1997; Nozaki-Taguchi and Yamamoto, 1998). However, we have previously reported that not all NSAIDs produce all of their effect through activation of this pathway, since L-NAME was not able to block antinociception produced by either indomethacin or acetaminophen in the uric acid-induced functional impairment model (López-Muñoz et al., 1996b). It has been recently reported that cyclic GMP accumulation due to the inhibition of phosphodiesterase or the direct activation of NO release by caffeine can explain its adjuvant effect on NSAID-induced antinociception (Aguirre-Bañuelos et al., 1999). This result is in line with the observation that L-NAME is able to block the potentiation of the antinociceptive effect of ketorolac by caffeine (López-Muñoz et al., 1996a) and also the meloxicam-induced antinociception (this study).

There are several observations indicating that the NO–cyclic GMP pathway plays a hyperalgesic rather than an antinociceptive role. It has been reported that either intraplantar or systemic administration of L-NAME, but not D-NAME, produces dose-dependent antinociception in the second phase of the formalin test (Haley et al., 1992; Malmberg and Yaksh, 1993; Aley et al., 1998). In contrast, we were not able to observe antinociception after L-NAME administration. Differences could be due to the different intensity of nociception used in this study (1% formalin). The nociceptive or inflammatory role of the NO–cyclic GMP pathway has been described for bradykinin, substance P and carrageenin (Kawabata et al., 1994). A possible explanation for these conflicting observations is that the role of this pathway varies among the groups of primary sensory neurons mobilized by different types and intensities of nociceptive stimuli, as proposed previously (Granados-Soto et al., 1997; Cunha et al., 1999). However, it is likely that other factors play a significant role in the observed differences.

In the present study, we were able to observe a dose-dependent antinociceptive effect in the formalin test. Since meloxicam preferentially inhibits cyclooxygenase-2 (Pairet et al., 1998), our results suggest a relevant participation of a peripheral cyclooxygenase-2 in the nociceptive and inflammatory process in the formalin test. However, since meloxicam is not a highly selective inhibitor of cyclooxygenase-2, it is likely that cyclooxygenase-1 also participates in this effect. Actually, the participation of cyclooxygenase-2 in the formalin test is contradictory. On the one hand, systemic or spinal administration of a non-selective cyclooxygenase-2/cyclooxygenase-1 inhibitor (ibuprofen), but not cyclooxygenase-2 selective inhibitors, such as celecoxib and *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS 398), produced antinociception in the formalin test (Dirig et al., 1997; Euchenhofer et al., 1998), indicating an important participation of cyclooxygenase-1, but not cyclooxygenase-2, in this test. On the other hand, there is evidence to support the participation of the cyclooxygenase-2 enzyme in formalin-induced acute nociception, as NS 398 was able to reduce the formalin-induced flinches (Yamamoto and Nozaki-Taguchi, 1996).

It has been suggested that the antinociceptive activity of meloxicam observed after systemic administration is mainly due to a peripheral action at or near the nociceptor endings (Laird et al., 1997). In this study, we observed a peripheral antinociceptive effect of meloxicam. This effect, however, was partially due to the activation of the NO–cyclic GMP pathway, but the participation of inhibition of cyclooxygenase-2/cyclooxygenase-1 cannot be ruled out with the present experiments. In contrast, it has been recently proposed that meloxicam has a central antinociceptive effect, since it inhibits spinal nociceptive reflexes and wind-up (López-Garcia and Laird, 1998). Those authors have proposed that meloxicam-induced antinociception may not be due to inhibition of the cyclooxygenase enzyme, but rather may involve actions on other systems. It would be interesting to assess if the spinal activation of the NO–cyclic GMP pathway plays a role in the action of meloxicam at these levels.

In this study, peripheral antinociception with no incidence of side effects was observed after local administration of meloxicam. Therefore, the current work emphasizes the significant peripheral antinociceptive effect of meloxicam, which supports the use of this drug in managing several pain states generated after tissue injury.

In summary, meloxicam produced peripheral antinociception in the formalin test in the rat. The antinociceptive effect of meloxicam was antagonized by L-NAME and ODQ. These results strongly suggest that, besides the inhibitory action on prostaglandin synthesis inhibition, the activation of the NO–cyclic GMP pathway plays an impor-

tant role in the peripheral antinociception of meloxicam in the formalin test.

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