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Short communication

Evidence for the involvement of nitric oxide in the antinociceptive effect of ketorolac

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Abstract

The involvement of nitric oxide in the antinociception produced by ketorolac was assessed using the pain-induced functional impairment model in the rat: 800 μg of N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthesis, or saline was injected intra-articularly in a hind limb joint previously injured with uric acid. Animals then received ketorolac, dipyrone or no drug. Ketorolac and dipyrone produced a significant antinociceptive effect which was reduced by pretreatment with N^G-nitro-L-arginine methyl ester, but not with saline. It is concluded that the antinociceptive effect of both drugs involves the local participation of nitric oxide.

Keywords: Ketorolac tromethamine; Nitric oxide (NO); N^G-Nitro-L-arginine methyl ester; Dipyrone

1. Introduction

Ketorolac is a non-steroidal antiinflammatory drug (NSAID) which has been shown to be effective in the treatment of moderate to severe pain (Bloomfield et al., 1986). As other NSAIDs, ketorolac is able to block prostaglandin synthesis (Rauk and Laifer, 1993). Nevertheless, since this drug exhibits greater potency and efficacy than other members of the group, it has been proposed that it possesses other mechanisms of action as well. Ketorolac does not bind to opioid receptors (López et al., 1987), therefore direct activation of opioid receptors can be discarded. Indirect activation by means of the release of endogenous opioids has been suggested (Domer, 1990; Uphouse et al., 1993). This possibility, however, seems unlikely since the analgesic activity of ketorolac is not blocked by naloxone (Uphouse et al., 1993).

It has been shown that the analgesic effect of certain NSAIDs, such as dipyrone and diclofenac, involves activation of the arginine-nitric oxide-cGMP pathway in addition to prostaglandin synthesis inhibition (Lorenzetti and Ferreira, 1985; Duarte et al., 1992; Ferreira et al., 1991). The purpose of the present work was to examine if local nitric oxide synthesis is involved in the analgesic effect of ketorolac assayed in the pain-induced functional impairment model in the rat.

2. Material and methods

2.1. Animals

Female Wistar rats, 180–220 g, from our own breeding facilities (Crl:(WI)BR) were used in this study. The animals were housed in a room with controlled temperature (22 ± 2° C) for at least 2 days before the study. Twelve hours before the initiation of experiments, food was withheld, but the animals had free access to drinking water. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmerman, 1983).

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2.2. Drugs

Ketorolac tromethamine was obtained from Syntex (Mexico City, Mexico). Dipyrone was obtained from Química Hoechst (Mexico City, Mexico). N^G-Nitro-L-arginine methyl ester and uric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Measurement of antinociceptive activity

Antinociceptive activity was measured using the pain-induced functional impairment model in the rat, also known as PIFIR (López-Muñoz et al., 1993). Pain was induced by the intra-articular injection of 0.05 ml of 30% uric acid suspended in mineral oil in the knee joint of the right hind limb, under light anaesthesia with ether. An electrode was attached to each hind paw between the plantar pad and the rats were allowed to recover from anaesthesia. The animals were then placed on a stainless steel cylinder of 30 cm diameter. The cylinder was rotated at 4 rpm forcing the rats to walk. The variable measured in this model was the time of contact between each of the rat's hind paws and the cylinder. When the electrode placed on the animal's paw made contact with the cylinder floor, a circuit was closed and the time that the circuit remained closed was recorded. The cylinder was rotated for 2-min periods, during which time recordings were made, allowing the rats to rest for 30 min between recording periods.

After uric acid injection, the rats developed a progressive dysfunction of the injured limb. This was recorded as a diminished time of contact between the right hind limb and the cylinder. The data are expressed as the functionality index, i.e. the time of contact of the injected limb divided by the time of contact of the control left limb multiplied by 100. After 2 h, the functionality index was zero, i.e. the injured limb made no contact with the cylinder. At this time, the rats received an intra-articular injection of 800 μg of N^G-nitro-L-arginine methyl ester dissolved in 0.05 ml of isotonic saline or of vehicle. One hour later, they received an oral ketorolac tromethamine dose (3.2 mg/kg) or a subcutaneous dose of dipyrone (316.2 mg/kg). The functionality index was determined every 30 min in the 4 h following. Additional groups of rats which were injected intra-articularly with either N^G-nitro-L-arginine methyl ester or saline, but did not receive any analgesic agent, served as controls. Recovery of the functionality index was considered as the expression of the antinociceptive effect.

2.4. Data and statistical analysis

The functionality index against time curves were constructed and the maximal antinociceptive effect (E_{max}^{obs}) was determined directly from these plots. The area under the functionality index against time curve (AUC_E), estimated using the trapezoidal rule, was taken as an expression of the overall antinociceptive activity during the 4-h observation period (López-Muñoz et al., 1993). The effects of either ketorolac or dipyrone in the presence and absence of local N^G-nitro-L-arginine methyl ester or saline injection were compared by means of Student's t-test for unpaired data. Differences were considered to reach statistical significance when $P < 0.05$.

3. Results

The functionality index recovery in rats injured with uric acid induced by ketorolac, dipyrone and by no analgesic agent is shown in Fig. 1. The expressions of antinociception, namely E_{max}^{obs} and AUC_E, are given in Table 1. When ketorolac was given to animals previously injected with intra-articular N^G-nitro-L-arginine methyl ester, the antinociceptive effect was significantly reduced compared to that observed in rats pretreated with saline. E_{max}^{obs} was reduced by about 40%, whereas AUC_E decreased about 66%. Dipyrone was also able to produce an antinociceptive effect in this model. As was the case for ketorolac, N^G-nitro-L-arginine methyl ester intra-articular injection decreased dipyrone-induced antinociception compared to saline pretreatment. E_{max}^{obs} and AUC_E were reduced by about 37 and 45% respectively.

The functionality index in rats pretreated with either saline or N^G-nitro-L-arginine methyl ester re-

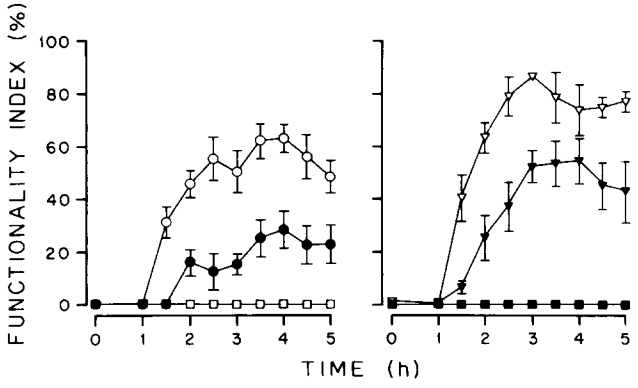

Fig. 1. Time course of the antinociceptive effect induced by 3.2 mg/kg of ketorolac tromethamine p.o (○,●), of 316.2 mg/kg of dipyrone s.c. (▽,▼) and of no analgesic agent (□,■), measured as functionality index recovery in rats submitted to pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind knee. Dark symbols correspond to antinociceptive activity determined in animals previously submitted to the intra-articular injection of N^G-nitro-L-arginine methyl ester (800 μg) in the injured joint. White symbols correspond to animals pretreated with intra-articular saline. Data are expressed as means ± S.E.M. for 6 animals.

Table 1
Antinociceptive effect of ketorolac (KET), dipyrone (DIP) and no analgesic agent in animals pretreated with an intra-articular injection of N^G-nitro-L-arginine methyl ester (800 μg) or saline in the pain-induced functional impairment model in the rat

Drug	E_{max}^{obs}(%)	AUC_E (%·h)
Saline	0.55 ± 0.32	0.31 ± 0.20
L-NAME	0.43 ± 0.22	0.29 ± 0.16
KET + saline	71.81 ± 6.84	195.76 ± 18.34
KET + L-NAME	43.38 ± 2.07 [a]	66.68 ± 7.33 [a]
DIP + saline	90.46 ± 2.51	267.74 ± 16.50
DIP + L-NAME	66.07 ± 5.66 [b]	148.37 ± 21.89 [b]

Antinociception is expressed as the maximal observed recovery of the functionality index (E_{max}^{obs}) and the area under the functionality index against time curve (AUC_E). The data are presented as means ± S.E.M. ($n = 6$). [a] Significantly different from the ketorolac + saline solution group ($P < 0.001$); [b] significantly different from the dipyrone + saline solution group ($P < 0.001$); as determined by Student's t-test.

mained at zero levels during the whole 4-h observation period when no analgesic agent was given. These results indicate that the antinociceptive effect of both ketorolac and dipyrone was solely due to the administration of these NSAIDs, since no spontaneous recovery of functionality was observed in their absence.

4. Discussion

There is evidence that prostaglandin synthesis inhibition is involved in the antinociceptive effect of ketorolac (Rauk and Laifer, 1993). It is, however, likely that other mechanisms of action are involved, given the high potency and efficacy of this drug. Ketorolac does not bind to opioid receptors (López et al., 1987), but indirect activation by release of endogenous opioids has been suggested (Domer, 1990; Uphouse et al., 1993).

Although Domer (1990) reported that ketorolac was active in the opioid-specific hot plate test, other authors did not observe any antinociceptive activity in this assay (Rooks et al., 1982; Uphouse et al., 1993). Moreover, the methods used by Domer (1990) have been questioned (Uphouse et al., 1993). Recently Uphouse et al. (1993) reported that ketorolac was effective to inhibit the abdominal stretching produced by p-phenylquinone injections, an effect that was blocked by nor-binaltorphimine, a kappa antagonist, but not by naloxone. Since naloxone antagonizes all known opioid receptors and nor-binaltorphimine is also able to block cannabinoid-induced antinociception (Welch, 1993), participation of opioid receptors in the analgesic effect of ketorolac is not clear.

Recently, Chávez and coworkers (1993) reported that ketorolac exhibits ionophoretic-like properties and they suggested that this might also be a mechanism of analgesic action, but showed no assay of antinociceptive activity. On the other hand, experimental evidence has been provided for a role of nitric oxide in the antinociceptive action of certain NSAIDs, such as dipyrone (Lorenzetti and Ferreira, 1985) and diclofenac (Tonussi and Ferreira, 1994).

Our results demonstrate that local administration of N^G-nitro-L-arginine methyl ester reduces the antinociceptive effect of both ketorolac and dipyrone in the pain-induced functional impairment model in the rat. Since it is well known that N^G-nitro-L-arginine methyl ester is an inhibitor of nitric oxide synthesis from L-arginine (Rees et al., 1990), our results provide evidence that the arginine-nitric oxide-cGMP pathway is involved in the antinociceptive activity of ketorolac. The participation of nitric oxide, however, appears to be only a component of the antinociceptive activity. Despite the fact that a high local N^G-nitro-L-arginine methyl ester dose (800 μg) was used, the antinociceptive effect was only partially reduced. In animals pretreated with N^G-nitro-L-arginine methyl ester, ketorolac and dipyrone were still able to induce a significant recovery of the functionality index. Hence, the antinociceptive effect observed in the presence of N^G-nitro-L-arginine methyl ester should be due to a different mechanism of action.

Our data allow us to conclude that local nitric oxide synthesis is involved in the analgesic effect of ketorolac, in addition to prostaglandin synthesis inhibition. The present results, however, do not allow us to discard the involvement of other mechanisms of action in the analgesic activity of this compound.

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