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Participation of the nitric oxide–cyclic GMP–ATP-sensitive K^+ channel pathway in the antinociceptive action of ketorolac

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Abstract

The involvement of nitric oxide (NO), cyclic GMP and ATP-sensitive K^+ channels in the antinociceptive effect of ketorolac was assessed using the formalin test in the rat. Local administration of ketorolac in a formalin-injured paw produced a dose-dependent antinociceptive effect due to a local action, as drug administration in the contralateral paw was ineffective. Pretreatment of the injured paw with N^G-L-nitro-arginine methyl ester (L-NAME, an NO synthesis inhibitor), 1*H*-(1,2,4)-oxadiazolo(4,2-a)quinoxalin-1-one (ODQ, a soluble guanylyl cyclase inhibitor) or glibenclamide (an ATP-sensitive K^+ channel blocker) prevented ketorolac-induced antinociception. However, pretreatment with saline or N^G-D-nitro-arginine methyl ester (D-NAME) did not block antinociception. Local administration of *S*-nitroso-*N*-acetylpenicillamine (SNAP, an NO donor) was inactive by itself, but increased the effect of ketorolac. The present results suggest that the antinociceptive effect of ketorolac involves activation of the NO–cyclic GMP pathway, followed by an opening of ATP-sensitive K^+ channels at the peripheral level. © 2001 Published by Elsevier Science B.V.

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

1. Introduction

Ketorolac is a non-steroidal anti-inflammatory drug (NSAID) which exhibits a potent analgesic activity (for review, see Flores-Murrieta and Granados-Soto, 1996; Gillis and Brogden, 1997), being effective in the treatment of moderate to severe pain (Stanski et al., 1990). Initially, it has been suggested that ketorolac is a highly potent cyclooxygenase inhibitor (Rooks et al., 1982). However, subsequent experimental observations show that ketorolac exhibits a potency similar to those of indomethacin and diclofenac in inhibiting cyclooxygenase-1 and cyclooxygenase-2 (Pallapies et al., 1995; Jett et al., 1999). Hence, to explain the high potency and efficacy observed in both experimental and clinical pain, other mechanisms of action, additional to prostaglandin synthesis inhibition, have been proposed to be involved in the antinociceptive effect of ketorolac.

Activation of opioid receptors, either directly or by an indirect mechanism involving the release of endogenous opioids, has been suggested to play a role in ketorolac-induced analgesia (Domer, 1990; Uphouse et al., 1993). Nonetheless, the information available at present does not support such assumption. Ketorolac does not bind to μ-, δ- or κ-opioid receptors, does not exhibit any significant activity in the hot-plate assay, and is not blocked by the non-selective opioid antagonist naloxone (Bustamante and Paeile, 1993; Granados-Soto et al., 1995a; López et al., 1987; Rooks et al., 1982; Yee and Waterbury, 1987). On the other hand, there is evidence that ketorolac-induced antinociception can be significantly reduced by local administration of the nitric oxide (NO) synthesis inhibitor N^G-L-nitro-arginine methyl ester (L-NAME), suggesting that NO production at the peripheral level plays a role in

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antinociception (Granados-Soto et al., 1995b). There are presently a number of studies which suggest that stimulation of the NO–cyclic GMP pathway has an important participation in the antinociceptive action of several NSAIDs (Tonussi and Ferreira, 1994; Lorenzetti and Ferreira, 1996; Islas-Cadena et al., 1999; Aguirre-Bañuelos and Granados-Soto, 2000). Thus, it appears that an increase in NO synthesis in the periphery is induced by ketorolac and that this mechanism plays a role in antinociception, in addition to prostaglandin synthesis inhibition.

Interestingly, it has been suggested that opioid agonists, such as morphine, also stimulate the NO–cyclic GMP pathway at the peripheral level (Duarte et al., 1990; Granados-Soto et al., 1997; Aguirre-Bañuelos and Granados-Soto, 1999). There are observations that glibenclamide, an ATP-sensitive K^+ channel blocker, is able to reduce the antinociceptive effect of morphine in rats and mice after peripheral, spinal and supraspinal administration (Ocaña et al., 1990, 1995; Raffa and Codd, 1994; Raffa and Martinez, 1995; Kang et al., 1997, 1998; Rodrigues and Duarte, 2000). Recently, Soares et al. (2000) have suggested a link between the activation of the NO–cyclic GMP pathway and the opening of ATP-sensitive K^+ channels, as glibeclamide was able to block the antinociceptive effect of sodium nitroprusside. Taken together, these data suggest that opioid agonists produce a peripheral antinociceptive action through the activation of the NO–cyclic GMP pathway followed by the opening of ATP-sensitive K^+ channels. Since we have previously reported that ketorolac is able to increase NO synthesis in the periphery, we decided to extend our observations by studying the activation of the NO–cyclic GMP pathway and the participation of ATP-sensitive K^+ channels in the antinociceptive effect of this drug using the formalin test in the rat.

2. Material and methods

2.1. Animals

Female Wistar rats aged 6–7 weeks (weight range, 160–180 g) from our own breeding facilities were used in this study. Female animals were used based on our observation that formalin injection produces the same pattern of flinching in either sex (unpublished observation). Animals had free access to food and drinking water before experiments. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (IASP, 1983). Additionally, the study was approved by the Institutional Animal Care Committee.

2.2. Measurement of antinociceptive activity

Antinociception was assessed using the formalin test. Rats were placed in open Plexiglass observation chambers for 30 min to allow them to accommodate to their surroundings; then they were removed for formalin administration. Fifty microliters of diluted formalin (1%) were injected s.c. into the dorsal surface of the right hind paw with a 30-gauge needle. Animals were then returned to the chambers and nocifensive behavior was observed immediately after formalin injection. Mirrors were placed to enable unhindered observation. Nocifensive behavior was quantified as the number of flinches of the injected paw during 1-min periods every 5 up to 60 min after injection (Wheeler-Aceto and Cowan, 1991; Malmberg and Yaksh, 1992; Aguirre-Bañuelos and Granados-Soto, 2000). Flinching was readily discriminated and was characterized as rapid and brief withdrawal or flexing of the injected paw. Formalin-induced flinching behavior is biphasic. 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 the rats were sacrificed in a CO_2 chamber.

2.3. Drugs

Ketorolac tromethamine was a gift of Roche–Syntex (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) and *S*-nitroso-*N*-acetylpenicillamine (SNAP) were purchased from Research Biochemicals International (Natick, MA, USA). Glibenclamide (glyburide) was purchased from Sigma (St. Louis, MO, USA). Ketorolac tromethamine, L-NAME, D-NAME and *S*-nitroso-*N*-acetylpenicillamine were dissolved in saline. Glibenclamide was dissolved in dimethylsulfoxide 20%. ODQ was dissolved in propylenglycol 50%.

2.4. Study design

Rats received an s.c. injection into the dorsal surface of the right hind paw of appropriate vehicle or increasing doses of ketorolac (25, 50 and 100 μg) 20 min before formalin injection. To determine if ketorolac-induced antinociception was mediated by the NO–cyclic GMP–ATP-sensitive K^+ channel pathway, the effect of L-NAME, ODQ and glibenclamide pretreatment on antinociception was assessed. Drugs were injected locally at 30 min before formalin injection. Another group of rats received an inactive ketorolac dose (25 μg/paw) and increasing doses of *S*-nitroso-*N*-acetylpenicillamine in order to assess if this NO donor is able to produce an antinociceptive effect, or to potentiate that of ketorolac. Rats in all groups were tested for possible side effects such as reduction in righting, stepping, corneal and pinna reflexes and catalepsy.

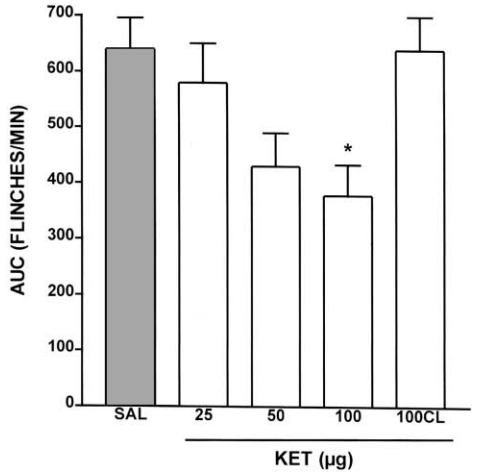

Fig. 1. Local antinociceptive effect of ketorolac in the formalin test. Rats were pretreated with saline or ketorolac into either the right (ipsilateral) or left (contralateral, CL) paw, before formalin injection. Data are expressed as the area-under-the-number-of-flinches against time curve (AUC). Bars are the means ± S.E.M. for seven to nine animals. * Significantly different from saline ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

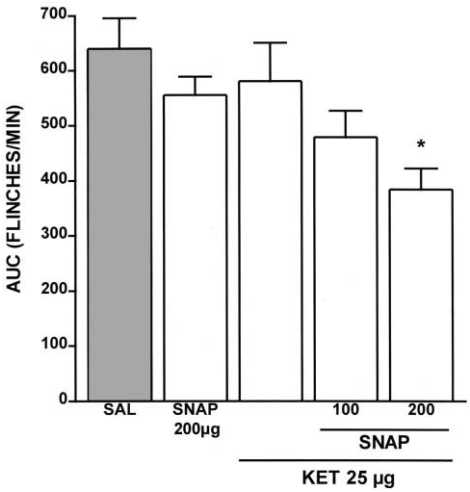

Fig. 3. Effect of *S*-nitroso-*N*-acetylpenicillamine (SNAP) on the peripheral antinociception produced by ketorolac. Rats were pretreated with *S*-nitroso-*N*-acetylpenicillamine and an ineffective dose of ketorolac into the right paw. Data are expressed as the area-under-the-number-of-flinches against time curve (AUC). Bars are the means ± S.E.M. for seven to nine animals. * Significantly different from the saline or ketorolac group ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

2.5. Data analysis and statistics

All results are presented as means ± S.E.M. for seven to nine animals per group. Curves were constructed potting the number of flinches as a function of 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 Tukey's test was used to compare differences between treatments. Differences were considered to reach statistical significance when $P < 0.05$.

3. Results

3.1. Peripheral antinociceptive effect of ketorolac

Formalin administration produced a typical pattern of flinching behavior. The first phase started immediately

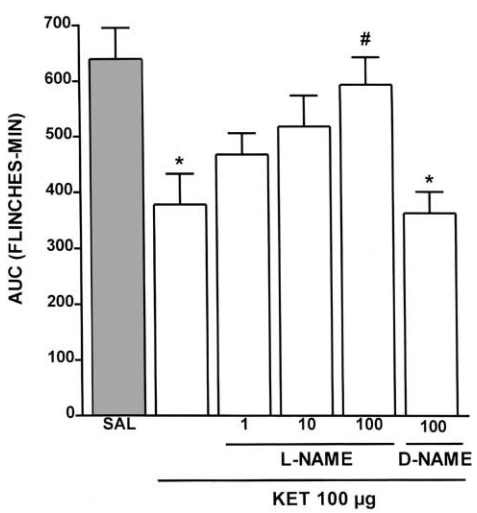

Fig. 2. Effect of L-NAME on the peripheral antinociception produced by ketorolac. Rats were pretreated with L-NAME or D-NAME and ketorolac into the right paw. Data are expressed as the area-under-the-number-of-flinches against time curve (AUC). Bars are the means ± S.E.M. for seven to nine animals. * Significantly different from the saline group ($P < 0.05$) and #significantly different from ketorolac ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

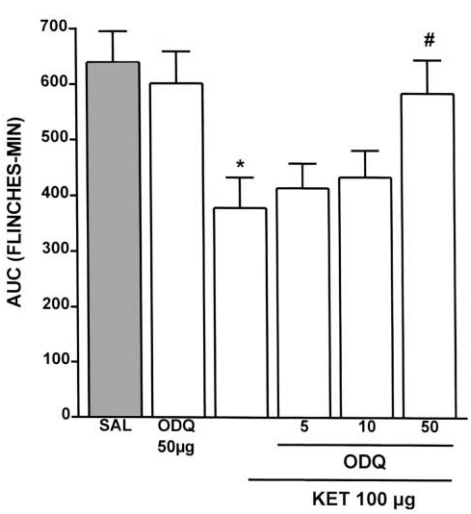

Fig. 4. Effect of ODQ on the peripheral antinociception produced by ketorolac. Rats were pretreated with ODQ and ketorolac into the right paw. Data are expressed as the area-under-the-number-of-flinches against time curve (AUC). Bars are the means ± S.E.M. for seven to nine animals. * Significantly different from the saline group ($P < 0.05$) and #significantly different from ketorolac ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

after administration of formalin and then diminished gradually in about 10 min. The second phase started at 15 min and lasted until 1 h (Aguirre-Bañuelos and Granados-Soto, 2000). Ipsilateral, but not contralateral, local administration of ketorolac produced a dose-dependent reduction in the flinching behavior, otherwise observed after formalin injection (Fig. 1). Ketorolac significantly reduced the number of flinches during phase two ($p < 0.05$), but not during phase one. No side effects were observed in either group, control or treated.

3.2. Effect of L-NAME and S-nitroso-N-acetylpenicillamine on the peripheral antinociceptive effect of ketorolac

Local pretreatment with the NO synthesis inhibitor L-NAME (100 μg/paw) did not produce any antinociceptive effect by itself (data not shown). However, L-NAME reversed the antinociception produced by ketorolac in a dose-dependent manner (Fig. 2). Administration of the inactive isomer, D-NAME, was not able to produce antinociception (data not shown), nor to reverse that produced by ketorolac (Fig. 2). Moreover, coadministration of an inactive dose of ketorolac (25 μg/paw) with the NO donor, *S*-nitroso-*N*-acetylpenicillamine (200 μg/paw), significantly reduced the nociceptive behavior ($P < 0.05$) during the second phase of the formalin test, despite the fact that *S*-nitroso-*N*-acetylpenicillamine, by itself, did not produce any antinociceptive effect. (Fig. 3).

3.3. Effect of ODQ and glibenclamide on the peripheral antinociceptive effect of ketorolac

Local pretreatment with the NO-sensitive guanylyl cyclase inhibitor ODQ (50 μg/paw) did not produce any antinociceptive effect by itself. However, pretreatment with ODQ, but not with saline, reversed ketorolac-induced antinociception in a dose-dependent fashion (Fig. 4). In a similar manner, local pretreatment with an ATP-sensitive K^+ channel inhibitor, glibenclamide (50 μg/paw), did not produce any antinociceptive effect, but significantly reversed ketorolac-induced antinociception in a dose-dependent manner (Fig. 5).

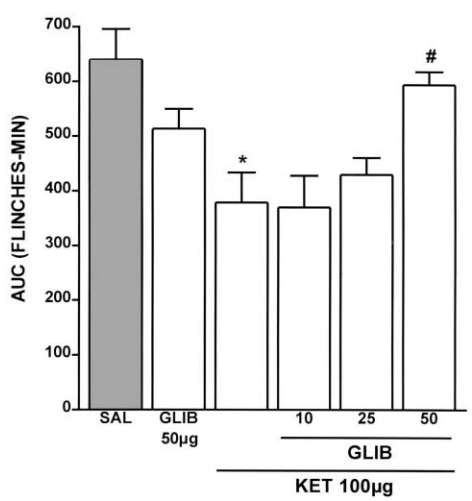

Fig. 5. Effect of glibenclamide on the peripheral antinociception produced by ketorolac. Rats were pretreated with glibenclamide and ketorolac into the right paw. Data are expressed as the area-under-the-number-of-flinches against time curve (AUC). Bars are the means ± S.E.M. for seven to nine animals. * Significantly different from the saline group ($P < 0.05$) and # significantly different from ketorolac ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

4. Discussion

In the present investigation, we were able to observe peripheral antinociception with ketorolac. The antinociceptive effect was not due to a systemic or a central action since the administration of ketorolac in the contralateral paw was inactive. The effect of ketorolac was blocked in a dose-dependent manner by the NO synthesis inhibitor, L-NAME, but not by saline nor by the inactive isomer of L-NAME, D-NAME. Moreover, coadministration of an NO donor with an otherwise inactive dose of ketorolac resulted in a significant antinociceptive effect. These results confirm our previous observations (Granados-Soto et al., 1995b) and support the hypothesis of the participation of NO synthesis at the peripheral level in the antinociceptive effect of several drugs (Duarte et al., 1990, 1992; Tonussi and Ferreira, 1994; Lorenzetti and Ferreira, 1996; Granados-Soto et al., 1997; Nozaki-Taguchi and Yamamoto, 1998; Islas-Cadena et al., 1999; Aguirre-Bañuelos and Granados-Soto, 2000). Furthermore, the antinociceptive effect of ketorolac was also blocked by ODQ, an inhibitor of NO-sensitive soluble guanylyl cyclase (Moro et al., 1996), suggesting that activation of the NO–cyclic GMP pathway plays an important role in ketorolac-induced antinociception in the formalin test.

We observed that glibenclamide, an ATP-sensitive K^+ channel blocker, was also able to diminish ketorolac-induced antinociception. It has been reported that glibenclamide specifically blocks ATP-sensitive K^+ channels, with no effect on Ca^{2+}- or voltage-dependent K^+ channels (Amoroso et al., 1990; Davies et al., 1991; Edwards and Weston, 1993). Therefore, our data suggest that the opening of ATP-sensitive K^+ channels is involved in the observed antinociceptive action of ketorolac. To our knowledge, this is the first report about the participation of the ATP-sensitive K^+ channel in the antinociceptive activity of a NSAID. Our results thus suggest that, in addition to prostaglandin synthesis inhibition, ketorolac activates the NO–cyclic GMP–ATP-sensitive K^+ channel pathway at the peripheral level, and that this mechanism plays a significant role in the antinociceptive response elicited by this compound. The evidence that NO can activate different types of K^+ channels in several tissues by an increase in cyclic GMP (Armstead, 1996; Carrier et al., 1997) is in line with our suggestion.

It has been suggested that activation of opioid receptors has a role in the stimulation of peripheral NO–cyclic GMP pathway by morphine (Duarte et al., 1990; Granados-Soto et al., 1997; Aguirre-Bañuelos and Granados-Soto, 1999). At present, there is evidence that the μ_3 opioid receptors are coupled to NO production (Stefano et al., 1995) and that glibenclamide is able to block the antinociceptive effect of morphine in rats and mice (Ocaña et al., 1990, 1995; Raffa and Codd, 1994; Raffa and Martinez, 1995; Kang et al., 1997, 1998; Rodrigues and Duarte, 2000). Moreover, it has been reported that glibenclamide blocks the antinociceptive effect of sodium nitroprusside, an NO donor (Soares et al., 2000). These observations strongly suggest that morphine activates the NO–cyclic GMP–ATP-sensitive K^+ channel pathway to produce an antinociceptive response. Our results show that ketorolac, a non-opioid drug, is also able to activate the NO–cyclic GMP–ATP-sensitive K^+ channel pathway in the periphery in a manner similar to morphine, and thus produce antinociception despite the fact that opioid receptors are not activated. The mechanism by which ketorolac activates the NO–cyclic GMP–ATP-sensitive K^+ channel pathway remains to be elucidated.

In summary, ketorolac produced peripheral antinociception in the formalin test in the rat. The antinociceptive effect of ketorolac was antagonized by L-NAME, ODQ and glibenclamide and potentiated by *S*-nitroso-*N*-acetylpenicillamine. These results strongly suggest that, besides prostaglandin synthesis inhibition, the activation of the NO–cyclic GMP–ATP-sensitive K^+ channel pathway plays an important role in the peripheral antinociceptive effect of ketorolac in the formalin test. The activation of this pathway may explain, at least partially, the marked efficacy of ketorolac observed in both experimental and clinical pain.

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