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The NO–cGMP–K^+ channel pathway participates in the antinociceptive effect of diclofenac, but not of indomethacin

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Abstract

The aim of this study was to examine if the peripheral antinociceptive effects of diclofenac and indomethacin involve the sequential participation of NO and cGMP synthesis followed by potassium channel opening. The peripheral antinociceptive effects of diclofenac, indomethacin, pinacidil (a potassium channel opener) and atrial natriuretic peptide (ANP, which increases cGMP content in a NO-independent manner) were assayed using the formalin test in the rat. All compounds produced significant local antinociception. Diclofenac effect was reverted by N^G-L-nitro-arginine methyl ester (L-NAME, an inhibitor of NO synthesis), by 1 H-(1,2,4)-oxadiazolo (4,2-a) quinoxalin-1-one (ODQ, an inhibitor soluble guanylyl cyclase), and by the potassium channel blockers glibenclamide, tolbutamide, charybdotoxin and apamin. Pinacidil effect was blocked by glibenclamide, tolbutamide, charybdotoxin and apamin, strongly suggesting that potassium channel opening results in antinociception. ANP effect was inhibited by the potassium channel blockers, but not by L-NAME, suggesting that potassium channel opening is a consequence of an increased cGMP content. Indomethacin was effective, but at doses higher than those of diclofenac, and could not be blocked by L-NAME nor by potassium channel blockers. The present results suggest that the L-arginine–NO–cGMP–potassium channel pathway is involved in the peripheral antinociceptive effect of diclofenac, but not of indomethacin, and thus provide evidence for differences in mechanisms of action among nonsteroidal antiinflammatory drugs (NSAIDs).

Keywords: Diclofenac; Indomethacin; Pinacidil; Atrial natriuretic peptide; Antinociception; Nitric oxide; Cyclic GMP; Potassium channels

1. Introduction

Diclofenac is a nonsteroidal antiinflammatory drug (NSAID) which exhibits potent analgesic and antiinflammatory properties. Diclofenac was introduced for the treatment of rheumatic and nonrheumatic disorders three decades ago (Todd and Sorkin, 1988) and is still extensively used (Suárez-Otero et al., 2002). Despite the long and widespread use of diclofenac, its exact mechanism of action has not yet been fully elucidated. It is known that diclofenac, as other nonselective NSAIDs, is able to impair prostaglandin synthesis by the inhibition the cyclooxygenase isozymes COX-1 and COX-2 in both, the injured tissue and the central nervous system (Vane and Botting, 1996; Vanegas, 2002; Warner et al., 1999). However, there is evidence that additional prostaglandin-independent mechanisms are involved in the antinociceptive action of diclofenac at both, the peripheral and central levels.

Björkman et al. (1992) demonstrated the analgesic effect of diclofenac after local injection into several brain regions and suggested that this effect is directly or indirectly due to the activation of opioid mechanisms, as it can be blocked by naloxone. Furthermore, it has been reported that diclofenac is able to increase β-endorphin levels in both plasma and brain (Martini et al., 1984; Sacerdote et al., 1985). It has also been suggested that diclofenac antinociceptive effect is

Abbreviations: AUC, area under the curve; cGMP, cyclic guanosine monophosphate; L-NAME, N^G-L-nitro-arginine methyl ester; NO, nitric oxide; NSAID, nonsteroidal antiinflammatory drug; ODQ, 1H-(1,2,4)-oxadiazolo(4,2-a)quinoxalin-1-one.

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mediated by descending inhibitory serotonin, opioid and/or other neurotransmitter systems interfering with nociceptive signals at the spinal level (Björkman, 1995). More recently, it has been reported that diclofenac increases the concentration of kynurenate, an endogenous antagonist of NMDA receptors, in the spinal cord and diencephalon preventing the pronociceptive actions of glutamate (Edwards et al., 2000).

There is also evidence for prostaglandin-independent mechanisms involved in the antinociceptive action of diclofenac at the peripheral level. Tonussi and Ferreira (1994) showed that local administration of diclofenac, but not of indomethacin, at the site of injury results in antinociception and that this effect could be blocked by local pretreatment with inhibitors of either nitric oxide (NO) or cyclic guanosine monophosphate (cGMP) synthesis. They thus proposed the participation of the L-arginine–NO–cGMP pathway in nociceptor desensitization. Further observations suggest that this pathway also participates in the peripheral antinociceptive action of several other NSAIDs, such as meloxicam, ketorolac, nimesulide, and metamizol (Aguirre-Bañuelos and Granados-Soto, 2000; Granados-Soto et al., 1995; Islas-Cadena et al., 1999; Lorenzetti and Ferreira, 1996). We have shown, using an experimental arthritis model, that local pretreatment at the injured joint with a NO synthesis inhibitor significantly reduces the antinociceptive effect of orally administrated diclofenac, but not of indomethacin or paracetamol (López-Muñoz et al., 1996a). These results suggest that it is likely that the peripheral activation of the L-arginine–NO–cGMP pathway is actually involved in the systemic effects of some, but not all, NSAIDs.

There is evidence that ion channels are also involved in diclofenac-induced peripheral antinociception. It has been reported that diclofenac is an inhibitor of H^+-gated channels in sensory neurons and that this action may explain the analgesic effect of topical drug application. Interestingly, indomethacin does not exhibit such effect (Voilley et al., 2001). Our group has provided evidence that potassium channels are also involved in the peripheral antinociceptive effect of diclofenac (Ortiz et al., 2002), as well as of ketorolac (Lázaro-Ibáñez et al., 2001). It has been suggested that potassium channels are opened as a consequence of the activation of the L-arginine–NO–cGMP pathway (Lázaro-Ibáñez et al., 2001) but, with the data available at present, it cannot be discarded that potassium channel opening may occur by a different mechanism. Therefore, the aim of the present work was to examine if the peripheral antinociceptive effects of diclofenac and indomethacin, two of the most widely used NSAIDs in therapeutics, involve the sequential participation of NO and cGMP synthesis followed by potassium channel opening. For this purpose, we examined the local antinociceptive effects of diclofenac, indomethacin, pinacidil and atrial natriuretic peptide (ANP) in the formalin test in the rat. Pinacidil was assayed as this drug is a nonspecific potassium channel opener (Bychkov et al., 1997; Khan et al., 1998). ANP is a heart-derived hormone involved in several physiological processes, including diuresis, natriuresis, vasodilation and blood pressure regulation (De Bold et al., 1991; De León et al., 1987). ANP binds to membrane receptors NPR-A and NPR-B coupled to particulate guanylyl cyclase catalyzing the formation of cGMP (Chinkers et al., 1989). Hence, ANP increases the intracellular levels of cGMP, but by a mechanism different from NO. Moreover, it is known that ANP induces a cGMP-dependent opening of potassium channels (White et al., 1993). Diclofenac, indomethacin, pinacidil and ANP were assayed in absence and presence of inhibitors of NO synthase and of NO-sensitive soluble guanylyl cyclase, as well as of potassium channel blockers.

2. Methods

2.1. Animals

Female Wistar rats aged 6–7 weeks (weight range, 180–200 g) from our own breeding facilities were used in this study. 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 Institutional Animal Care Committee approved the study.

2.2. Drugs

Diclofenac sodium was a gift of Novartis Farmacéutica (Mexico City). Indomethacin was a gift of Laboratorios Silanes (Mexico City). ANP, pinacidil, glibenclamide (glyburide), tolbutamide, charybdotoxin, and apamin were purchased from Sigma (St. Louis, MO, USA). N^G-L-nitroarginine methyl ester (L-NAME) and 1 H-(1,2,4)-oxadiazolo (4,2-a) quinoxalin-1-one (ODQ) were purchased from RBI (Natick, MA, USA). Diclofenac, ANP, charybdotoxin, apamin and L-NAME were dissolved in saline. Pinacidil, glibenclamide and tolbutamide were dissolved in dimethyl sulfoxide (20%). ODQ was dissolved in propylenglycol (20%). Indomethacin was dissolved in saline alkalinized with sodium bicarbonate.

2.3. Measurement of antinociceptive activity

Antinociception was assessed using the formalin test. Rats were placed in open Plexiglas 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 subcutaneously into the dorsal surface of the right hind paw with a 30-gauge needle. Animals were then returned to the chambers and nociceptive behaviour was observed immediately after formalin injection. Mirrors were

placed behind each chamber to enable unhindered observation of the formalin-injected paw. Nociceptive behaviour 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; Aguirre-Bañuelos and Granados-Soto, 2000). Flinching was readily identified and characterised as a rapid and brief withdrawal or flexing the injected paw. At the end of the experiment the rats were sacrificed in a CO_2 chamber.

2.4. Study design

Rats received a local injection of vehicle (50 μl/paw), diclofenac (100 μg/paw), or increasing doses of pinacidil (5–50 μg/paw), ANP (100–500 ng/paw) or indomethacin (50–800 μg/paw) in their right hind paw 20 min before formalin injection into the same paw. To determine whether the antinociceptive effect was due to a local action, an additional set of rats was injected with the tested drugs in the left (contralateral) paw 20 min before formalin was injected into the right paw.

To determine the participation of the L-arginine–NO–cGMP pathway in the peripheral antinociceptive effect of the tested drugs, rats were pretreated with L-NAME, a NO synthase inhibitor (Rees et al., 1990) or with ODQ, a NO-sensitive soluble guanylyl cyclase inhibitor (Moro et al., 1996). These inhibitors were locally injected into the formalin-injured paw 30 min before the insult, that is 10 min before the antinociceptive agent. To determine the participation of potassium channels in the antinociceptive response, the potassium channel blockers glibenclamide, tolbutamide, charybdotoxin and apamin were injected into the formalin-injured paw 10 min before the insult, that is 10 min after the antinociceptive agent. Glibenclamide and tolbutamide block ATP-sensitive potassium channels (Edwards and Weston, 1993), whereas charybdotoxin and apamin block large- and small-conductance Ca^{2+}-activated potassium channels, respectively (Sah, 1996).

Inhibitors and blockers were injected in a vehicle volume of 50 μl. Doses and drug administration schedules were selected based on previous reports (Ocaña et al., 1990; Rodrigues and Duarte, 2000; Soares and Duarte, 2001; Soares et al., 2000) and on pilot experiments in our laboratory. The observer was unaware of the treatment in each animal. Rats in all groups were tested for possible side effects such as reduction in righting, stepping, corneal and pinna reflexes, as previously described (Malmberg and Yaksh, 1992).

2.5. Data analysis and statistics

All results are presented as mean ± S.E.M. for six animals per group. Curves were made plotting the number of flinches against time. The area under the number of flinches against time curve (AUC) was calculated by the trapezoidal rule. Analysis of variance followed by the Tukey's test was used to compare the differences between treatments. Differences were considered to achieve statistical significance when $P < .05$.

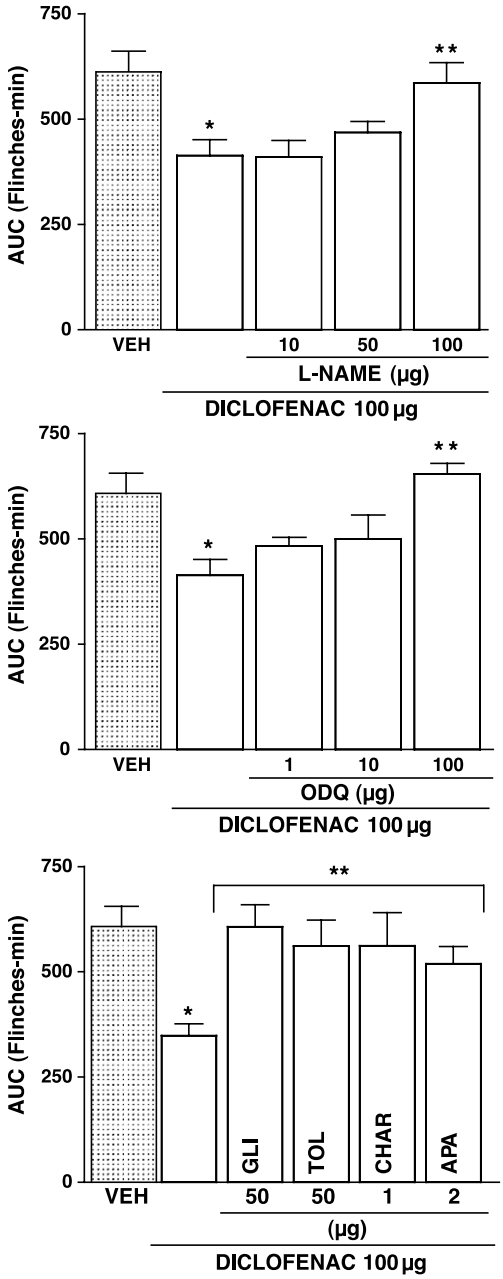

Fig. 1. Local antinociceptive effect of diclofenac in the formalin test. Diclofenac was assayed in absence and presence of increasing doses of the inhibitor of NO synthase L-NAME (upper panel) and of the inhibitor of soluble guanylyl cyclase ODQ (middle panel), as well as of the potassium channel blockers glibenclamide (GLI), tolbutamide (TOL), charybdotoxin (CHAR) and apamin (APA) (lower panel). Data are expressed as the area under the number of flinches against time curve (AUC) corresponding to the second phase of the formalin test. Bars indicate the mean ± S.E. of six animals. * Significantly different from vehicle ($P < .05$), and ** significantly different from diclofenac ($P < .05$) as determined by analysis of variance followed by the Tukey's test.

3. Results

3.1. Peripheral antinociceptive effect of diclofenac

Local injection of 1% formalin produced a typical flinching behaviour. Flinching behaviour was biphasic. The first phase started immediately after formalin injection, decreasing gradually in about 10 min. The second phase started at 15 min and lasted until 60 min (Lázaro-Ibáñez et al., 2001; Ortiz et al., 2002). Ipsilateral, but not contralateral, local administration of diclofenac significantly reduced flinching behaviour otherwise observed after formalin injection, but only during the second phase of the assay, being inactive on Phase 1 (Ortiz et al., 2002). Therefore, only the data from Phase 2 were submitted for further analysis. Pretreatment with L-NAME was able to significantly prevent the local effect of diclofenac in a dose-dependent manner (Fig. 1, upper panel). ODQ exhibited a similar pattern, being also able to significantly inhibit diclofenac-induced antinociception in a dose-dependent manner (Fig. 1, middle panel). The potassium channel blockers glibenclamide, tolbutamide, charybdotoxin and apamin significantly abolished antinociception due to diclofenac (Fig. 1, lower panel). Local administration of L-NAME, ODQ, glibenclamide, tolbutamide, charybdotoxin or apamin, in absence of diclofenac was not able to significantly modify formalin-induced flinching behaviour (Fig. 2). No side effects were observed in any of the studied groups of animals.

3.2. Peripheral antinociceptive effect of pinacidil

Ipsilateral, but not contralateral, local administration of the potassium channel opener pinacidil significantly reduced flinching behaviour in a dose-dependent manner during the second phase of the formalin test (Fig. 3, upper panel). Pinacidil, as diclofenac, failed to reduce flinching during the first phase of the assay (data not shown). The antinociceptive effect of pinacidil in Phase 2 was significantly inhibited by glibenclamide, tolbutamide, charybdotoxin and apamin (Fig. 3, lower panel). No side effects were observed in any of the studied groups of animals.

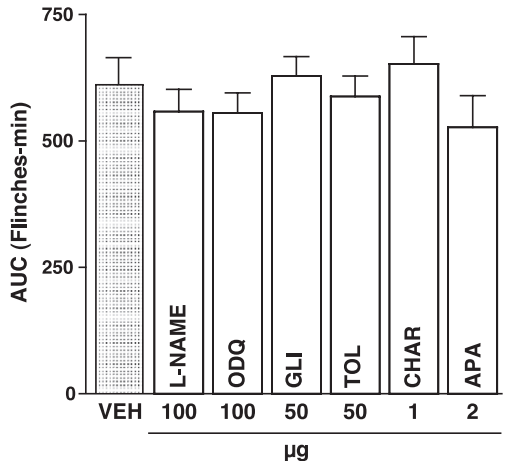

Fig. 2. Lack of local effects of the inhibitor of NO synthase L-NAME, the inhibitor of soluble guanylyl cyclase ODQ, and the potassium channel blockers glibenclamide (GLI), tolbutamide (TOL), charybdotoxin (CHAR) and apamin (APA) in the formalin test. Data are expressed as the area under the number of flinches against time curve (AUC) corresponding to the second phase of the formalin test. Bars indicate the mean ± S.E. of six animals.

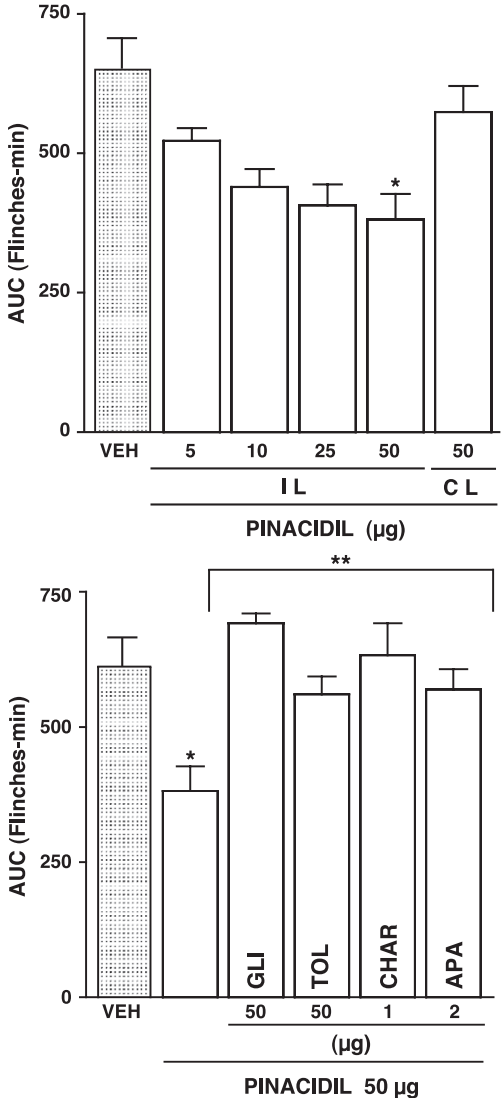

Fig. 3. Upper panel: local antinociceptive effect of the potassium channel opener pinacidil in the formalin test after ipsilateral (IL) and contralateral (CL) administration. Lower panel: effect of the IL administration of pinacidil assayed in absence and presence of the potassium channel blockers glibenclamide (GLI), tolbutamide (TOL), charybdotoxin (CHAR) and apamin (APA). Data are expressed as the area under the number of flinches against time curve (AUC) corresponding to the second phase of the formalin test. Bars indicate the mean ± S.E. of six animals. * Significantly different from vehicle ($P < .005$), and ** significantly different from pinacidil ($P < .05$) as determined by analysis of variance followed by the Tukey's test.

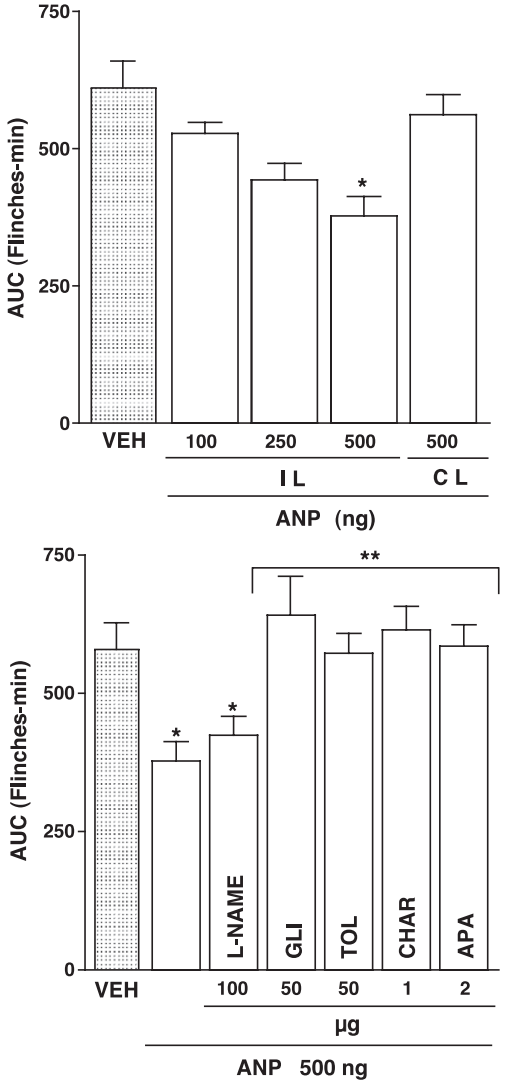

Fig. 4. Upper panel: local antinociceptive effect of atrial natriuretic peptide (ANP) in the formalin test after ipsilateral (IL) and contralateral (CL) administration. Lower panel: effect of the IL administration of ANP assayed in absence and presence the inhibitor of NO synthase L-NAME and of the potassium channel blockers glibenclamide (GLI), tolbutamide (TOL), charybdotoxin (CHAR) and apamin (APA). Data are expressed as the area under the number of flinches against time curve (AUC) corresponding to the second phase of the formalin test. Bars indicate the mean ± S.E. of six animals. * Significantly different from vehicle ($P < .005$), and * * significantly different from ANP ($P < .05$) as determined by analysis of variance followed by the Tukey's test.

3.3. Peripheral antinociceptive effect of ANP

Ipsilateral, but not contralateral, local administration of ANP significantly reduced flinching behaviour in a dose-dependent manner during Phase 2 of the formalin test (Fig. 4, upper panel). ANP, as diclofenac and pinacidil, failed to reduce flinching during the first phase of the assay (data not shown). The antinociceptive effect of ANP was significantly abolished by the potassium channel blockers glibenclamide, tolbutamide, charybdotoxin and apamin (Fig. 4, lower panel). Notwithstanding, the inhibitor of NO synthase, L-NAME, failed to significantly inhibit the antinociceptive effect of ANP at 100 µg/paw, dose that reverted the antinociceptive effect of diclofenac. No side effects were observed in any of the studied groups of animals.

3.4. Peripheral antinociceptive effect of indomethacin

Ipsilateral, but not contralateral, administration of indomethacin produced a dose-dependent reduction of formalin-induced flinching behaviour in the second phase of the formalin test (Fig. 5, upper panel). However, indomethacin was not as active as diclofenac, since 800 µg/paw was

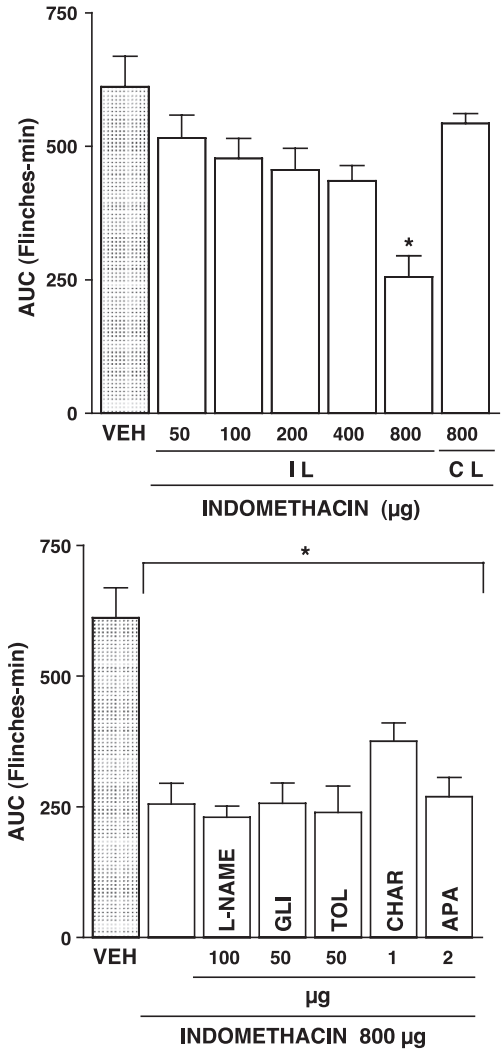

Fig. 5. Upper panel: local antinociceptive effect of indomethacin in the formalin test after ipsilateral (IL) and contralateral (CL) administration. Lower panel: effect of the IL administration of indomethacin assayed in absence and presence the inhibitor of NO synthase L-NAME and of the potassium channel blockers glibenclamide (GLI), tolbutamide (TOL), charybdotoxin (CHAR) and apamin (APA). Data are expressed as the area under the number of flinches against time curve (AUC) corresponding to the second phase of the formalin test. Bars indicate the mean ± S.E. of six animals. * Significantly different from vehicle ($P < .005$), as determined by analysis of variance followed by the Tukey's test.

required to achieve a statistically significant antinociceptive response. Diclofenac was able to significantly reduce flinching at 100 μg/paw. As diclofenac, pinacidil and ANP, indomethacin failed to reduce flinching during the Phase 1 of the formalin test (data not shown). The antinociceptive effect of indomethacin could not be abolished by the inhibitor of NO synthase L-NAME, nor by the potassium channel blockers glibenclamide, tolbutamide, charybdotoxin and apamin, at doses at which these compounds were able to significantly abolish the antinociceptive effects of diclofenac (Fig. 5, lower panel). No side effects were observed in any of the studied groups of animals.

4. Discussion

It is well known that diclofenac and indomethacin are nonselective inhibitors of prostaglandin synthesis by COX-1 and COX-2 (Vane and Botting, 1996; Warner et al., 1999). Prostaglandin synthesis inhibition is likely involved in the antiinflammatory and antinociceptive effects of these compounds, but additional mechanisms of action cannot be excluded (Björkman, 1995; Tonussi and Ferreira, 1994). Prostaglandin-independent mechanisms have been described for some NSAIDs at both, the peripheral and central levels (Aguirre-Bañuelos and Granados-Soto, 2000; Björkman, 1995; Edwards et al., 2000; Martini et al., 1984; Ortiz et al., 2002; Sacerdote et al., 1985; Tonussi and Ferreira, 1994; Voilley et al., 2001). Since the NSAID group is remarkably heterogeneous from a structural point of view, it is likely that there are differences in the mechanisms of antinociceptive action among its individual members. For example, differences in the mechanisms of peripheral antinociception between diclofenac and indomethacin have been observed by Tonussi and Ferreira (1994) and then confirmed by our group (López-Muñoz et al., 1996a).

Our group has been interested in the prostaglandin-independent mechanisms of NSAID-induced antinociception at the peripheral level, particularly on the activation of the L-arginine–NO–cGMP pathway in the primary afferent neurons. The role of this pathway is supported by several observations. It has been described that NO donors are able to induce a dose-dependent antinociceptive effect (Soares et al., 2000) and to potentiate the antinociceptive response of some NSAIDs (Islas-Cadena et al., 1999; Lázaro-Ibáñez et al., 2001). Local administration of membrane permeable analogs of cGMP are also able to induce antinociception (Soares and Duarte, 2001) and to potentiate the effect of caffeine–ketorolac combinations (Aguirre-Bañuelos et al., 1999). Moreover, sildenafil, an inhibitor of phosphodiesterase 5, induces peripheral antinociception (Jain et al., 2001) and is able to potentiate the local antinociceptive effect of diclofenac (Asomoza-Espinosa et al., 2001). However, the role of the L-arginine–NO–cGMP pathway as a mechanism of antinociception, is controversial. There are observations documenting that an increase in NO in the periphery is pronociceptive rather than antinociceptive (Alley et al., 1998; Meller et al., 1994). It then appears that activation of the L-arginine–NO–cGMP pathway can produce contrasting effects depending on the experimental pain model used. Kawabata et al. (1994) have proposed that the role of NO in peripheral nociception depends on the tissue level. Thus, it is likely that there is a heterogeneous population of primary neurons in the periphery, with regard to the role of the L-arginine–NO–cGMP pathway. This is the case of the spinal cord, where different subsets of neurons have been identified. Activation of this pathway appears to induce an excitatory effect on the majority of spontaneously active neurons in lamina X, whereas neurons in superficial lamina I/II are predominantly inhibited (Pehl and Schmid, 1997). Notwithstanding, it is also possible that the contrasting effects of the activation of the L-arginine–NO–cGMP pathway depend on different mechanisms of action triggered by high or low cGMP intracellular content. This has been described for spinal neurons, and it appears that antinociceptive mechanisms predominate as they require much less cGMP than pronociceptive processes (Tegeder et al., 2002).

There is evidence that the opening of potassium channels is involved in the peripheral antinociceptive effects produced by certain NSAIDs, such as ketorolac and diclofenac (Lázaro-Ibáñez et al., 2001; Ortiz et al., 2002), and by morphine (Rodrigues and Duarte, 2000). It has been reported that glibenclamide, an ATP-sensitive potassium channel blocker, reduces the antinociceptive effects of the NO donor sodium nitroprusside (Soares et al., 2000), suggesting a link between the activation L-arginine–NO–cGMP pathway and potassium channel opening. In this study, we observed that diclofenac induced a local (peripheral) antinociceptive effect in the formalin test which could be abolished by inhibitors of NO synthase and NO-sensitive soluble guanylyl cyclase, as well as by potassium channel blockers, observations that are in line with such assumption. L-NAME, ODQ, glibenclamide, tolbutamide, charybdotoxin and apamin, by themselves, did not produce any significant alteration in formalin-induced flinching behaviour. These results allow excluding the possibility that the prevention of diclofenac antinociception was due to a hyperalgesic or nociceptive effect. Furthermore, the observation that pinacidil was able to mimic the antinociceptive response of diclofenac, and that its effect could be blocked by glibenclamide, tolbutamide, charibdotoxin and apamin, strongly supports the participation of potassium channels in the antinociceptive response. The fact that pinacidil effect could be reverted by blockers of ATP-sensitive and large- and small- conductance Ca^{2+}-activated potassium channels indicates that this compound is a nonselective potassium channel opener, as it has been previously pointed by other investigators (Bychkov et al., 1997; Khan et al., 1998).

ANP, as diclofenac and pinacidil, produced a significant antinociceptive effect. Antinociception appeared to be due to a local action, as ANP injection in the contralateral paw did not produce any significant modification in formalin-

induced flinching behaviour. To our knowledge, this is the first report on the ability of ANP to produce peripheral antinociception. It has been well established that ANP activates particulate guanylyl cyclase (Chinkers et al., 1989) and modulates various ion channels, including Ca^{2+}-activated, ATP-sensitive, inwardly rectifying, and outwardly rectifying potassium channels (Kourie and Rive, 1999; Waldman and Murad, 1987). In the present study, we observed that the local antinociceptive effect of ANP was inhibited by ATP-sensitive and large- and small-conductance Ca^{2+}-activated potassium channel blockers, but not by the inhibitor of NO synthase L-NAME. These results suggest that ANP produces antinociception by a mechanism similar to that participating in its vasodilator effect, that is a cGMP-induced opening of potassium channels (Waldman and Murad, 1987). The failure of L-NAME to inhibit the effect of ANP is explained by the fact that the increase in intracellular cGMP content by this cardiac peptide does not imply the participation of NO-sensitive guanylyl cyclase.

It is well known that NSAIDs exhibit a significant antinociceptive effect only on Phase 2 of the formalin test, being ineffective on the first phase (Malmberg and Yaksh, 1992). This is due to the fact that the two phases of this assay involve different mechanisms of nociception. It has been suggested that the first phase is due to a direct effect on nociceptors while the second phase corresponds to inflammatory pain (Hunskaar and Hole, 1987). In the present study, we observed that pinacidil and ANP were ineffective on the first phase of the assay, suggesting that these compounds, as NSAIDs, exert their peripheral antinociceptive action only on inflammatory pain. This fact is consistent with a common effector antinociceptive mechanism, shared by diclofenac, pinacidil and ANP.

Taken together, the results obtained with the local administration of diclofenac, pinacidil and ANP give strong support to the hypothesis that antinociception in the periphery can be obtained by activation of the L-arginine–NO–cGMP–potassium channel pathway in a sequential manner (Lázaro-Ibáñez et al., 2001). It has been previously shown that this pathway participates in other physiological processes such as vasodilation (Archer et al., 1994; Sampson et al., 2001; Wu et al., 2001) and the relaxation of colonic smooth muscle (Koh et al., 1995). There is evidence that ANP produces potassium channel stimulation through cGMP-dependent dephosphorilation (White et al., 1993). Therefore, it seems likely that potassium channel opening produced by an increased intracellular concentration of cGMP is the final effector mechanism of the antinociceptive response produced by diclofenac and ANP. Riedel and Neeck (2001) have pointed that, depending on the expression of cGMP-controlled ion channels in the neurons involved, the response can be nociception or antinociception. Our data suggest the presence in subcutaneous tissue nociceptors of ATP-sensitive and large- and small-conductance Ca^{2+}-activated potassium channels controlled by cGMP. Potassium channel opening results in an outward potassium leakage causing the hyperpolarization, and thus desensitization, of the primary neuron. Our results thus show that ANP appears to be a useful tool for the characterization of the participation of cGMP-stimulated potassium channel opening in antinociception.

The L-arginine–NO–cGMP–potassium channel pathway, however, does not appear to be a mechanism involved in the peripheral effects of all NSAIDs since L-NAME, glibenclamide, tolbutamide, charybdotoxin and apamin were not able to significantly inhibit the local antinociceptive response of indomethacin. Our observations in the formalin test confirm previous reports that inhibition of NO and cGMP synthesis do not affect the antinociceptive effect of indomethacin in other experimental pain models (López-Muñoz et al., 1996a; Tonussi and Ferreira, 1994). As mentioned above, it is well known that both, diclofenac and indomethacin are nonselective inhibitors of prostaglandin synthesis inhibition, and that this mechanism of action plays an important role in its antinociceptive actions (Vane and Botting, 1996; Warner et al., 1999). It is therefore likely that indomethacin is a "pure" prostaglandin synthesis inhibitor, while diclofenac, and other NSAIDs such as ketorolac, metamizol, nimesulide and meloxicam (Aguirre-Bañuelos and Granados-Soto, 2000; Granados-Soto et al., 1995; Islas-Cadena et al., 1999; Lorenzetti and Ferreira, 1996) exhibit additional mechanisms of action. In line with this assumption, we have observed that the antinociceptive effect of ketorolac in an experimental arthritis model can be partially, but not completely, abolished by the local injection of L-NAME in the injured joint (Lopez-Muñoz et al., 1996b). The remaining effect is probably due to prostaglandin synthesis inhibition, although the involvement of other mechanisms cannot be ruled out. The information available at present thus suggests that there are important differences in the mechanisms of antinociceptive action among NSAIDs, and particularly between diclofenac and indomethacin. Diclofenac is able to activate the L-arginine–NO–cGMP–potassium channel pathway and to inhibit H^+-gated channels (Voilley et al., 2001), while indomethacin does not. This is consistent with the fact that, in the present study, the dose of indomethacin required to achieve a significant local antinociceptive effect in the formalin test was about four times higher than that of diclofenac.

In summary, the L-arginine–NO–cGMP–potassium channel pathway is involved in the peripheral antinociceptive effect of diclofenac. Potassium channel opening appears to be the consequence of an increase in the intracellular content of cGMP at the nociceptor. Augmented cGMP concentrations can be achieved by activation of NO-sensitive soluble guanylyl cyclase, as it is the case of diclofenac, or by activation of NO-insensitive particulate guanylyl cyclase, as it is the case of ANP. Direct opening of potassium channels, as it is the case of pinacidil, also results in antinociception. Unlike diclofenac, the peripheral antinociceptive effect of indomethacin does not imply the participation of the L-arginine–NO–cGMP–potassium

channel pathway. The present results provide evidence for differences in the mechanisms of peripheral antinociceptive action among NSAIDs.

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