ELSEVIER

European Journal of Pharmacology 438 (2002) 85–91

www.elsevier.com/locate/ejphar

Pharmacological evidence for the activation of K^+ channels by diclofenac

Mario I. Ortiz [a,b], Jorge E. Torres-López [c,d], Gilberto Castañeda-Hernández [a], Rodolfo Rosas [e], Guadalupe C. Vidal-Cantú [d], Vinicio Granados-Soto [d,*]

[a] *Sección Externa de Farmacología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México, D.F., Mexico*
[b] *Area Académica de Medicina del Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Pachuca, Hidalgo, Mexico*
[c] *Centro de Investigación y Posgrado, División Académica de Ciencias de la Salud, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, Mexico*
[d] *Departamento de Farmacobiología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Czda. de los Tenorios 235, Col. Granjas Coapa, 14330 México, D.F., Mexico*
[e] *Novartis Farmacéutica, México, D.F., Mexico*

Received 14 January 2002; accepted 18 January 2002

Abstract

The involvement of K^+ channels in the antinociceptive action of diclofenac was assessed in the formalin test. Local administration of diclofenac produced a dose-dependent antinociceptive effect due to a local action because drug administration in the contralateral paw was ineffective. Pretreatment of the injured paw with glibenclamide and tolbutamide (ATP-sensitive K^+ channel inhibitors), charybdotoxin and apamin (large- and small-conductance Ca^{2+}-activated K^+ channel blockers, respectively), 4-aminopyridine or tetraethylammonium (voltage-dependent K^+ channel inhibitors) prevented diclofenac-induced antinociception. Given alone, K^+ channel inhibitors did not modify formalin-induced nociceptive behavior. Pinacidil (an ATP-sensitive K^+ channel opener) also produced antinociception which was blocked by glibenclamide. The peripheral antinociceptive effect of morphine (positive control) was blocked by glibenclamide and 4-aminopyridine but not by charybdotoxin or apamin. The results suggest that the peripheral antinociceptive effect of diclofenac may result from the activation of several types of K^+ channels, which may cause hyperpolarization of peripheral terminals of primary afferents.

Keywords: Diclofenac; Apamin; Charybdotoxin; Glibenclamide; Pinacidil; Morphine

1. Introduction

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) which has strong anti-inflammatory, antipyretic and analgesic activities (Menassé et al., 1978; Todd and Sorkin, 1988). This drug has been proven to be effective in the treatment of rheumatic and non-rheumatic conditions (Todd and Sorkin, 1988). However, it has been suggested that in addition to the in vitro and in vivo inhibition of cyclooxygenase (Oliw et al., 1978), diclofenac apparently possesses additional mechanisms of action (Attal et al., 1988). Some reports have suggested a central analgesic action of diclofenac mediated by endogenous opioids in some brain stem nuclei related to the control of nociception (Sacerdote et al., 1985; Vescovi et al., 1986; Björkman, 1995). However, there is evidence that naloxone (an opioid receptor antagonist) and *N*-methyl-nalorphine (a peripheral opioid receptor antagonist) are able to block the antinociception produced by morphine but not that produced by diclofenac (Tonussi and Ferreira, 1994). Thus, these results demonstrate that the action of diclofenac is not due to the peripheral or central release of an opioid-like substance.

It has been reported that the inhibition of serotonergic transmission by pretreatment with methiotepin, ritanserin, parachlorophenylalanine or 5,7-dihydroxytryptamine reduces the antinociceptive effect of diclofenac (Björkman, 1995), suggesting a direct relationship between central serotonergic mechanisms and the antinociceptive effect of diclofenac. In addition, diclofenac is able to block in a dose-dependent manner the hyperalgesia induced by intrathecal *N*-methyl-D-aspartate (NMDA), but not that induced by intrathecal substance P or DL-α-NH_2-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid (AMPA). This effect is

* Corresponding author. Tel.: +52-5-483-2868; fax: +52-5-483-2863.
E-mail address: vgranados@prodigy.net.mx (V. Granados-Soto).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01288-8

reversed by L-arginine but not by D-arginine. These data suggest that diclofenac is able to interfere with the pronociceptive activity of the nitric oxide (NO) system at the spinal level (Björkman, 1995).

In contrast, in the periphery, diclofenac-induced antinociception can be partially diminished by NO synthesis inhibition (Tonussi and Ferreira, 1994; López-Muñoz et al., 1996). This is consistent with several studies showing that the NO–cyclic GMP pathway plays an important role in the peripheral antinociception induced by some NSAIDs (Lorenzetti and Ferreira, 1996; López-Muñoz et al., 1996; Aguirre-Bañuelos and Granados-Soto, 2000).

We have found recently that ketorolac-induced antinociception can be blocked by glibenclamide (Lázaro-Ibáñez et al., 2001), suggesting that this drug can activate ATP-sensitive K^+ channels in order to produce its antinociceptive effect. Therefore, this work was undertaken to determine whether specific and non-specific K^+ channel blockers have any effect on the peripheral antinociception induced by diclofenac. For this purpose, we tested the actions of glibenclamide and tolbutamide (ATP-sensitive K^+ channel blockers; Edwards and Weston, 1993), charybdotoxin (a selective blocker of large-conductance Ca^{2+}-activated K^+ channels; Miller et al., 1985; Stretton et al., 1992), apamin (a selective blocker of small-conductance Ca^{2+}-activated K^+ channels; Romey et al., 1984), 4-aminopyridine and tetraethylammonium (voltage-dependent K^+ channel blockers; Cook and Quast, 1990).

2. Material and 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 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 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%) was 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 nociceptive behavior was observed immediately after formalin injection. Mirrors were placed to enable unhindered observation. Nociceptive behavior was quantified as the number of flinches of the injected paw during 1-min periods every 5 min up to 60 min after injection (Wheeler-Aceto and Cowan, 1991; Aguirre-Bañuelos and Granados-Soto, 2000). Flinching was readily identified and 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 killed in a CO_2 chamber.

2.3. Drugs

Diclofenac sodium was a gift of Novartis Farmacéutica (Mexico City). Morphine was obtained from Secretaría de Salud (Mexico City). Glibenclamide (glyburide), tolbutamide, charybdotoxin, apamin, pinacidil, 4-aminopyridine and tetraethylammonium were purchased from Sigma (St. Louis, MO, USA). Diclofenac sodium, morphine, 4-aminopyridine, tetraethylammonium, charybdotoxin and apamin were dissolved in saline. Glibenclamide, tolbutamide and pinacidil were dissolved in dimethyl sulfoxide (20%).

2.4. Study design

Rats received appropriate vehicle (50 μl) or increasing doses of diclofenac either ipsilaterally (10–200 μg/paw) or contralaterally (200 μg/paw) 20 min before formalin injection. To assess if the antinociceptive effect of drugs was due to a local action, formalin was administered in one paw and the test drug in the contralateral paw (s.c.). Doses were selected on the basis of previous pilot studies with our model. The observer was unaware of the treatment in each animal. To determine whether diclofenac-induced antinociception was mediated by K^+ channels, the effect of glibenclamide, tolbutamide, apamin, charybdotoxin, 4-aminopyridine and tetraethylammonium (-10 min) on the antinociceptive effect induced by diclofenac was assessed. Other groups received pinacidil in order to assess the participation of ATP-sensitive K^+ channels in antinociception in the formalin test. In addition, morphine was used as positive control because there is evidence that its antinociceptive effect can be blocked by ATP-sensitive, but not Ca^{2+}-activated, K^+ channel inhibitors (Rodrigues and Duarte, 2000). Rats in all groups were tested for possible side effects such as reduction of righting, stepping and corneal reflexes.

2.5. Data analysis and statistics

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

3. Results

3.1. Peripheral antinociceptive effect of diclofenac

Formalin administration produced a typical pattern of flinching behavior (Lázaro-Ibáñez et al., 2001). The first phase started immediately after the administration of formalin and then diminished gradually in about 10 min. The second phase started at about 15 min and lasted until 1 h. Ipsilateral, but not contralateral, local administration of diclofenac produced a dose-dependent reduction in the flinching behavior otherwise observed after formalin injection (Fig. 1). Diclofenac 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.

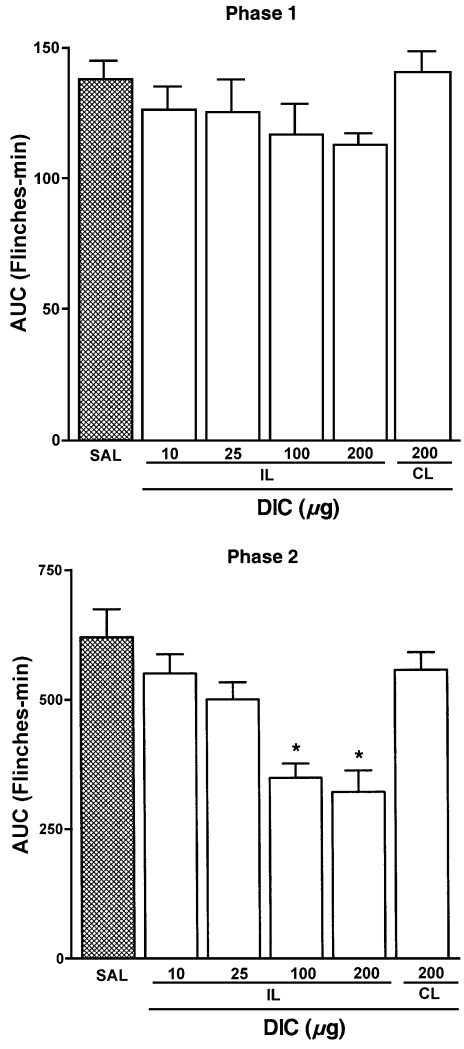

Fig. 1. Local antinociceptive effect of diclofenac (DIC) in the formalin test. Rats were pretreated with s.c. injection of saline or diclofenac into either the right (ipsilateral, IL) or left (contralateral, CL) paw before formalin injection. Data are expressed as the area under the number of flinches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

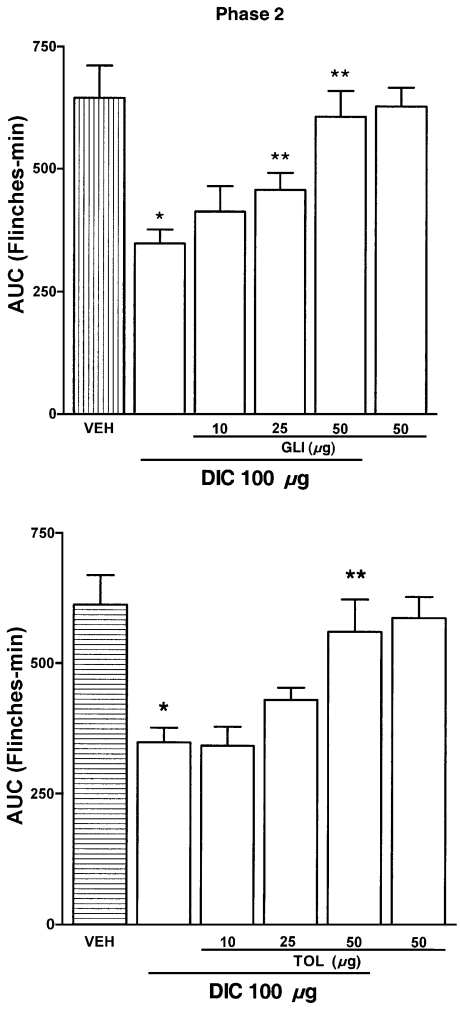

Fig. 2. Effect of ATP-sensitive K^+ channel inhibitors glibenclamide (GLI, top panel) and tolbutamide (TOL, bottom panel) on the peripheral antinociception produced by diclofenac (DIC) during the second phase of the formalin test. Rats were pretreated with s.c. injection of glibenclamide or tolbutamide plus diclofenac into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean ± S.E.M. for six animals. * Significantly different from the vehicle group ($P<0.05$) and ** significantly different from the diclofenac group ($P<0.05$) as determined by the analysis of variance followed by Tukey's test.

3.2. Effect of glibenclamide, tolbutamide, charybdotoxin, apamin, 4-aminopyridine and tetraethylammonium on the peripheral antinociceptive effect of diclofenac

Local pretreatment with the ATP-sensitive K^+ channel inhibitors glibenclamide and tolbutamide significantly reversed in a dose-dependent manner ($P<0.05$) diclofenac-induced antinociception (Fig. 2). Charybdotoxin and apamin (large- and small-conductance Ca^{2+}-activated K^+ channel blockers, respectively) (Fig. 3) as well as 4-aminopyridine and tetraethylammonium (voltage-dependent K^+

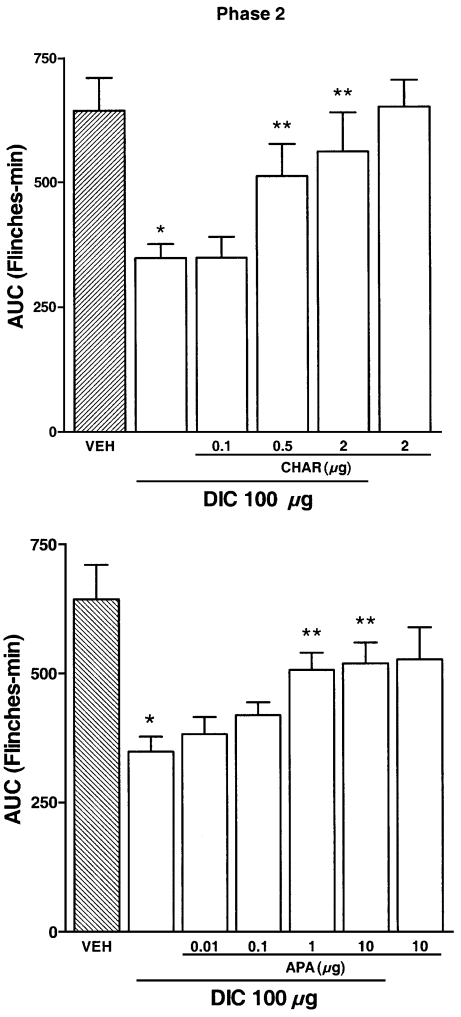

Fig. 3. Effect of large- and small-conductance Ca^{2+}-activated K^+ channel blockers charybdotoxin (CHAR, top panel) and apamin (APA, bottom panel), respectively, on the peripheral antinociception produced by diclofenac (DIC) during the second phase of the formalin test. Rats were pretreated with s.c. injection of charybdotoxin or apamin plus diclofenac into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean ± S.E.M. for at least six animals. * Significantly different from the vehicle group ($P<0.05$) and * * significantly different from the diclofenac group ($P<0.05$) as determined by the analysis of variance followed by Tukey's test.

channel blockers) (Fig. 4) also antagonized the antinociception produced by diclofenac. Given alone, K^+ channel blockers did not modify the formalin-induced nociceptive behavior.

3.3. Effect of glibenclamide on the peripheral antinociceptive effect of pinacidil

Under the same conditions, pinacidil (an ATP-sensitive K^+ channel opener) produced dose-dependent antinociception during the second phase of the formalin test (Fig. 5). The antinociceptive effect of pinacidil was reversed by the ipsilateral administration of glibenclamide (Fig. 5). Contralateral administration of K^+ channels blockers did not modify diclofenac- or pinacidil-induced antinociception (data not shown).

3.4. Effect of glibenclamide, 4-aminopyridine, charybdotoxin and apamin on the peripheral antinociception induced by morphine

A previous study has shown that the ipsilateral administration of morphine (1.25–10 μg/paw) produces a dose-dependent peripheral antinociceptive effect during the second phase of the formalin test (Mixcoatl-Zecuatl et al., 2000). In this study, the local administration of morphine

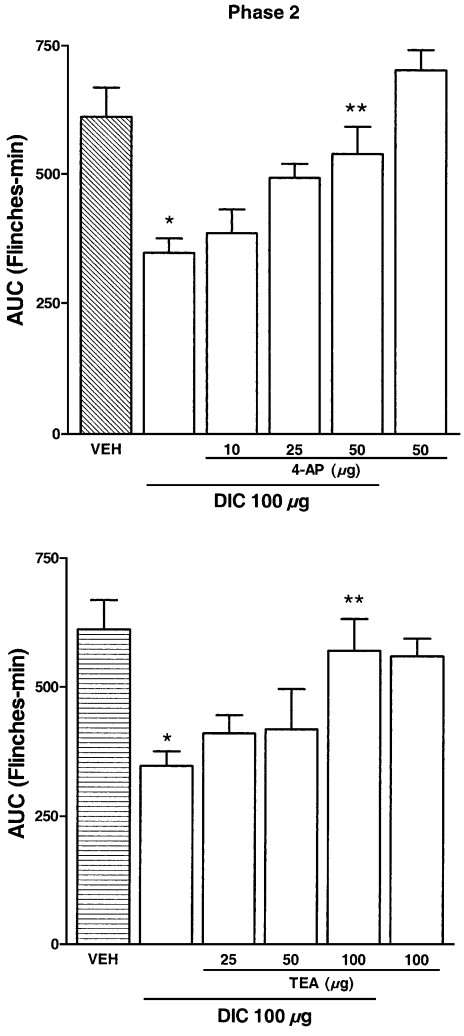

Fig. 4. Effect of voltage-gated K^+ channel blockers 4-aminopyridine (4-AP, top panel) and tetraethylammonium (TEA, bottom panel) on the peripheral antinociception produced by diclofenac (DIC) during the second phase of the formalin test. Rats were pretreated with s.c. injection of 4-AP or TEA plus diclofenac into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean ± S.E.M. for at least six animals. * Significantly different from the vehicle group ($P<0.05$) and * * significantly different from the diclofenac group ($P<0.05$) as determined by the analysis of variance followed by Tukey's test.

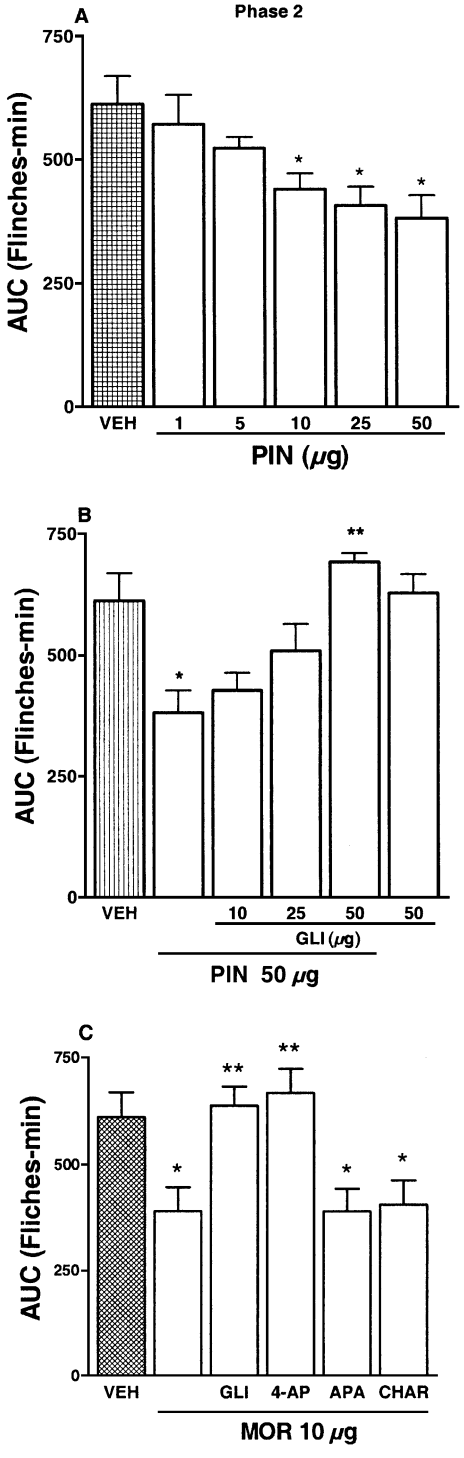

Fig. 5. Antinociceptive effect of pinacidil (PIN) during the second phase of the formalin test (A) and its blockade by glibenclamide (B). Effect of glibenclamide 50 μg (GLI), 4-aminopyridine 50 μg (4-AP), charybdotoxin 2 μg (CHAR) or apamin 10 μg (APA) on the peripheral antinociception produced by morphine 10 μg (MOR 10 μg) during the second phase of the formalin test (C). Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean ± S.E.M. for at least six animals. * Significantly different from the vehicle group ($P<0.05$) and ** significantly different from the pinacidil or morphine group ($P<0.05$) as determined by the analysis of variance followed by Tukey's test.

(10 μg/paw) produced a significant reduction of formalin-induced flinching behavior as compared to vehicle (Fig. 5). Glibenclamide and 4-aminopyridine, but not charybdotoxin or apamin, blocked the antinociception produced by morphine. The K^+ channel blockers, by themselves, did not affect the nociceptive behavior induced by formalin.

4. Discussion

The results reported here indicate that the modulation of primary afferent neuronal K^+ channels represents an important step in the induction of peripheral antinociception by diclofenac. Our data demonstrate that the local administration of K^+ channel blockers prevented the diclofenac-induced peripheral antinociception. In addition, the ATP-sensitive K^+ channel openers pinacidil and morphine (positive controls) were also able to produce antinociception in the formalin test, indicating K^+ channel functionality.

At the concentrations used in this work, the K^+ channel blockers (glibenclamide, tolbutamide, charybdotoxin, apamin, 4-aminopyridine and tetraethylammonium) used did not modify the flinching behavior of rats in comparison with that of control rats. The lack of effect of the K^+ channel blockers is consistent with the results of studies in which these compounds did not modify the nociceptive activity of thermal noxious stimuli and mechanical hyperalgesia (Welch and Dunlow, 1993; Rodrigues and Duarte, 2000), thus excluding the possibility that the prevention of diclofenac antinociception could be due to a hyperalgesic or nociceptive effect of the K^+ channel blockers used. The lack of modification of the flinching behavior by the K^+ channel modulators at concentrations that are able to prevent diclofenac antinociception might also indicate that the K^+ channels of primary afferent neurons involved in the modulation of pain are not tonically activated.

The data presented suggest the participation of ATP-sensitive K^+ channels in diclofenac-induced antinociception. The fact that glibenclamide and tolbutamide specifically block 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), suggests that diclofenac could produce its peripheral antinociceptive effect through the activation of ATP-sensitive K^+ channels. Since glibenclamide was also able to block pinacidil-induced antinociception, our data suggest that the opening of ATP-sensitive K^+ channels underlies the antinociception induced by diclofenac.

This study also provides pharmacological evidence for the involvement of Ca^{2+}-activated K^+ channels in the antinociceptive effect induced by diclofenac. Local administration of large- and small-conductance Ca^{2+}-activated K^+ channel blockers (charybdotoxin and apamin, respectively) prevented the antinociceptive effect produced by diclofenac, indicating the participation of both large- and small-conductance Ca^{2+}-activated K^+ channels in the

modulation by diclofenac of inflammatory pain at the primary afferent neuron. A non-selective inhibitor (tetraethylammonium) of Ca^{2+}-activated K^+ channels (Cook and Quast, 1990; Halliwell, 1990) also prevented diclofenac-induced antinociception, which further supports the participation of these channels in the peripheral mechanism of action of diclofenac. The participation of the intermediate-conductance Ca^{2+}-activated K^+ channels cannot be discounted on the basis of the present experiments because charybdotoxin is able to inhibit some of these channels (Reinhart et al., 1989).

Our results provide evidence for the involvement of voltage-dependent K^+ channels in the peripheral mechanism of action of diclofenac. The local administration of tetraethylammonium and 4-aminopyridine (blockers of voltage-dependent K^+ channels; Cook and Quast, 1990; Halliwell, 1990; Mathie et al., 1998) prevented the antinociception induced by diclofenac. Since 4-aminopyridine is a delayed rectifier K^+ channel inhibitor (Rosati et al., 1998), the blockade of diclofenac-induced antinociception by 4-aminopyridine also suggests the participation of this channel. However, since these drugs are non-selective compounds, the possibility that the observed effect could be due to actions on other K^+ channels cannot be discounted.

In this study, we used morphine as the positive control since there is evidence that the peripheral administration of this drug produces antinociception through the opening of ATP-sensitive K^+ channels (Rodrigues and Duarte, 2000) but not voltage- or small- and large-conductance Ca^{2+}-activated K^+ channels. Morphine (10 μg/paw) produced a significant antinociceptive effect compared to saline, and this effect was completely blocked by glibenclamide and 4-aminopyridine (50 μg/paw) but not by charybdotoxin (2 μg/paw) or apamin (10 μg/paw). These results confirm previous observations indicating that morphine produces its antinociceptive effect by opening ATP-sensitive K^+ channels but not small- and large-conductance Ca^{2+}-activated K^+ channels (Ocaña et al., 1990, 1995; Wild et al., 1991; Raffa and Martinez, 1995; Kang et al., 1998; Rodrigues and Duarte, 2000). Surprisingly, 4-aminopyridine (a delayed rectifier K^+ channel and non-selective voltage-dependent K^+ channel inhibitor) blocked morphine antinociception in the formalin test. At millimolar concentrations, 4-aminopyridine can inhibit ATP-sensitive K^+ channels (Haworth et al., 1989). In contrast, glibenclamide also blocks an ATP-independent K^+ current in a neuroblastoma cell line (Reeve et al., 1992) and a delayed rectifier K^+ current in neural and cardiac cells (Rosati et al., 1998). Therefore, it is possible that at the administered concentrations, both drugs, glibenclamide and 4-aminopyridine, block ATP-sensitive K^+ channels as well as delayed rectifier K^+ channels.

There is evidence to support the activation of K^+ channels by NSAIDs. Fenamates are able to activate a voltage-dependent K^+ current from jejunal smooth muscle (Ferrugia et al., 1993). In addition, fenamates activate the large-conductance Ca^{2+}-activated K^+ channels of coronary artery and rabbit portal vein smooth cells (Ottolia and Toro, 1994; Greenwood and Large, 1995). Based on the fact that the relaxant effect of fenamates is blocked by iberiotoxin and 4-aminopyridine, it has been reported that the relaxant activity of flufenamic and tolfenamic acids involves the opening of large-conductance Ca^{2+}-dependent and delayed rectifier K^+ channels (Li et al., 1999). However, these actions were associated with the relaxant effect but not with the antinociceptive action of fenamates. Recently, we have shown that glibenclamide is able to block the antinociception induced by ketorolac (Lázaro-Ibáñez et al., 2001), suggesting that this drug could produce its peripheral antinociceptive effect through the opening of ATP-sensitive K^+ channels. Therefore, our results for diclofenac confirm our previous observation about the participation of ATP-sensitive K^+ channels in the mechanism of action of ketorolac. In addition, in the present study, we demonstrated for the first time that the antinociceptive effect of diclofenac is associated with the opening of different types of K^+ channels (ATP-sensitive, large- and small-conductance Ca^{2+}-activated and likely voltage-dependent K^+ channels). A similar profile has been reported for the central antinociception induced by amitriptyline and clomipramine (Galeotti et al., 1997, 2001) in the mouse hot-plate test.

In summary, diclofenac produced peripheral antinociception in the formalin test. The antinociceptive effect of diclofenac was antagonized by glibenclamide, tolbutamide, charybdotoxin, apamin, 4-aminopyridine and tetraethylammonium. These results strongly suggest that besides the inhibitory action on prostaglandin synthesis, the opening of several K^+ channels at the primary afferent neuron plays an important role in the peripheral antinociception of diclofenac.

Acknowledgements

Mario I. Ortiz and Jorge E. Torres-López are PROMEP fellows. Authors greatly appreciate the bibliographic assistance of H. Vázquez.

References

Aguirre-Bañuelos, P., Granados-Soto, V., 2000. Evidence for the participation of the nitric oxide–cyclic GMP pathway in the antinociceptive action of meloxicam in the formalin test. Eur. J. Pharmacol. 395, 9–13.

Amoroso, S., Schmid-Antomarch, H., Fosset, M., Ladzunky, M., 1990. Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. Science 247, 852–854.

Attal, N., Kayser, V., Eschalier, A., Benoist, J., Guilbaud, G., 1988. Behavioral and electrophysiological evidence for an analgesic effect of a non-steroidal anti-inflammatory agent sodium diclofenac. Pain 35, 341–348.

Björkman, R., 1995. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol: experimental studies in the rat. Acta Anaesthesiol. Scand. 103, 1–44 (Suppl).

Cook, N.S., Quast, U., 1990. Potassium channel pharmacology. In: Cook, N.S. (Ed.), Potassium Channels: Structure, Classification, Function and Therapeutic Potential. Ellis Horwood, Chichester, pp. 181–255.

Davies, N.W., Standen, N.B., Stanfield, P.R., 1991. ATP-dependent K^+ channels of muscle cells—their properties, regulation, and functions. J. Bioenerg. Biomembr. 23, 509–535.

Edwards, G., Weston, A.H., 1993. The pharmacology of ATP-sensitive K^+ channels. Annu. Rev. Pharmacol. Toxicol. 33, 597–637.

Ferrugia, G., Rae, J.L., Szuszewski, J.H., 1993. Characterization of an outward potassium current in canine jejunal circular smooth muscle and its activation by fenamates. J. Physiol. 468, 297–310.

Galeotti, N., Ghelardini, C., Capaccioli, S., Quattrone, A., Bartolini, A., 1997. Blockade of clomipramine and amitriptyline analgesia by an antisense oligonucleotide to mKv1.1, a mouse Shaker-like potassium channel. Eur. J. Pharmacol. 330, 15–25.

Galeotti, N., Ghelardini, C., Bartolini, A., 2001. Involvement of potassium channels in amitriptyline and clomipramine analgesia. Neuropharmacology 40, 75–84.

Greenwood, I.A., Large, W.A., 1995. Comparison of the effects of the fenamates on Ca^{2+}-activated chloride and potassium currents in rabbit portal vein smooth muscle cells. Br. J. Pharmacol. 116, 2939–2948.

Halliwell, J.V., 1990. K^+ channels in the central nervous system. In: Cook, N.S. (Ed.), Potassium Channels: Structure, Classification, Function and Therapeutic Potential. Ellis Horwood, Chichester, pp. 348–381.

Haworth, R.A., Goknur, A.B., Berkoff, H.A., 1989. Inhibition of ATP-sensitive potassium channels of adult rat heart cells by anti-arrhythmic drugs. Circ. Res. 65, 1157–1160.

IASP, 1983. Ethical guidelines for investigations on experimental pain in conscious animals. Pain 16, 109–110.

Kang, Y., Zhang, Z., Yang, S., Qiao, J., Dafny, N., 1998. ATP-sensitive K^+ channels are involved in the mediation of intrathecal norepinephrine- or morphine-induced antinociception at the spinal level: a study using EMG planimetry of flexor reflex in rats. Brain Res. Bull. 45, 269–373.

Lázaro-Ibáñez, G.G., Torres-López, J.E., Granados-Soto, V., 2001. Participation of the nitric oxide–cyclic GMP–ATP-sensitive K^+ channel pathway in the antinociceptive action of ketorolac. Eur. J. Pharmacol. 426, 41–46.

Li, L., Vaali, K., Vapaatalo, H., Kankaanranta, H., 1999. Effects of K^+ channel inhibitors on relaxation induced by flufenamic and tolfenamic acids in guinea-pig trachea. Eur. J. Pharmacol. 383, 169–176.

López-Muñoz, F.J., Castañeda-Hernández, G., Torres-López, J.E., Picazo, Y.F., Flores-Murrieta, F.J., Granados-Soto, V., 1996. Differences in the mechanism of antinociceptive action of non-steroidal anti-inflammatory drugs in the rat. Pharm. Sci. 2, 189–190.

Lorenzetti, B.B., Ferreira, S.H., 1996. Activation of the arginine–nitric oxide pathway in primary sensory neurons contributes to dipyrone-induced spinal and peripheral analgesia. Inflammation Res. 45, 308–311.

Mathie, A., Wooltorton, J.R.A., Watkins, C.S., 1998. Voltage-activated potassium channels in mammalian neurons and their block by novel pharmacological agents. Gen. Pharmacol. 30, 13–24.

Menassé, R., Hedwell, P., Kraetz, J., Pericin, C., Riesterer, L., Sallmann, A., Ziel, R., Jaques, R., 1978. Pharmacological properties of diclofenac sodium and its metabolites. Scand. J. Rheumatol., Suppl. 22, 5–16.

Miller, C., Moczydlowski, E., Latorre, R., Phillips, M., 1985. Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. Nature 313, 316–318.

Mixcoatl-Zecuatl, T., Aguirre-Bañuelos, P., Granados-Soto, V., 2000. Sildenafil produces antinociception and increases morphine antinociception in the formalin test. Eur. J. Pharmacol. 400, 81–87.

Ocaña, M., Del Pozo, E., Barrios, M., Baeyens, J.M., 1990. An ATP-dependent K^+ channel blocker antagonizes morphine analgesia. Eur. J. Pharmacol. 186, 377–378.

Ocaña, M., Del Pozo, E., Barrios, M., Baeyens, J.M., 1995. Subgroups among μ-opioid receptor agonists distinguished by ATP-sensitive K^+ channel-acting drugs. Br. J. Pharmacol. 114, 1296–1302.

Oliw, E., Lunden, I., Anggard, E., 1978. In vivo inhibition of prostaglandin synthesis in rabbit kidney by non-steroidal anti-inflammatory drugs. Acta Pharmacol. Toxicol. 42, 179–184.

Ottolia, M., Toro, L., 1994. Potentiation of large conductance K_{Ca} channels by niflumic, flufenamic, and mefenamic acids. Biophys. J. 67, 2272–2279.

Raffa, R.B., Martinez, R.P., 1995. The "glibenclamide-shift" of centrally acting antinociceptive agents in mice. Brain Res. 677, 277–282.

Reeve, H.L., Vaughan, P.F.T., Peers, C., 1992. Glibenclamide inhibits a voltage-gated K^+ currents in the human neuroblastoma cell line SH-SY5Y. Neurosci. Lett. 135, 37–40.

Reinhart, P.H., Chung, S., Levitan, I.B., 1989. A family of calcium-dependent potassium channels from rat brain. Neuron 2, 1031–1041.

Rodrigues, A.R.A., Duarte, I.D.G., 2000. The peripheral antinociceptive effect induced by morphine is associated with ATP-sensitive K^+ channels. Br. J. Pharmacol. 129, 110–114.

Romey, G., Hughes, M., Schmid-Antomarchi, H., Lazduns-Ki, M., 1984. Apamin: a specific toxin to study a class of Ca^{2+}-dependent K^+ channels. J. Physiol. (Paris) 79, 259–264.

Rosati, B., Rochetti, M., Zaza, A., Wanke, E., 1998. Sulfonylureas blockade of neural and cardiac HERG channels. FEBS Lett. 440, 125–130.

Sacerdote, P., Monza, G., Mantegazza, P., Panerai, A.E., 1985. Diclofenac and pirprofen modify pituitary hypothalamic beta-endorphin concentrations. Pharmacol. Res. Commun. 17, 679–684.

Stretton, D., Miura, M., Bevisi, M.G., Barnes, P.J., 1992. Calcium-activated potassium channels mediate prejunctional inhibition of peripheral sensory nerves. Proc. Natl. Acad. Sci. U.S.A. 9, 1325–1329.

Todd, P.A., Sorkin, E.M., 1988. Diclofenac sodium: a reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs 35, 244–285.

Tonussi, C.R., Ferreira, S.H., 1994. Mechanism of diclofenac analgesia: direct blockade of inflammatory sensitization. Eur. J. Pharmacol. 251, 173–179.

Vescovi, P., Passeri, M., Gerra, G., 1986. Naloxone inhibits the early phase of diclofenac analgesia in man. Pain Clin. 19, 151–155.

Welch, S.P., Dunlow, L.D., 1993. Antinociceptive activity of intrathecally administered potassium channel openers and opioid agonists, a common mechanism of action? J. Pharmacol. Exp. Ther. 267, 390–399.

Wheeler-Aceto, H., Cowan, A., 1991. Standardization of the rat paw formalin test for evaluation of analgesics. Psychopharmacology 104, 35–44.

Wild, K.D., Vanderah, T., Mosberg, H.I., Porreca, F., 1991. Opioid δ receptor subtypes are associated with different potassium channels. Eur. J. Pharmacol. 193, 135–141.