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Possible involvement of potassium channels in peripheral antinociception induced by metamizol: lack of participation of ATP-sensitive K^+ channels

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Abstract

The present work assessed the possible participation of K^+ channels in the peripheral antinociceptive action of metamizol in the 1% formalin test. Ipsilateral, but not contralateral, local peripheral administration of metamizol produced a dose-dependent antinociception only during the second phase of the formalin test. K^+ channel blockers alone did not modify formalin-induced nociceptive behavior. However, local peripheral pretreatment of the paw with charybdotoxin and apamin (large- and small-conductance Ca^{2+}-activated K^+ channel blockers, respectively), 4-aminopyridine and tetraethylammonium (voltage-dependent K^+ channel inhibitors), but not glibenclamide or tolbutamide (ATP-sensitive K^+ channel inhibitors), dose-dependently prevented metamizol-induced antinociception. The above results suggest that metamizol could open large- and small-conductance Ca^{2+}-activated K^+ channels, but not ATP-sensitive K^+ channels, in order to produce its peripheral antinociceptive effect in the formalin test. The participation of voltage-dependent K^+ channels was also suggested, but since nonselective inhibitors were used, the data await further confirmation.

Keywords: Metamizol; Antinociception; K^+ channels; Apamin; Charybdotoxin; 4-Aminopyridine; Tetraethylammonium; Glibenclamide; Tolbutamide

1. Introduction

Metamizol (Dipyrone, Aventis Pharma) is a nonsteroidal anti-inflammatory drug (NSAID), which has strong antipyretic, analgesic and spasmolitic actions but small anti-inflammatory activity (Brune and Alpermann, 1983; Tatsuo et al., 1994; Muriel-Villoria et al., 1995). Metamizol reduces prostaglandin synthesis in both the periphery and the central nervous system (Abbate et al., 1990; Shimada et al., 1994) by the inhibition of both cyclooxygenase-1 and cyclooxygenase-2 (Campos et al., 1999). However, because metamizol is able to induce a significant antinociceptive action in the absence of an anti-inflammatory response, an important participation of central mechanisms in the antinociceptive activity of this drug has been suggested. Several observations support this suggestion. Metamizol microinjection into the periaqueductal gray matter (PAG) diminishes the response of spinal ascending axons subsequent to activation of peripheral C-fibers and produces antinociception in the tail-flick model (Carlsson and Jurna, 1987; Hernández and Vanegas, 2001). These antinociceptive effects of metamizol are due to the activation of descending opioidergic pain control systems, because it can be partially reduced by naloxone given either systemically or microinjected into the spinal cord, PAG or the rostral ventromedial medulla (Hernández and Vanegas, 2001). Besides, it has also been reported that metamizol is able to stimulate the release of pituitary and hypothalamic β-endorphins (Vlaskovska et al., 1989). In addition, a possible interference of metamizol with the nociceptive activity of glutamate at the spinal level also has been suggested (Beirith et al., 1998).

Metamizol apparently possesses an additional mechanism of action. There is evidence that metamizol-induced peripheral antinociception can be diminished by either the

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nitric oxide (NO) synthase or soluble guanylyl cyclase inhibition (Granados-Soto et al., 1995; Lorenzetti and Ferreira, 1996). There are now a number of studies which suggest that this pathway has indeed an important participation in the peripheral antinociception induced by some NSAIDs (Duarte et al., 1992; Lorenzetti and Ferreira, 1996; Aguirre-Bañuelos and Granados-Soto, 2000).

Recent evidence suggests that ketorolac- or diclofenac-induced antinociception involves the modulation of the NO–cyclic GMP pathway (Granados-Soto et al., 1995; Tonussi and Ferreira, 1994). Furthermore, we have found that the antinociception induced by these drugs can be prevented by K^+ channel inhibitors (Lázaro-Ibáñez et al., 2001; Ortiz et al., 2002), suggesting that both drugs could activate the NO–cyclic GMP–K^+ channel pathway in order to produce their peripheral antinociceptive effect. Like diclofenac and ketorolac, metamizol modulates the NO–cyclic GMP pathway at the periphery (Granados-Soto et al., 1995; Lorenzetti and Ferreira, 1996). Therefore, it is likely that metamizol could also modulate K^+ channels. This work was undertaken to determine whether specific and nonspecific K^+ channel blockers have any effect on the peripheral antinociception induced by metamizol. 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 intermediate- and large-conductance Ca^{2+}-activated K^+ channels; Stretton et al., 1992), apamin (a selective blocker of small-conductance Ca^{2+}-activated K^+ channels; Romey et al., 1984) and voltage-dependent K^+ channel blockers (4-aminopyridine and tetraethylammonium; Cook and Quast, 1990).

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 (Zimmermann, 1983). Additionally, the study was approved by the Institutional Animal Care and Use Committee (Departamento de Farmacobiología, Mexico, D.F., Mexico).

2.2. Measurement of antinociceptive activity

Antinociception was assessed using the formalin test. A moderate dose of formalin (1%) was used in order to produce maximal sensitivity to metamizol. Rats were placed in open Plexiglas observation chambers for 20 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 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 min up to 60 min after injection (Wheeler-Aceto and Cowan, 1991). 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

Metamizol sodium was a gift of Aventis Pharma (Mexico City). Glibenclamide (glyburide), tolbutamide, charybdotoxin, apamin, 4-aminopyridine and tetraethylammonium were purchased from Sigma (St. Louis, MO, USA). Metamizol, tetraethylammonium, 4-aminopyridine, charybdotoxin and apamin were dissolved in saline. Glibenclamide and tolbutamide were dissolved in dimethylsulfoxide 20%.

2.4. Experimental design

Rats were injected subcutaneously into the dorsal surface of the right hind paw with appropriate vehicle (saline of dimethylsulfoxide 20%) or increasing doses of metamizol (50–400 μg/paw in 50 μl) 20 min before formalin injection into the same paw. To determine whether metamizol acted

Table 1
Effect of the vehicles of several K^+ channel inhibitors and metamizol (MET) during the second phase of the formalin test

Treatment	AUC (flinches/min) second phase
Saline[a] + VEH[b]	592.5 ± 43.7
Saline + Gli-50	627.9 ± 38.6
Saline + Tol-50	587.9 ± 40.4
MET + VEH	372.5 ± 26.1[c]
Saline + saline[a]	604.3 ± 46.9
Saline + CHAR-2	649.1 ± 38.0
Saline + APA-10	526.6 ± 62.7
Saline + 4-AP-50	703.7 ± 39.2
Saline + TEA-100	560.8 ± 34.8
MET + saline	345.2 ± 24.3[c]

Rats were pretreated with K^+ channel inhibitors or metamizol (200 μg) into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Doses are in micrograms (μg) and effect is expressed as the mean ± S.E.M. for at least six animals.

[a] Saline was used as vehicle in these experimental series because metamizol (MET), charybdotoxin (CHAR), apamin (APA), 4-aminopyridine (4-AP) and tetraethylammonium (TEA) were dissolved in this solvent.

[b] A solution of DMSO 20% was used as vehicle (VEH) since glibenclamide (Gli) and tolbutamide (Tol) were dissolved in this solvent.

[c] Significantly different from the vehicle group ($P < .05$), as determined by analysis of variance followed by Tukey's test.

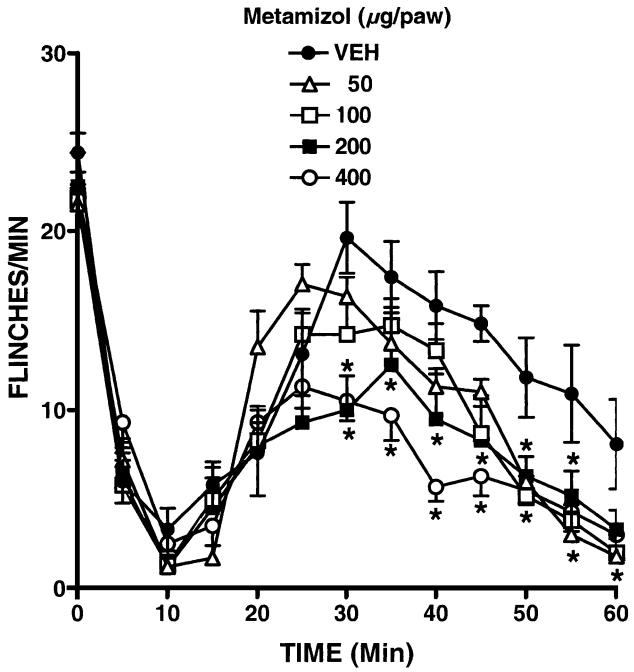

Fig. 1. Time course of the local antinociceptive effect of metamizol in the formalin test. Rats were pretreated with a subcutaneous injection of vehicle or metamizol into the dorsal part of the right (ipsilateral) paw 20 min before formalin injection. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean ± S.E.M. for six animals. * Significantly different from vehicle group ($P < .05$), as determined by analysis of variance followed by Tukey's test.

locally, metamizol was administered to the left (contralateral) paw 20 min before formalin was injected into the right paw, and the effect was assessed. To determine if metamizol-induced antinociception was mediated by the K^+ channels activation, rats were treated first with metamizol (200 μg) 20 min before formalin injection. Ten minutes later, rats were treated with glibenclamide (10–50 μg), tolbutamide (10–50 μg), apamin (0.1–10 μg), charybdotoxin (0.1–2 μg), tetraethylammonium (50–100 μg) and 4-aminopyridine (10–50 μg) and then, 10 min later, animals received the formalin injection. Drugs were injected in a volume of 50 μl. Each rat received three injections and appropriate controls for multiple injections were performed before starting the formal study (Table 1). Doses of K^+ channel inhibitors for peripheral administration were selected based on previous reports (Soares and Duarte, 2001) and on pilot experiments in our laboratory. Observer was unaware of the treatment in each animal. Rats in all groups were tested for possible side effects such as reduction of righting, stepping and corneal reflexes before and at 30 min after formalin injection.

2.5. Data analysis and statistics

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

3. Results

3.1. Peripheral antinociceptive effect of metamizol

Formalin administration produced a typical pattern of flinching behavior. The first phase started immediately after administration of formalin and then diminished gradually in

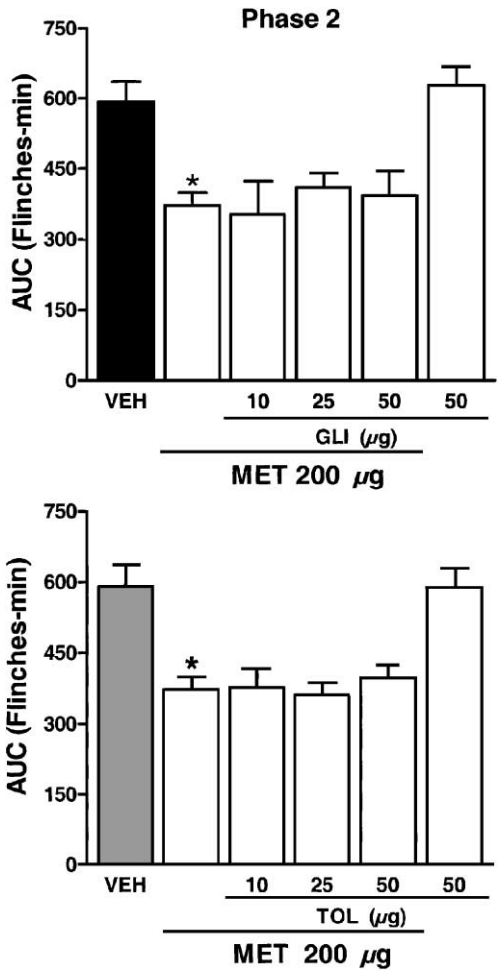

Fig. 2. Effect of ATP-sensitive K^+ channel inhibitors glibenclamide (GLI, top panel) and tolbutamide (TOL, bottom panel) on the peripheral antinociception produced by metamizol 200 μg (MET) during the second phase of the formalin test. Rats were pretreated with a subcutaneous injection of metamizol (− 20 min) plus glibenclamide or tolbutamide (− 10 min) into the right paw. At time 0 rats received the formalin injection in the same 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 ($P < .05$), as determined by analysis of variance followed by Tukey's test.

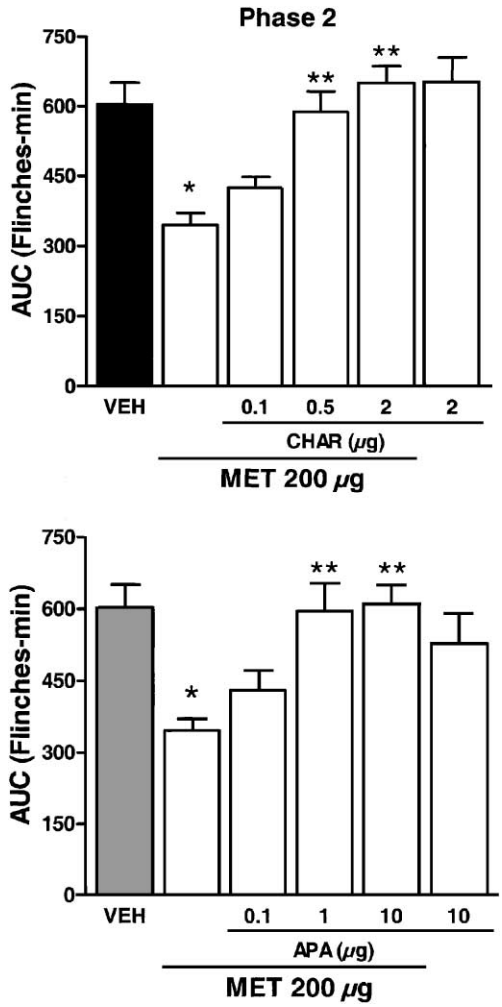

Fig. 3. Effect of large- and small-conductance Ca^{2+}-activated K^+ channel blockers charybdotoxin (CHAR, top panel) and apamin (APA, bottom panel), respectively, on the peripheral antinociception produced by metamizol 200 μg (MET) during the second phase of the formalin test. Rats were pretreated with a subcutaneous injection of metamizol (−20 min) plus charybdotoxin or apamin (−10 min) into the right paw. At time 0 rats received the formalin injection in the same 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 ($P<.05$) and ** significantly different from the metamizol group ($P<.05$), as determined by analysis of variance followed by Tukey's test.

about 10 min. The second phase started at about 15 min and lasted until 1 h. Ipsilateral (50–400 μg/paw), but not contralateral (400 μg/paw), local peripheral administration of metamizol produced a dose-dependent reduction in the flinching behavior otherwise observed after formalin injection (Fig. 1). Metamizol significantly reduced the number of flinches during Phase 2 ($P<.05$), but not during Phase 1. The highest dose of metamizol (400 μg) did not significantly increase the antinociceptive effect compared to metamizol at 200 μg, therefore this dose was selected to carry out the experiments with K^+ channel inhibitors. No side effects were observed in either group, control or treated.

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

Local peripheral pretreatment with ATP-sensitive K^+ channel inhibitors glibenclamide and tolbutamide (10–50 μg/paw) did not prevent metamizol-induced antinociception in the formalin test (Fig. 2). Interestingly, charybdotoxin (0.1–2 μg/paw) and apamin (0.1–10 μg/paw) (large- and small-conductance Ca^{2+}-activated K^+ channel blockers, respectively) prevented metamizol-induced antinociception (Fig. 3). Furthermore, 4-aminopyridine (10–50 μg/paw) and tetraethylammonium (50–100 μg/paw) (voltage-dependent

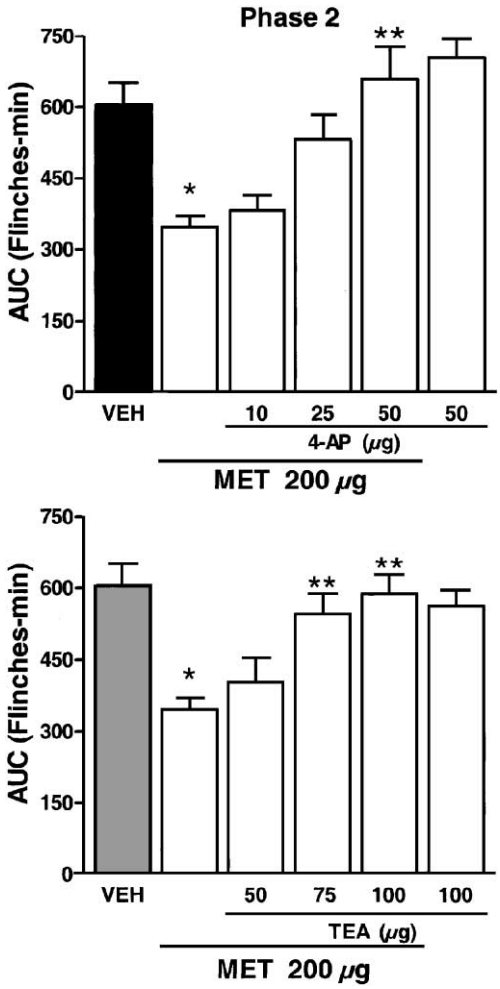

Fig. 4. Effect of voltage-dependent K^+ channel blockers 4-aminopyridine (4-AP, top panel) and tetraethylammonium (TEA, bottom panel) on the peripheral antinociception produced by metamizol 200 μg (MET) during the second phase of the formalin test. Rats were pretreated with a subcutaneous injection of metamizol (−20 min) plus 4-AP or TEA (−10 min) into the right paw. At time 0 rats received the formalin injection in the same 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 ($P<.05$) and ** significantly different from the metamizol group ($P<.05$), as determined by analysis of variance followed by Tukey's test.

K^+ channel blockers) also prevented the antinociception produced by metamizol in the 1% formalin test (Fig. 4). K^+ channel blockers, by themselves, were not able to modify formalin-induced nociceptive behavior.

4. Discussion

Peripheral administration of metamizol produced a dose-dependent antinociceptive effect in our model. This drug, however, was not able to completely block the formalin-induced nociceptive behavior. These results confirm a limited antinociceptive efficacy of local metamizol (Lorenzetti and Ferreira, 1996; Aguirre-Bañuelos and Granados-Soto, 1999) and it supports a significant participation of a peripheral component in the action of this drug. The peripheral antinociceptive activity of metamizol could be due to its action as inhibitor of prostaglandin synthesis (Brune and Alpermann, 1983; Abbate et al., 1990; Campos et al., 1999) as well as to the modulation of the NO–cyclic GMP pathway (Granados-Soto et al., 1995; Lorenzetti and Ferreira, 1996). Central actions of metamizol can be discarded, because the drug was given locally. Moreover, contralateral administration of metamizol did not produce any effect, suggesting that the observed effect was restricted to drug action at the local level.

In the present study, the ATP-sensitive K^+ channel blockers glibenclamide and tolbutamide were not able to block the antinociceptive action of metamizol at the same doses that blocked the antinociception produced by ketorolac (Lázaro-Ibáñez et al., 2001), diclofenac (Ortiz et al., 2002), morphine or dibutyryl-cyclic GMP (Rodrigues and Duarte, 2000; Soares and Duarte, 2001). It is interesting to note that the same doses of ATP-sensitive K^+ channel blockers are also able to reverse the antinociception induced by gallic acid ethyl ester and sodium nitroprusside (Santos et al., 1999; Soares et al., 2000). Then, the data presented do not support the possible participation of ATP-sensitive K^+ channels in metamizol-induced antinociception. The lack of effect of the ATP-sensitive K^+ channel blockers is consistent with studies in which metamizol-induced relaxation in phenylephrine-precontracted rabbit thoracic aorta smooth muscle (Ergun et al., 1999) and the spinal or supraspinal antinociception caused by metamizol in the formalin and other tests are not influenced by glibenclamide (Beirith et al., 1998).

This study provides pharmacological evidence for the involvement of Ca^{2+}-activated K^+ channels in the antinociceptive effect induced by metamizol. The local peripheral administration of large- and small-conductance Ca^{2+}-activated K^+ channel blockers (charybdotoxin and apamin, respectively) prevented the antinociceptive effect produced by metamizol, suggesting the participation of both large- and small-conductance Ca^{2+}-activated K^+ channels in the modulation of inflammatory pain at the primary afferent. A nonselective inhibitor (tetraethylammonium) of large-conductance Ca^{2+}-activated K^+ channels (Cook and Quast, 1990; Halliwell, 1990) also prevented metamizol-induced antinociception, further supporting the participation of these channels in the peripheral mechanism of action of metamizol.

Our results also provide pharmacological evidence for the involvement of voltage-dependent K^+ channels in the peripheral mechanism of action of metamizol. Local peripheral administration of tetraethylammonium and 4-aminopyridine (blockers of voltage-dependent K^+ channels; Cook and Quast, 1990; Halliwell, 1990; Grissmer et al., 1994; Mathie et al., 1998) prevented metamizol-induced antinociception. However, as these drugs are nonselective compounds, the possibility that the observed effect could be due to the actions on other K^+ channels cannot be discounted.

At the concentrations used in this work, the K^+ channel blockers (charybdotoxin, apamin, 4-aminopyridine and tetraethylammonium) used did not modify the flinching behavior of rats in comparison with control groups (Table 1). The lack of effect of the K^+ channel blockers agrees with results of studies in which these compounds did not modify the nociceptive activity of thermal noxious stimuli, the nociceptive behavior induced by formalin and mechanical hyperalgesia (Welch and Dunlow, 1993; Rodrigues and Duarte, 2000; Ortiz et al., 2002), excluding that the prevention of metamizol-mediated antinociception could be due to a hyperalgesic effect of the K^+ channel blockers used.

Evidence suggests that metamizol, besides inhibiting prostaglandin synthesis (Abbate et al., 1990; Campos et al., 1999), modulates the NO–cyclic GMP pathway at the periphery and at the spinal cord (Granados-Soto et al., 1995; Lorenzetti and Ferreira, 1996). Previous studies have shown that drugs (morphine, diclofenac, ketorolac, sodium nitroprusside) that modulate the NO–cyclic GMP pathway also modulate K^+ channels (Granados-Soto et al., 1995; Rodrigues and Duarte, 2000; Soares et al., 2000; Lázaro-Ibáñez et al., 2001; Ortiz et al., 2002). Our results suggest that metamizol is able to modulate peripheral Ca^{2+}-activated K^+ channels. Therefore, it is tempting to speculate that metamizol could modulate the NO–cyclic GMP pathway, which in turn would activate Ca^{2+}-activated K^+ channels to hyperpolarize the primary afferent neuron to finally produce antinociception. The activation of K^+ channels directly by NO (Bolotina et al., 1994) or through a phosphorylation by a cyclic GMP-activated protein kinase G (Fukao et al., 1999) is in line with our suggestion.

Taken together, the present results suggest that, besides the inhibitory action on prostaglandin synthesis, the activation of small- and large-conductance Ca^{2+}-activated, but not ATP-sensitive, K^+ channels at the primary afferent neuron plays an important role in the peripheral antinociception of metamizol in the 1% formalin test. The participation of voltage-dependent K^+ channels was also suggested, however, since nonselective inhibitors were used, the data await further confirmation.

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