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Involvement of ATP-sensitive K^+ channels in the peripheral antinociceptive effect induced by dipyrone

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Abstract

We evaluated the effect of several K^+ channel blockers on the peripheral antinociception induced by dipyrone using the rat paw pressure test, in which sensitivity is increased by intraplantar injection (2 μg) of prostaglandin E_2. Dipyrone administered locally into the right hindpaw (50, 100 and 200 μg) elicited a dose-dependent antinociceptive effect which was demonstrated to be local, since only higher doses produced an effect when injected in the contralateral paw. The specific blockers of ATP-sensitive K^+ channels glibenclamide (40, 80 and 160 μg/paw) and tolbutamide (80, 160 and 320 μg/paw) antagonized the peripheral antinociception induced by dipyrone (200 μg/paw). Charybdotoxin (2 μg/ paw), a blocker of large conductance Ca^{2+}-activated K^+ channels, and dequalinium (50 μg/paw), a selective blocker of small conductance Ca^{2+}-activated K^+ channels, did not modify the effect of dipyrone. This effect was also unaffected neither by intraplantar administration of non-specific voltage-dependent K^+ channel blockers tetraethylammonium (1700 μg) and 4-aminopyridine (100 μg) nor cesium (500 μg), a non-specific K^+ channel blocker. These results suggest that the peripheral antinociceptive effect of dipyrone may result from activation of ATP-sensitive K^+ channels, while other K^+ channels appear not to be involved in the process. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: K^+ channel blocker; Antinociception; Dipyrone; K^+ channel

1. Introduction

Dipyrone is a nonsteroidal anti-inflammatory drug (NSAID) widely used. It is generally believed that the analgesic activity of NSAIDs is related to their inhibitory actions on peripherally released prostaglandins (Vane, 1971; Ferreira, 1972; Ferreira et al., 1973). Despite it have passed 100 years since dipyrone was synthesized, its mode and site of action remain a matter of controversy. According to Nikolova et al. (1980), the profile of pharmacological effects of dipyrone is certainly different from that of NSAIDs.

In 1985, Lorenzetti and Ferreira suggested that dipyrone-induced analgesia results from a direct action upon the hyperalgesic event and not only from interference with the action of a specific mediator such as prostaglandins. Duarte et al. (1992) described the involvement of the L-arginine/nitric oxide/cGMP pathway in peripheral antinociception induced by intraplantar injection of dipyrone. It was shown by Ferreira et al. (1991) that the L-arginine/NO/cGMP pathway is also involved in peripheral morphine antinociception.

Nitric oxide can activate different types of K^+ channels in different types of tissues by an increase in cGMP (Thornbury et al., 1991; Kubo et al., 1994; Murphy and Brayden, 1995; Carrier et al., 1997). Recently, Soares et al. (2000) showed that the activation of ATP-sensitive K^+ channels could be the mechanism by which nitric oxide, donated by sodium nitroprusside, induces peripheral antinociception. Rodrigues and Duarte (2000) demonstrated that the peripheral antinociceptive effect induced by morphine is associated with ATP sensitive K^+ channels activation.

The above observations led us to suppose that nociceptor desensitization may occur through the activation of K^+ channels, leading to an increase of the neuronal threshold to pain.

The present study was undertaken to determine whether specific and non-specific K^+ channel blockers have any effect on the peripheral antinociception induced by dipyrone. For this purpose, we tested the effects of glibenclamide and tolbutamide, sulphonylureas that specifically block ATP-sensitive K^+ channels (Edwards and Weston, 1993);

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charybdotoxin (ChTx), a blocker of large conductance Ca^{2+}-activated K^+ channels (Miller et al., 1985); dequalinium (DQ), a selective blocker of small conductance Ca^{2+}-activated K^+ channels (Dunn, 1994); non-selective voltage-dependent K^+ channel blockers, 4-aminopyridine (4-AP) and tetraethylammonium (TEA), and cesium, a non-specific K^+ channel blocker (Cook and Quast, 1990).

2. Materials and methods

2.1. Animals

The experiments were performed on 160–200 g male Wistar rats from CEBIO-UFMG. The animals were housed in a temperature-controlled room (23 ± 1 °C) on an automatic 12-h light/dark cycle (06:00–18:00 h). All tests were conducted during the light phase (08:00–17:00 h). Food and water were freely available until the beginning of the experiments. Naive animals were used throughout.

2.2. Measurement of hyperalgesia

Hyperalgesia was induced by a subcutaneous injection of prostaglandin E_2 (2 μg) into the plantar surface of rat's hindpaw and measured according to the paw pressure test described by Randall and Selitto (1957). An analgesy-meter (Ugo Basile, Italy) with a cone-shaped paw presser with a rounded tip, which applies a linearly increasing force to the rat's right hindpaw was used. The weight in grams (g) required to elicit nociceptive responses, such as paw flexion or struggle, was determined as the nociceptive threshold. A cut-off value of 300 g was used to prevent damage to the paws. The nociceptive threshold was determined by the average of three consecutive trials recorded before (0 time) and 3 h after prostaglandin E_2 injection. The results were calculated as the difference between these two averages (Δ of the nociceptive threshold) and expressed as grams.

2.3. Experimental protocol

Dipyrone was administered subcutaneously in the right hindpaw 2 h after local injection of prostaglandin E_2. In the protocol used to determine whether dipyrone was acting outside the injected paw, prostaglandin E_2 was injected into both hindpaws, while dipyrone was administered 2 h later into the left or right paw. The nociceptive threshold was always measured in the right hindpaw. All the K^+ channel blockers were injected subcutaneously into the right hindpaw 5 min before dipyrone (Rodrigues and Duarte, 2000; Soares et al., 2000).

2.4. Drug administration

The drug used as hyperalgesic agent was prostaglandin E_2 (Calbiochem, USA), and dipyrone (Sigma, USA) was used as the antinociceptive drug. The K^+ channel blockers and their supplies were: glibenclamide (Sigma), tolbutamide (ICN Biomedicals, USA), charybdotoxin (Sigma), 4-aminopyridine (Sigma), tetraethylammonium chloride (Sigma), dequalinium chloride (Calbiochem) and cesium (Mitsuwa's Pure Chemicals, Japan). Dipyrone and prostaglandin E_2 were dissolved in isotonic saline and injected in a volume of 100 μl/paw. The K^+ channel blockers were dissolved in demineralized water, with exception of sulphonylureas that were dissolved in Tween 80 vehicle (2% in saline), immediately before use and injected in a volume of 50 μl/paw.

2.5. Statistical analysis

The statistical analysis were carried out by one-way analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons. Probabilities less than 5% ($P < 0.05$) were considered statistically significant.

3. Results

3.1. Antinociceptive effect of dipyrone

The administration of dipyrone (50, 100 and 200 μg) into the right hind paw produced an antinociceptive response against the hyperalgesia induced by prior local injection of prostaglandin E_2 (2 μg/paw) in a dose-dependent manner (Fig. 1). Higher doses of dipyrone (400 or 800 μg/paw) were not statistically different from the dose of 200 μg in counteract the hyperalgesia induced by prostaglandin E_2 (data not

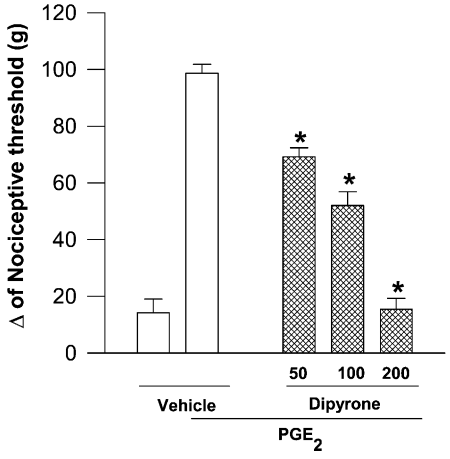

Fig. 1. Effect of dipyrone on the nociceptive threshold in rats with prostaglandin E_2-induced hyperalgesia. Dipyrone (μg/paw) was administered 2 h after local administration of 100 μl of prostaglandin E_2 (2 μg). The antinociceptive response was measured in the paw pressure test as described in Section 2. Each column represents the mean ± S.E.M. ($n = 5$). * indicates a significant difference from the PGE_2 + vehicle-injected control ($P < 0.05$, ANOVA + Bonferroni's test).

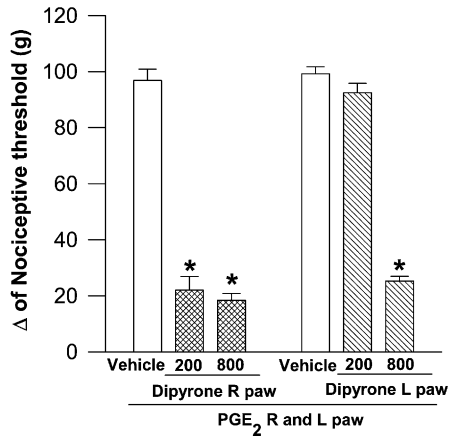

Fig. 2. Exclusion of outside paw antinociceptive effect of dipyrone (200 μg). Dipyrone (μg) was administered into the right (R) or left (L) paw 2 h after prostaglandin E_2 (2 μg) administration into both hind paws. The antinociceptive response of the right (R) hindpaw was measured in the paw pressure test as described in Section 2. Each column represents the mean ± S.E.M. ($n = 5$). * indicates a significant difference from the PGE_2 + vehicle-injected control ($P < 0.05$, ANOVA + Bonferroni's test).

shown). Dipyrone at the dose of 200 μg, when administered into the left paw, did not produce an antinociceptive effect in the right paw, whereas dipyrone at the dose of 800 μg when injected into the left paw induced a potent antinociceptive effect in the contralateral paw (Fig. 2).

3.2. Antagonism of dipyrone-induced antinociception by glibenclamide and tolbutamide

Glibenclamide (40, 80 and 160 μg/paw) significantly reduced the dipyrone-induced peripheral antinociception

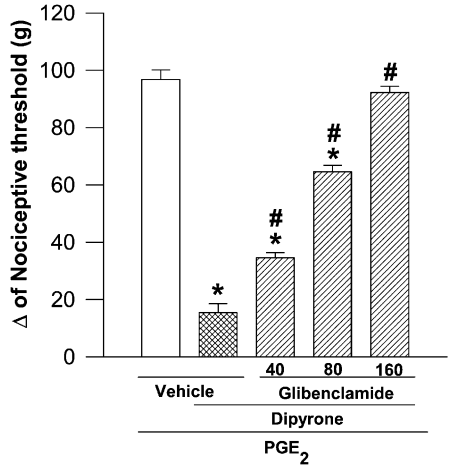

Fig. 3. Antagonism induced by intraplantar administration of glibenclamide of the peripheral antinociception produced by dipyrone in hyperalgesic paws (PGE_2, 2 μg). Glibenclamide (μg) was administered 5 min before dipyrone (200 μg/paw). Each column represents the mean ± S.E.M. ($n = 5$). * and # indicate a significant difference compared to (PGE_2 + vehicle + vehicle) and (PGE_2 + dipyrone + vehicle)-injected controls, respectively ($P < 0.05$, ANOVA + Bonferroni's test).

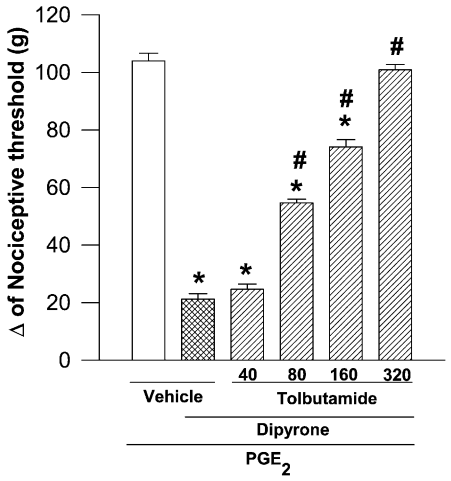

Fig. 4. Antagonism induced by intraplantar administration of tolbutamide of the peripheral antinociception produced by dipyrone in hyperalgesic paws (PGE_2, 2 μg). Tolbutamide (μg) was administered 5 min before dipyrone (200 μg/paw). Each column represents the mean ± S.E.M. ($n = 5$). * and # indicate a significant difference compared to (PGE_2 + vehicle + vehicle) and (PGE_2 + dipyrone + vehicle)-injected controls, respectively ($P < 0.05$, ANOVA + Bonferroni's test).

(200 μg/paw) in a dose-dependent manner (Fig. 3). As shown in Fig. 4, the other sulphonylurea tested, tolbutamide, at the doses of 80, 160 and 320 μg/paw also

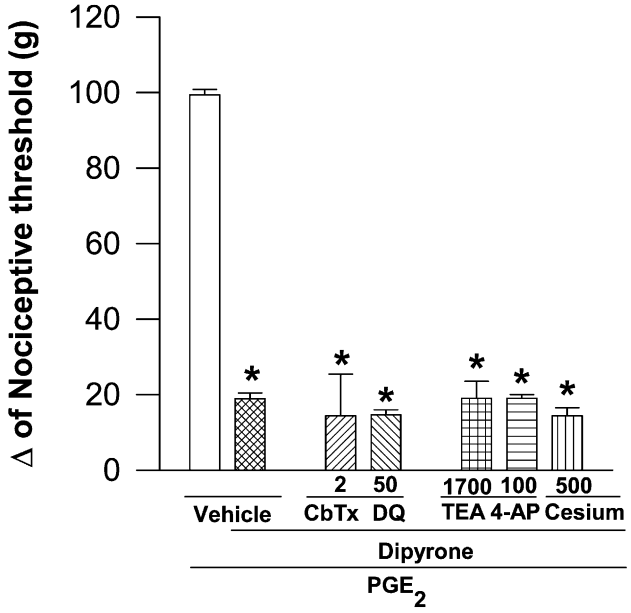

Fig. 5. Effect of intraplantar administration of charybdotoxin (ChTx), dequalinium (DQ), tetraethylammonium (TEA), 4-aminopyridine (4-AP) and cesium on the peripheral antinociception induced by dipyrone in hyperalgesic paws (PGE_2, 2 μg). ChTx, DQ, TEA, 4-AP and cesium (μg) were administered 5 min before dipyrone (200 μg/paw). Each column represents the mean ± S.E.M. ($n = 4–8$). No statistically significant difference between the groups treated with PGE_2 + Dipyrone + vehicle and PGE_2 + Dipyrone + ChTx, DQ, TEA, 4-AP or Cesium was found in any case. * indicates a significant difference from the PGE_2 + vehicle + vehicle-injected control ($P < 0.05$, ANOVA + Bonferroni's test).

significantly inhibited the dipyrone-induced antinociceptive effect. Neither sulphonylurea tested significantly modified the nociceptive threshold in control animals or induced any overt behavioural effect at the doses used (data not shown).

3.3. Effect of charybdotoxin, dequalinium, tetraethylammonium, 4-aminopyridine and cesium on dipyrone-induced antinociception

Charybdotoxin (2 μg) and dequalinium (50 μg) injected into the paw did not significantly reduce the peripheral antinociception induced by dipyrone. Fig. 5 also shows that tetraethylammonium (1700 μg/paw), 4-aminopyridine (100 μg/paw) and cesium (500 μg/paw) failed to significantly counteract the antinociception induced by dipyrone.

4. Discussion

In the present series of experiments we have examined, the hypothesis that the final mechanism by which dipyrone exerts its peripheral antinociceptive effect may be the activation of K^+ channels, which would cause a hyperpolarization of peripheral terminals of primary afferents, leading to a decrease in action potential generation.

Dipyrone produced a well-defined dose-dependent peripheral antinociceptive effect in the rat paw prostaglandin E_2-induced hyperalgesia test. Although some studies suggest a central action for dipyrone (Carlsson et al., 1986; Akman et al., 1996), this drug is believed to inhibit the peripheral synthesis of cyclooxygenase metabolites, thus preventing the nociceptor sensitization (Vane, 1971). The use of a prostaglandin E_2-induced hyperalgesia test eliminates the possibility that the peripheral effect of dipyrone results from a blockade of the release of prostaglandins produced during the inflammatory process. Our results are in agreement with a previous study (Lorenzetti and Ferreira, 1985) that supports that the analgesic effect of dipyrone results from a direct blockade of hyperalgesia and not only from prevention of the release of prostaglandin E_2 in inflamed tissues. In order to exclude the possibility that dipyrone at the dose of 200 μg/paw produced analgesia by acting at sites outside the paw, we used the strategy of evaluating the efficacy of ipsi versus contralateral paw administration. We chose this strategy because the route and site of injection would be the same. Prostaglandin E_2 was administered into both hind paws, thus creating the same tissue conditions and equal possibility that the agents tested would reach sites outside the injected paw. Remembering that the nociceptive threshold was always measured in the right hind paw, dipyrone at the dose of 200 μg was uneffective when administered into the contralateral paw, suggesting that at this dose, dipyrone has a peripheral site of action.

Our results demonstrated that the sulphonylureas glibenclamide and tolbutamide could prevent the peripheral antinociceptive effect induced by dipyrone in a dose-dependent manner. The sensitivity to sulphonylureas is commonly used to characterize the ATP-sensitive K^+ channels (Babenko et al., 1998). These drugs specifically block ATP-sensitive K^+ channels, with no effect on Ca^{2+}-activated K^+ channels or voltage-dependent K^+ channels (Amoroso et al., 1990; Nichols and Lederer, 1991; Edwards and Weston, 1993). Previous studies carried out in our laboratory using the rat paw pressure test, showed that the sulphonylureas tested do not cause any hyperalgesic or antinociceptive effect when administered alone. These blockers were also effective in counteract the antinociception induced by dipyrone even when they were injected 45 min after this antinociceptive drug, suggesting that glibenclamide and tolbutamide are able to prevent as well as to revert ATP-sensitive K^+ channels activation in our experimental model (data not shown). Our results also agree with those who described glibenclamide as more potent in blocking ATP-sensitive K^+ channels than tolbutamide (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990; Nichols and Lederer, 1991).

In contrast, charybdotoxin, a toxin that blocks large conductance Ca^{2+}-activated K^+ channels (Miller et al., 1985) and dequalinium, a selective blocker of small conductance Ca^{2+}-activated K^+ channels (Dunn, 1994), failed to antagonize the peripheral antinociceptive effect induced by dipyrone. Dequalinium, at equivalent dose, as well as apamin, was able to significantly antagonize the current associated with the afterhyperpolarization in neurons of preoptic area, suggesting an involvement of Ca^{2+}-activated K^+ channels in this effect, using an ovariectomized, female guinea pig model (Wagner et al., 2001). Our results showed that 4-aminopyridine, tetraethylammonium and cesium administered intraplantarly had no significant effect on the dipyrone-induced antinociception. These results were still negative even when these blockers were applied 45 min after the antinociceptive agent (results not shown). Although tetraethylammonium and 4-aminopyridine, even in lower doses than those used in the present study, had been able to block baclofen-induced antinociception in mice (Ocaña and Baeyens, 1993), Ocaña et al. (1995), studying the central antinociceptive effect of morphine and fentanyl; Rodrigues and Duarte (2000), in a study of the peripheral antinociceptive action of morphine and Soares et al. (2000), in a study of the mechanism of peripheral antinociceptive action of sodium nitroprusside also observed that these blockers failed in reverse the action of the antinociceptive substances tested. The lack of antagonism of the dipyrone effect by Ca^{2+}-activated K^+ channel blockers and voltage dependent K^+ channel blockers is interesting, because it suggests that this analgesic drug do not act in an unspecific and indiscriminate way, on the contrary, its peripheral antinociceptive effect appears to be specifically related to ATP-sensitive K^+ channels.

Dipyrone-mediated antinociception was shown to be related to the activation of the L-arginine/NO/cGMP pathway in primary sensory neurons (Duarte et al., 1992). There are many other studies in which an antinociceptive effect, via activation of the L-arginine/NO/cGMP, was demonstrated (Germany et al., 1996; Ahn et al., 1998). It was suggested that the molecular mechanism of peripheral (Ferreira et al., 1991) and central (Duarte and Ferreira, 1992) analgesia induced by morphine involves the activation of L-arginine/NO/cGMP. Morphine was shown by Rodrigues and Duarte (2000) to exert its peripheral antinociceptive effect by activating ATP-sensitive K^+ channels. A recent study carried out in our laboratory demonstrated that the peripheral antinociceptive action of the nitric oxide donor, sodium nitroprusside, is associated with ATP-sensitive K^+ channels, thus establishing a link between the participation of NO/cGMP pathway in the analgesia induced by certain drugs and the activation of ATP-sensitive K^+ channels (Soares et al., 2000).

In conclusion, our results support the suggestion that the peripheral antinociceptive effect of dipyrone is associated with ATP-sensitive K^+ channels activation, possibly involving the stimulation of the L-arginine/NO/cGMP pathway in sensory neurons. The mechanisms by which this second messenger system evokes that K^+ channel activation still needs to be understood.

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References

Ahn, D.K., Kim, Y.S., Park, J.S., 1998. Central NO is involved in the antinociceptive action of intracisternal antidepressants in freely moving rats. Neurosci. Lett. 243, 105–108.

Akman, H., Aksu, F., Gültekin, I., Özbek, H., Oral, U., Doran, F., Baysal, F., 1996. A possible central antinociceptive effect of dipyrone in mice. Pharmacology 53, 71–78.

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

Babenko, A.P., Aguilar-Bryan, L., Bryan, J., 1998. A view of Sur/K_{IR} 6.X, K_{ATP} channels. Annu. Rev. Physiol. 60, 667–687.

Carlsson, K.H., Helmreich, J., Jurna, I., 1986. Activation of inhibition from the periaqueductal grey matter mediates central analgesic effect of metamizol (dipyrone). Pain 27, 373–390.

Carrier, G.O., Fuchs, L.C., Winecoff, A.P., Giulumian, A.D., White, R.E., 1997. Nitrovasodilatador relax mesenteric microvessels by cGMP-induced stimulation of Ca^{2+}-activated K^+ channels. Am. J. Physiol. 273, H76–H83.

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.

Duarte, I.D.G., Ferreira, S.H., 1992. The molecular mechanism of central analgesia induced by morphine or carbachol and the L-arginine-nitric oxide-cGMP pathway. Eur. J. Pharmacol. 321, 171–174.

Duarte, I.D.G., Dos Santos, I.R., Lorenzetti, B.B., Ferreira, S.H., 1992. Analgesia by direct antagonism of nociceptor sensitization involves the arginine-nitric oxide-cGMP pathway. Eur. J. Pharmacol. 217, 225–227.

Dunn, P.M., 1994. Dequalinium, a selective blocker of the slow afterhyperpolarization in rat sympathetic neurones in culture. Eur. J. Pharmacol. 252, 189–194.

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

Ferreira, S.H., 1972. Prostaglandins, aspirin like drugs and analgesia. Nature (New Biol.) 240, 200–203.

Ferreira, S.H., Moncada, S., Vane, J.R., 1973. Prostaglandins and the mechanism of analgesia produced by aspirin like drugs. Br. J. Pharmacol. 49, 86–97.

Ferreira, S.H., Duarte, I.D.G., Lorenzetti, B.B., 1991. The molecular mechanism of action of peripheral morphine analgesia: stimulation of the cGMP system via nitric oxide release. Eur. J. Pharmacol. 201, 121–122.

Germany, A., González, P., Contreras, E., 1996. Possible role of nitric oxide in the antinociceptive action of intraventricular bradykinin in mice. Eur. J. Pharmacol. 310, 123–127.

Kubo, M., Nakaya, Y., Matsuoka, S., Saito, K., Kuroda, Y., 1994. Atrial natriuretic factor and isosorbide dinitrate modulate the gating of ATP-sensitive K^+ channel in cultured vascular smooth muscle cells. Circ. Res. 74, 470–476.

Lorenzetti, B.B., Ferreira, S.H., 1985. Mode of analgesic action of dipyrone: direct antagonism of inflammatory hyperalgesia. Eur. J. Pharmacol. 114, 375–381.

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.

Murphy, M.E., Brayden, J.E., 1995. Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive K^+ channels. J. Physiol. (London) 486, 47–58.

Nichols, C.G., Lederer, J.W., 1991. Adenosine triphosphate-sensitive K^+ channels in the cardiovascular system. Am. J. Physiol. 261, H1675–H1690.

Nikolova, M., Stefanova, D., Nikolov, R., Daleva, L., 1980. Comparative pharmacological study of dipyrone (analgin) and acetylsalicylic acid: analgesic effects. In: Ovtcharov, R., Pola, W. (Eds.), Proceedings of Dipyrone Symposium Moscow, 1978. F.K. Schattwer Verlag, Stuttgart, p. 91.

Ocaña, M., Baeyens, M., 1993. Differential effects of K^+ channel blockers on antinociception induced by α_2-adrenoceptor, GABA B and k-opioid receptor agonists. Br. J. Pharmacol. 110, 1049–1054.

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.

Randall, L.O., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissues. Arch. Int. Pharmacodyn. 111, 409–419.

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.

Schmid-Antomarchi, H., Amoroso, S., Fosset, M., Lazdunski, M., 1990. K^+ channel openers activate brain sulphonylureas-sensitive K^+ channels and block neurosecretion. Proc. Natl. Acad. Sci. U. S. A. 87, 3489–3492.

Soares, A.C., Leite, R., Tatsuo, M.A.K.F., Duarte, I.D.G., 2000. Activation of ATP-sensitive K^+ channels: mechanism of peripheral antinociceptive action of the nitric oxide donor, sodium nitroprusside. Eur. J. Pharmacol. 400, 67–71.

Thornbury, K.D., Ward, S.M., Dalziel, H.H., Carl, A., Westfall, D.P., Sanders, K.M., 1991. Nitric oxide and nitrocysteine mimic nonadrenergic,

noncholinergic hyperpolarization in canine proximal colon. Am. J. Physiol. 26, G553–G557.

Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nature (New Biol.) 231, 232–235.

Wagner, E.J., Ronnekleiv, O.K., Kelly, M.J., 2001. The noradrenergic inhibition of an apamin-sensitive, small-conductance Ca^{2+}-activated K^{+} channel in hypothalamic gamma-aminobutyric acid neurons: pharmacology, estrogen sensitivity, and relevance to the control of the reproductive axis. J. Pharmacol. Exp. Ther. 299, 21–30.