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Activation of ATP-sensitive K^+ channels: mechanism of peripheral antinociceptive action of the nitric oxide donor, sodium nitroprusside

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Abstract

Using the rat paw pressure test, in which sensitivity is increased by intraplantar injection of prostaglandin E_2 (PGE_2), we conducted a study using several K^+ channel blockers. The objective was to determine what types of K^+ channels could be involved in the peripheral antinociceptive action of the nitric oxide donor sodium nitroprusside (SNP). SNP elicited a dose-dependent (250 and 500 μg/paw) peripheral antinociceptive effect, which was considered local, since only higher doses produced an effect in the contralateral paw. The effect of SNP (500 μg/paw) was dose-dependently antagonized by intraplantar administration of the sulfonylureas tolbutamide (20, 40 and 160 μg) and glibenclamide (40, 80 and 160 μg), selective blockers of ATP-sensitive K^+ channels. Charybdotoxin (2 μg/paw), a selective blocker of high conductance Ca^{2+}-activated K^+ channels, and apamin (10 μg/paw), a selective blocker of low conductance Ca^{2+}-activated K^+ channels, did not modify the peripheral antinociception induced by SNP. Tetraethylammonium (2 mg/paw), 4-aminopyridine (200 μg/paw) and cesium (800 μg/paw) also had no effect. Based on this experimental evidence, we conclude that the activation of ATP-sensitive K^+ channels could be the mechanism by which nitric oxide, donated by SNP, induces peripheral antinociception, and that Ca^{2+}-activated K^+ channels and voltage-dependent K^+ channels appear not to be involved in the process.

Keywords: cGMP; Sodium nitroprusside; K^+ channel; Antinociception

1. Introduction

Cholinergic drugs have been reported to produce central and peripheral analgesia. Pain inhibiting effects have been observed after intracerebroventricular (Metys et al., 1969; Pedigo et al., 1975) and intrathecal administration (Yaksh et al., 1985; Iwamoto and Marion, 1993), as well as after microinjection of these drugs into discrete brain regions (Brodie and Proudfit, 1984; Katayama et al., 1984). Systemically administered acetylcholine receptor agonists also cause analgesia (Metys et al., 1969; Dayton and Garrett, 1973; Duarte et al., 1990b). In addition to these spinal and supraspinal antinociceptive sites of action, Ferreira and Nakamura (1979) described a peripheral analgesic effect of cholinergic agents. Because dibutyryl cGMP mimicked acetylcholine-induced analgesia, they suggested that cholinergic agents might cause analgesia by increasing cGMP at the nociceptor level.

Duarte et al. (1990a) demonstrated that intraplantar injection of acetylcholine and sodium nitroprusside (SNP) induced antinociception in the rat paw made hyperalgesic with prostaglandin E_2 (PGE_2). The antinociceptive effect induced by acetylcholine was blocked by L-N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of nitric oxide synthase (NOS). The antinociceptive actions of both acetylcholine and SNP were blocked by methylene blue, an inhibitor of guanylate cyclase, and were potentiated by MY5445, an inhibitor of cGMP phosphodiesterase. In 1992, Duarte and Ferreira described the involvement of the L-arginine/NO/cGMP pathway in central morphine-induced antinociception.

It is known that 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; Armstead, 1996; Zhuo et al., 1997; Carrier et al., 1997). More recently, Rodrigues and Duarte (2000) demonstrated the participation of ATP-sensitive K^+ channels in the peripheral antinociception induced by morphine.

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

The aim of the present study was to verify the possible relation between increased intracellular levels of NO/cGMP induced by the nitric oxide donor SNP and activation of K^+ channels causing neuronal desensitization, and to determine what types of K^+ channels may be involved in this effect.

2. Material and methods

2.1. Animals

The experiments were performed on 180–250 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 (0600–1800 h). All testings were done during the light phase (0900–1700 h). Food and water were freely available until the beginning of the experiments. Naive animals were used throughout.

2.2. Measurement of the hyperalgesia

Hyperalgesia was induced by a subcutaneous injection of PGE_2 (2 μg) into the plantar surface of the rat's hindpaw. The hyperalgesia was measured according to the paw pressure test described by Randall and Selitto (1957). An analgesymeter was used (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. The weight in grams 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 from the average of three consecutive trials recorded before (zero time) and 3 h after PGE_2 injection. The results were calculated as the difference between these two averages (Δ of nociceptive threshold) and expressed as grams.

2.3. Experimental protocol

SNP was administered subcutaneously in the right hindpaw 2 h after the local injection of PGE_2. In the protocol used to determine whether SNP was acting outside the injected paw, PGE_2 was injected into both hindpaws, while SNP was administered 2 h later in the left paw. The nociceptive threshold was always measured in the right hindpaw. All the K^+ channel blockers were injected subcutaneously into the right hindpaw. The sulfonylureas (glibenclamide and tolbutamide) were administered 5 min before SNP, while all the other K^+ channel blockers were injected 45 min after SNP (Wild et al., 1991; Ocaña and Baeyens, 1993; Yonehara and Takiuchi, 1997).

2.4. Drug administration

The drug used as hyperalgesic agent was PGE_2 (Sigma), and SNP (Sigma) was used as the antinociceptive drug. The K^+ channel blockers were glibenclamide (Sigma), tolbutamide (ICN Biomedicals), charybdotoxin (Sigma), apamin (Sigma), tetraethylammonium chloride (Sigma), 4-aminopyridine (Sigma) and Cesium (Mitsuwa's Pure Chemicals). All drugs were dissolved in isotonic saline, with exception of the sulphonylureas, which were dissolved in Tween 80 (2% in saline) and injected in a volume of 100 μl/paw.

2.5. Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) with post-hoc Bonferroni's test for multiple comparisons. Probabilities less than 5% ($P < 0.05$) were considered statistically significant.

3. Results

3.1. Antinociceptive action of SNP

Fig. 1 shows that intraplantar administration of SNP (250 and 500 μg) antagonized the hyperalgesic effect of PGE_2 (2 μg/paw) in a dose-dependent manner. SNP at the dose of 500 μg/paw, when administered in the left hindpaw, did not produce antinociception in the right hindpaw, whereas SNP at doses of 750 or 1000 μg, when injected into the left hindpaw, induced a potent antinociceptive effect in the contralateral paw (Fig. 2).

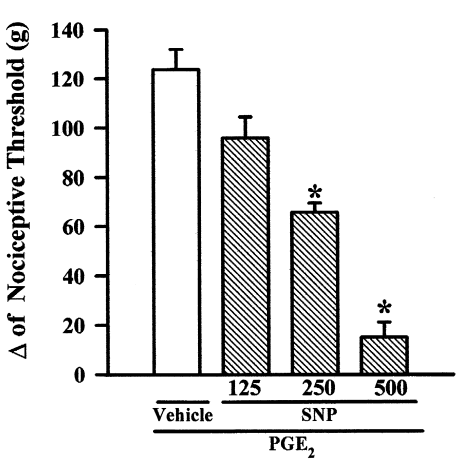

Fig. 1. Effect of SNP on the nociceptive threshold in rats with PGE_2-induced hyperalgesia. SNP (μg/paw) was administered 2 h after local administration of 100 μl of PGE_2. The antinociceptive response was measured in the paw pressure test as described in Section 2. Each column represents the mean ± S.E.M. ($n = 10$). * indicates a significant difference from the PGE_2 + vehicle injected control ($P < 0.05$, ANOVA + Bonferroni's test).

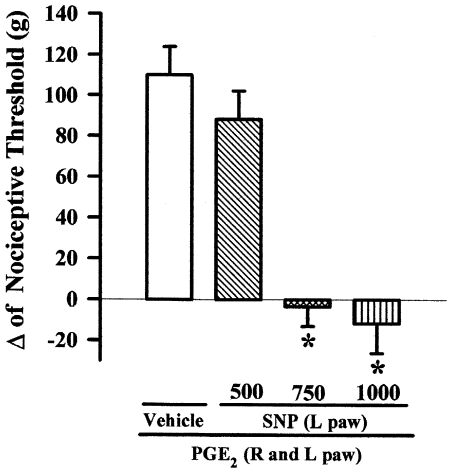

Fig. 2. Exclusion of outside paw antinociceptive effect of SNP. SNP (μg) was administered in the left (L) paw 2 h after PGE_2 administration in both hindpaws. The analgesic response of the right (R) hindpaw was measured in the paw pressure test as described in Section 2. Each column represents the mean ± S.E.M. ($n = 10$). * indicates a significant difference from the PGE_2 + vehicle-injected control ($P < 0.05$, ANOVA + Bonferroni's test).

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

The intraplantar injection of tolbutamide (20, 40 and 160 μg) reduced in a dose-dependent manner the peripheral antinociception induced by SNP (500 μg/paw; Fig. 3). The other sulfonylurea tested, glibenclamide (40, 80 and 160 μg/paw), also significantly inhibited the SNP-induced peripheral antinociceptive effect (Fig. 4). Neither sulphonylurea tested significantly modified the nociceptive threshold in control animals (data not show) or induced any overt behavioral effect at the doses used.

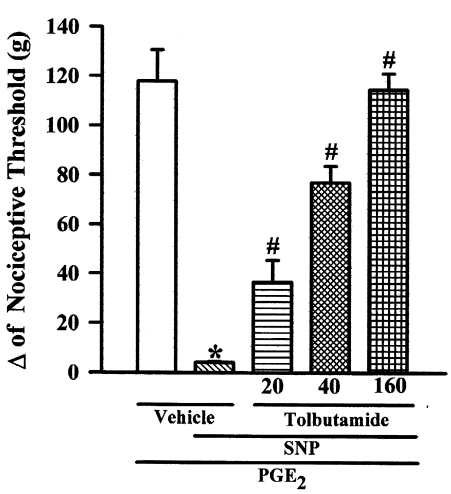

Fig. 3. Antagonism induced by intraplantar administration of tolbutamide of the peripheral antinociception produced by SNP in hyperalgesic paws. Tolbutamide (μg/paw) was administered 5 min before SNP (500 μg/paw). Each column represents the mean ± S.E.M. ($n = 5$). * and # indicates a significant difference compared to (PGE_2 + vehicle) and (PGE_2 + SNP + vehicle)-injected controls, respectively ($P < 0.05$, ANOVA + Bonferroni's test).

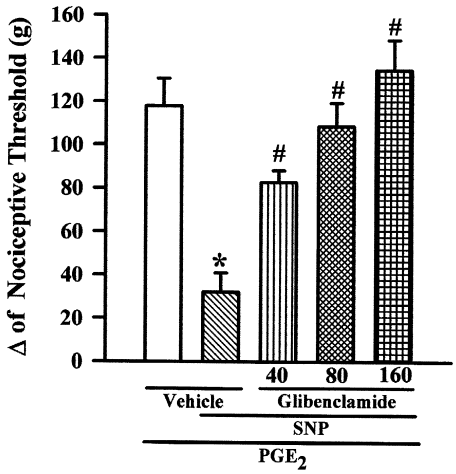

Fig. 4. Antagonism induced by intraplantar administration of glibenclamide of the antinociception produced by SNP in hyperalgesic paws. Glibenclamide (μg/paw) was administered 5 min before SNP (500 μg/paw). Each column represents the mean ± S.E.M. ($n = 5$). * and # indicates a significant difference compared to (PGE_2 + vehicle) and (PGE_2 + SNP + vehicle)-injected controls, respectively ($P < 0.05$, ANOVA + Bonferroni's test).

3.3. Effect of charybdotoxin and apamin on SNP-induced antinociception

Charybdotoxin (2 μg) injected into the paw did not significantly reduce the peripheral antinociception induced by SNP (500 μg/paw). Apamin (10 μg/paw) also did not modify significantly the antinociception induced by SNP (Fig. 5).

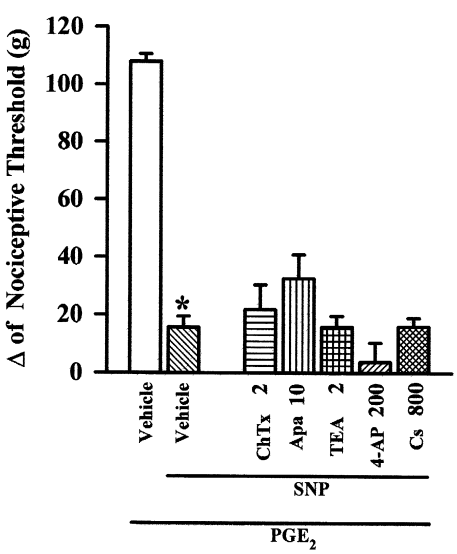

Fig. 5. Effect of intraplantar administration of apamin (Apa), charybdotoxin (ChTx), 4-aminopyridine (4-AP), tetraethylammonium (TEA) and cesium (Cs) on the peripheral antinociception induced by SNP in hyperalgesic paws. Drugs were administered 45 min after SNP (500 μg/paw). Each column represents the mean ± S.E.M. ($n = 5$). No statistically significant difference was found between the groups treated with PGE_2 + SNP + vehicle and PGE_2 + SNP + Apa or ChTx or 4-AP or TEA or Cs. * indicates a significant difference from the PGE_2 + vehicle-injected control ($P < 0.05$, ANOVA + Bonferroni's test).

3.4. Effect of tetraethylammonium, 4-aminopyridine and cesium on the SNP-induced antinociception

Fig. 5 also shows that tetraethylammonium (2 mg/paw), 4-aminopyridine (200 μg/paw) and Cs (800 μg/paw) did not modify significantly the antinociception induced by SNP.

4. Discussion

In the present experiments, SNP induced a dose-dependent antinociceptive effect on PGE_2-induced hyperalgesia. This result agrees with data reported in several other studies, in which an antinociceptive effect, via activation of the L-arginine/NO/GMP_C, was also demonstrated. The possible participation of this pathway in the antinociception induced by intracerebroventricular administration of bradykinin has been proposed in view of the fact that this action was antagonized by prior administration of N^G-nitro-L-arginine methyl ester (L-NAME) and methylene blue (Germany et al., 1996). Ahn et al. (1998) also demonstrated the participation of nitric oxide in the supraspinal antinociceptive action of antidepressant drugs, which was blocked by prior administration of L-NAME.

Interesting results have been recently obtained with topical application of nitroglycerin in patches that release a concentration of 0.2–1.0 mg/h of nitric oxide for the treatment of primary dysmenorrhea. This NO donor proved to be highly efficient in relieving pain in 90% of the patients studied (Ali et al., 1997). Christensen et al. (1997) also reported a therapeutic action of nitroglycerin at the dose of 0.5 mg administered sublingually for the relief of pancreatitis pain.

The possibility that SNP at the dose of 500 μg/paw produced analgesia by acting at sites outside the paw was excluded since its administration into the left paw did not alter the hyperalgesia in the contralateral paw. In these experiments, PGE_2 was administered in the left paw, so that this site of administration would be similar to that in the right paw, with an equal possibility that these agents would reach receptors outside the injected paw.

The sulfonylureas tolbutamide and glibenclamide reversed the peripheral antinociceptive effect of SNP in a dose-dependent manner. These drugs specifically block ATP-sensitive K^+ channels, with no effect on Ca^{2+}-activated or voltage-dependent K^+ channels (Amoroso et al., 1990; Davies et al., 1991; Nichols and Lederer, 1991; Edwards and Weston, 1993).

The central antinociceptive action of various substances, such as R-*N*-phenylisopropyl adenosine (R-PIA), an agonist of the adenosine A_1 receptor (Ocaña and Baeyens, 1994), prolactin (Shewade and Ramaswany, 1995) and various agonists of the 5-HT_{1A} receptor (Robles et al., 1996) also appears to be related to the activation of ATP-sensitive K^+ channels. Intracerebroventricular administration of glibenclamide (Ocaña et al., 1995) antagonized the central antinociceptive effect of morphine in mice, as measured in the hot-plate algesimetric test (Ocaña et al., 1990). Some studies using the tail-flick test have reported similar results, i.e., reversal of the central antinociceptive effect of morphine by glibenclamide.

In a study carried out in our laboratory using the rat paw compression test, Rodrigues and Duarte (2000) demonstrated that glibenclamide and tolbutamide reversed the peripheral antinociceptive action of an μ-opioid receptor agonist (morphine) in a dose-dependent manner.

The peripheral antinociceptive action of SNP seems to occur only through specific activation of ATP-sensitive K^+ channels, since blockers of other types of K^+ channels, such as Ca^{2+}-dependent (apamin and charybdotoxin) and voltage-dependent (4-aminopyridine, tetraethylammonium and cesium) channels did not reverse this action. The results were still negative when these blockers were applied according to the same protocol as for the sulphonylureas, 5 min before SNP (results not shown). Ocaña et al. (1995), in a study of the central antinociceptive effect of morphine and fentanyl, and Rodrigues and Duarte (2000), in a study of the peripheral antinociceptive effect of the morphine, also observed that 4-aminopyridine and tetraethylammonium did not reverse the effect of these agonists. Similarly, neither 4-aminopyridine nor tetraethylammonium reversed the antinociceptive action of R-PIA (Ocaña and Baeyens, 1994) and of agonists of the 5-HT_{1A} receptor (Robles et al., 1996).

It is important to point out that, as mentioned earlier, on the one hand, there are several studies demonstrating the participation of the NO–GMPc pathway in the analgesia induced by certain drugs, such as some opioids; however, there are also various studies that associate analgesia with the activation of ATP-sensitive K^+ channels. Thus, our results establish a link between these data by indicating that ATP-sensitive K^+ channels are involved in the peripheral antinociceptive effect of SNP. This suggestion is based on the finding that tolbutamide and glibenclamide reversed the peripheral antinociceptive effect of K^+ channel blockers. Thus, we propose that the activation of these channels after nitric oxide release at the nociceptive terminal may induce its desensitization.

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