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RESEARCH PAPER

Baclofen, an agonist at peripheral $GABA_B$ receptors, induces antinociception *via* activation of TEA-sensitive potassium channels

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Background and Purpose: Central anti-nociceptive actions of baclofen involve activation of K^+ channels. Here we assessed what types of K^+ channel might participate in the peripheral anti-nociception induced by baclofen.
Experimental approach: Nociceptive thresholds to mechanical stimulation in rat paws treated with intraplantar prostaglandin E_2.(PGE_2) to induce hyperalgesia were measured 3h after PGE_2 injection. Other agents were also given by intraplantar injection
Key results: Baclofen elicited a dose-dependent (15 - 240 μg per paw) anti-nociceptive effect. An intermediate dose of baclofen (60 μg) did not produce antinociception in the contralateral paw, showing its peripheral site of action. The $GABA_B$ receptor antagonist saclofen (12.5 - 100 μg per paw) antagonized, in a dose-dependent manner, peripheral antinociception induced by baclofen (60 μg), suggesting a specific effect. This antinociceptive action of baclofen was unaffected by bicuculline, $GABA_A$ receptor antagonist (80 μg per paw), or by (1,2,5,6 tetrahydropyridin-4-yl) methylphosphinic acid, $GABA_C$ receptor antagonist (20 μg per paw). The peripheral antinociception induced by baclofen (60 μg) was reversed, in a dose-dependent manner, by the voltage-dependent K^+ channel blockers tetraethylammonium (7.5 - 30 μg per paw) and 4-aminopyridine (2.5 - 10 μg per paw). The blockers of other K^+ channels, glibenclamide (160 μg), tolbutamide (320 μg), charybdotoxin (2 μg), dequalinium (50 μg) and caesium (500 μg) had no effect.
Conclusions and Implications: This study provides evidence that the peripheral antinociceptive effect of the $GABA_B$ receptor agonist baclofen results from the activation of tetraethylammonium-sensitive K^+ channels. Other K^+ channels appear not to be involved.
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Keywords: baclofen; K^+ channel; peripheral antinociception; 4-aminopyridine; tetraethylammonium; sulphonylureas; saclofen; $GABA_B$ receptor

Abbreviations: PGE_2, prostaglandin E_2; TEA, tetraethylammonium; TPMPA, (1,2,5,6 tetrahydropyridin-4-yl) methylphosphinic acid

Introduction

GABA is the major inhibitory neurotransmitter in the vertebrate central nervous system. GABA receptors have been classified into three distinct subtypes $GABA_A$, $GABA_B$ and $GABA_C$. Both $GABA_A$ and $GABA_C$ receptors form ligand-gated chloride channels, while the $GABA_B$ receptor belongs to the G-protein-coupled receptor family whose activation causes a decrease in Ca^{++} and increase in K^+ membrane conductance (Sigel *et al*., 1983; Johnston, 1997; Bowery and Enna, 2000).

Baclofen (β-[4-chlorophenyl] GABA) is a stereospecifically active agonist at the $GABA_B$ receptor (Bowery *et al*., 1981). It has been used therapeutically as a muscle relaxant and for the treatment of trigeminal neuralgia (Korolkovas, 1999).

In a variety of animal pain models, baclofen has been reported to produce antinociception following systemic (Shafizadeh *et al*., 1997; Sabetkasai *et al*., 1999), supraspinal (Sawynok, 1987; Shafizadeh *et al*., 1997; Potes *et al*., 2006) and spinal (Aran and Hammond, 1991; Sawynok, 1987; Malan *et al*., 2002) administration. In addition, studies have demonstrated the presence of $GABA_A$ and $GABA_B$ receptors in the periphery (Carlton *et al*., 1999; Calver *et al*., 2000).

Several reports have demonstrated that the opening of different potassium channels underlies the antinociceptive effect induced by activation of G-protein-coupled receptors such as the μ/δ-opioid and $GABA_B$ receptors. In the central nervous system, the opening of ATP-sensitive K^+ channels seems to play a role in antinociception induced by morphine and [D-pen^{2,5}]-enkephalin, since the sulphonylureas,

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glibenclamide and tolbutamide antagonize the antinociceptive effect of these drugs (Ocaña *et al*., 1990; Wild *et al*., 1991; Ocaña and Baeyens, 1993, 1994). Similarly, the peripheral antinociception induced by morphine and SNC80 is also antagonized by glibenclamide and tolbutamide (Rodrigues and Duarte 2000; Pacheco and Duarte, 2005). On the other hand, the central antinociception induced by baclofen in the tail-flick test is specifically antagonized by K^+ channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA) (Ocaña and Baeyens, 1993).

In this context, the aim of the present study was to verify the possible peripheral antinociceptive effect of baclofen using the rat paw pressure test. The specificity of baclofen in $GABA_B$ receptors was also tested through intraplantar administration of saclofen, a $GABA_B$ receptor antagonist. Furthermore, the possible involvement of K^+ channels was evaluated using specific K^+ channel blockers, especially those for tetraethylammonium (TEA)-sensitive K^+ channels, as they were associated with the central antinociception induced by GABA (Ocaña and Baeyens, 1993).

Methods

Animals

The experiments were performed on 160–200 g male Wistar rats ($N=4$–15 per group) from CEBIO-UFMG (The Animal Centre of the Federal University of Minas Gerais). The rats were housed in a temperature-controlled room ($23\pm1^{\circ}C$) on an automatic 12-h light/dark cycle (0600–1800 h of light phase). All testing was carried out during the light phase (0800–1500). Food and water were freely available until the beginning of the experiments. Naive rats were used throughout. All the experiments were approved by the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais.

Measurement of hyperalgesia

Hyperalgesia was induced by a subcutaneous (s.c.) injection of prostaglandin E_2 (PGE_2, 2 μg) into the plantar surface of the rat's hindpaw and measured by the paw pressure test described by Randall and Selitto (1957). The rat was carefully kept in a horizontal/normal position, by one hand of the researcher while the test paw was submitted to pressure. An analgesimeter (Ugo-Basile, Italy) with a cone-shaped paw-presser with a rounded tip was used to apply a linearly increasing force to the rat's right hindpaw. The weight in grams required to elicit nociceptive paw response was determined as the nociceptive threshold. A cutoff value of 300 g was used to prevent damage to the paws. The nociceptive threshold was measured in the right paw and determined by the average of three consecutive trials recorded before (zero time) and 3 h after PGE_2 injection (peak of effect). The results were calculated by the difference between these two averages (Δ of nociceptive threshold) and expressed as grams. To reduce stress, the rats were habituated to the apparatus 1 day before the experiments.

Experimental protocol

Baclofen was administered s.c. in the right hindpaw (R paw) 2.5 h after the local injection of PGE_2 (Figure 1a).

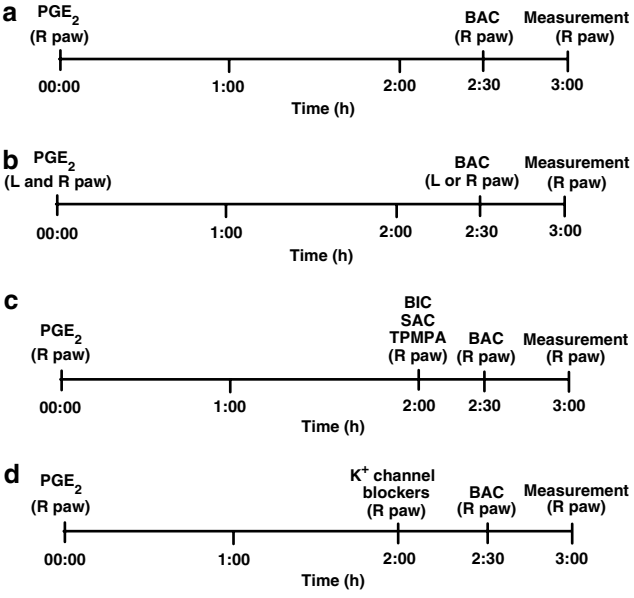

Figure 1 Schedules of drug administration: (**a**) baclofen; (**b**) exclusion of a systemic antinociceptive effect of baclofen; (**c**) GABA receptor antagonists; (**d**) K^+ channels blockers.

In the protocol used to determine whether baclofen was acting outside the injected paw, PGE_2 was injected into both hindpaws, while baclofen was administered into the left or right paw (L or R paw) (Figure 1b).

Saclofen, bicuculline and 1,2,5,6 tetrahydropyridin-4-yl) methylphosphinic acid (TPMPA) were administered s.c. in the right paw 5 min before baclofen (Figure 1c).

All the K^+ channel blockers were injected s.c. into the right hindpaw 30 min before baclofen (Figure 1d).

The nociceptive threshold was always measured in the right hindpaw. The protocol above was assessed in pilot experiments to determine the best moment of injection of each substance.

Statistical analysis

The data were analysed statistically by one-way analysis of variance (ANOVA) using the Bonferroni test *post hoc* for multiple comparisons. Probabilities less than 5% ($P<0.05$) were considered statistically significant.

Chemicals

The following drugs and chemicals were used: PGE_2 (Sigma, USA), baclofen (Novartis AG, Switzerland), saclofen (ToCris, EUA), bicuculline (Sigma), TPMPA (Sigma), glibenclamide (Sigma, USA), tolbutamide (ICN Biomedicals, USA), charybdotoxin (Sigma, USA), dequalinium (Calbiochem, USA), TEA (Sigma, USA), 4-AP (Sigma, USA) and caesium (Mitsuwa's Pure Chemical, Japan). PGE_2 (ethanol 8% in saline), baclofen, saclofen, bicuculline, TPMPA, TEA, 4-AP were dissolved in isotonic saline. The K^+ channel blockers charybdotoxin, caesium and dequalinium were dissolved in demineralized water, while the sulphonylureas glibenclamide and tolbutamide were dissolved in Tween 80 vehicle (2% in saline). All drugs were dissolved immediately before

use and injected in a volume of 100 μl per paw, with exception of K^+ channel blockers and bicuculline, saclofen and TPMPA which were injected in a volume of 50 μl. Control naïve rats (not hyperalgesic) were injected with the vehicle for PGE_2 (ethanol 8% in saline).

Results

Peripheral antinociceptive effect of baclofen

Figure 2a shows that intraplantar administration of baclofen (15, 30, 60, 120 and 240 μg) in the right paw antagonized the hyperalgesic effect of PGE_2 (2 μg per paw) in a dose-dependent manner. Baclofen, at a dose of 60 μg injected into the left paw, produced no antinociceptive effect in the right paw, whereas at a dose of 120 μg injected into the left paw, baclofen did induce an antinociceptive effect in the contralateral paw (Figure 2b).

Antagonism of baclofen-induced antinociception by saclofen

The intraplantar injection of saclofen (12.5, 25, 50 and 100 μg) reduced the peripheral antinociception induced by baclofen (60 μg per paw; Figure 3a) in a dose-dependent manner. Saclofen did not modify the nociceptive threshold in control animals (vehicle only) nor induce any overt behavioural effects at the doses used.

Effect of bicuculline and TPMPA on baclofen-induced antinociception

As shown in Figure 3b neither bicuculline (80 ng per paw) nor TPMPA (20 μg per paw) reduced the antinociceptive effects of baclofen (60 μg per paw). These drugs caused no effects when used alone (data not shown).

Antagonism of baclofen-induced antinociception by TEA and 4-AP

TEA (7.5, 15 and 30 μg per paw) and 4-AP (2.5, 5 and 10 μg per paw) significantly reduced the baclofen-induced peripheral antinociception (60 μg per paw) in a dose-dependent manner (Figures 4a and b). Neither of the K^+ channel blockers tested significantly modified the nociceptive threshold in control animals nor did they induce any overt behavioural effects.

Effect of glibenclamide, tolbutamide, charybdotoxin, dequalinium and caesium on baclofen-induced antinociception

Glibenclamide (160 μg) and tolbutamide (320 μg) injected into the paw did not reduce baclofen-induced antinociception (Figure 5). Charybdotoxin (2 μg), dequalinium (50 μg) and caesium (500 μg) also failed to counteract the antinociception induced by baclofen (Figure 5). These drugs did not induce hyperalgesia or antinociception by themselves (data not shown).

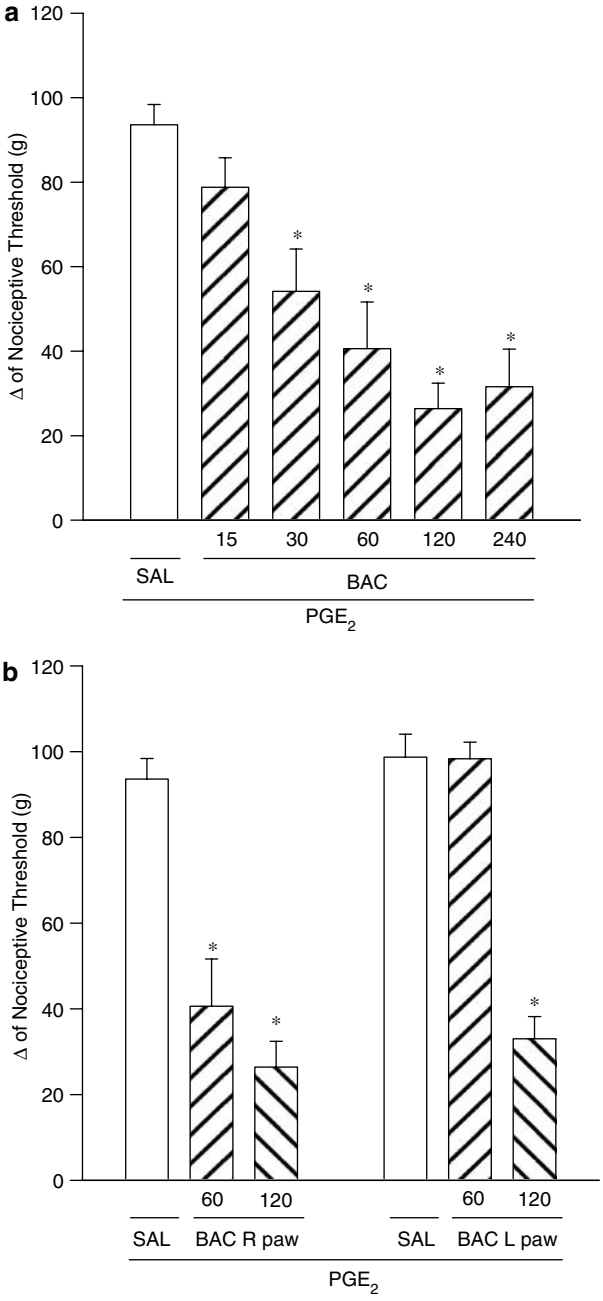

Figure 2 Dose-dependent effect of baclofen on the nociceptive threshold in PGE_2-induced hyperalgesia in rats (**a**) and exclusion of systemic antinociceptive effect of baclofen at dose of 60 μg (**b**). (**a**) Baclofen (15–240 μg per paw) was given 2.5 h after local administration of 100 μl of PGE_2 (2 μg). (**b**) Baclofen (60 or 120 μg per paw) was administered into the right (R) or left (L) paw 2 h and 30 min after PGE_2 (2 μg) administration into both hind paws. The antinociceptive response was measured in the paw pressure test as described in Methods. Each column represents the mean $\pm$s.e.m. for 5–10 rats per group. *Indicates a significant difference from the PGE_2 + saline (SAL) injected control ($P<0.05$, ANOVA + Bonferroni test).

Discussion and conclusions

The participation of the GABAergic system in pain modulation has been extensively studied at both systemic and central levels. However, there are only a few studies associating GABA and peripheral antinociception. In the present study, baclofen, a $GABA_B$ receptor agonist, induced a dose-dependent and peripheral antinociceptive effect on PGE_2-induced hyperalgesia.

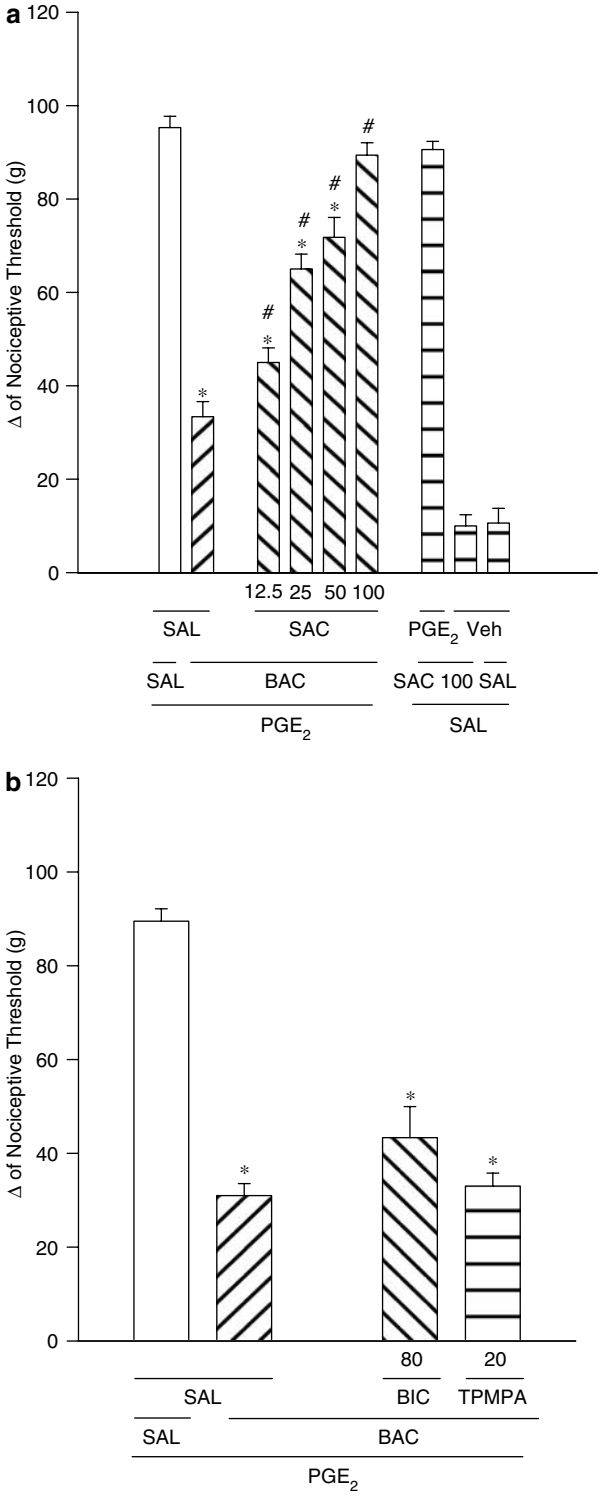

Figure 3 Effect of intraplantar administration of (**a**) saclofen (SAC) and in (**b**), bicuculline (BIC) and 1,2,5,6 tetrahydropyridin-4-yl) methylphosphinic acid (TPMPA) on the peripheral antinociception induced by baclofen in hyperalgesic paws (PGE_2, 2 μg). Saclofen (12.5–100 μg), BIC (80 ng) and TPMPA (20 μg), were administered 5 min before baclofen (60 μg per paw). Each column represents the mean $\pm$ s.e.m. for 5–8 rats per group. $^{*,\#}$Indicate significant differences compared to PGE_2 + SAL + SAL- and PGE_2 + BAC + SAL-injected groups, respectively ($P<0.05$, ANOVA + Bonferroni test). Veh = vehicle (ethanol 8% in saline).

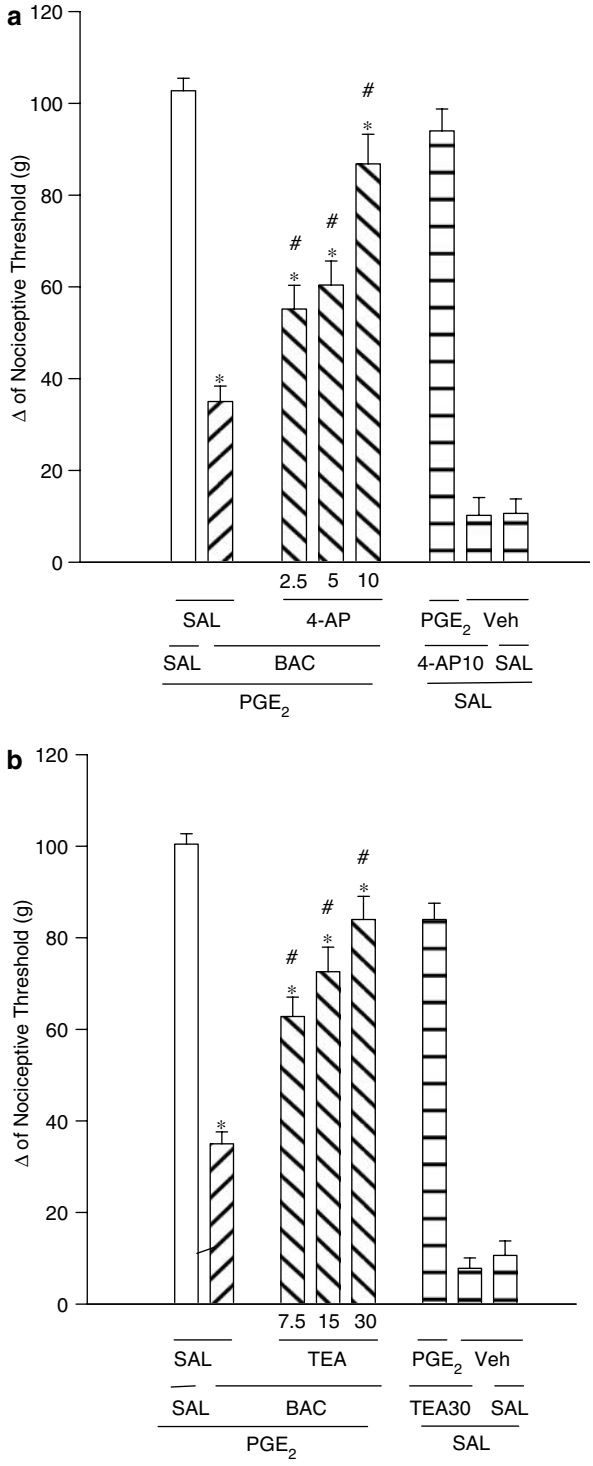

Figure 4 Antagonism induced by intraplantar administration of (**a**) 4-AP and (**b**) TEA of the peripheral antinociception produced by baclofen in hyperalgesic paws (PGE_2, 2 μg). 4-AP (2.5–10 μg) or TEA (7.5–30 μg) were administered 30 min before baclofen (60 μg per paw). Each column represents the mean $\pm$ s.e.m. for 5–15 rats per group. $^{*,\#}$Indicate significant differences compared to PGE_2 + SAL + SAL- and PGE_2 + BAC + SAL-injected groups, respectively ($P<0.05$, ANOVA + Bonferroni test). Veh = vehicle (ethanol 8% in saline).

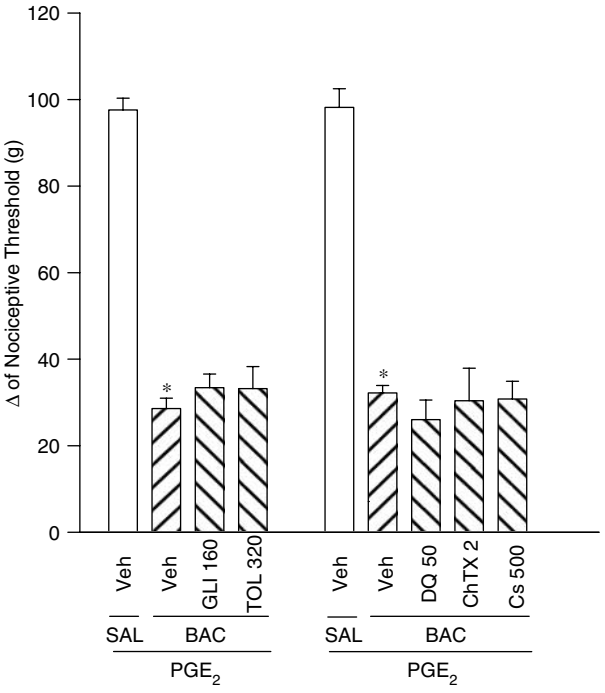

Figure 5 Effect of intraplantar administration of glibenclamide (GLI), tolbutamide (TOL) charybdotoxin (ChTX), dequalinium DQ0 and caesium (Cs) on the peripheral antinociception produced by baclofen in hyperalgesic paws (PGE_2, 2 μg). Drugs (the doses shown are μg per paw) were administered 30 min before baclofen (60 μg per paw). Each column represents the mean ± s.e.m. for 4–7 rats per group. * and # Indicate significant differences compared to PGE_2 + SAL + SAL- and PGE_2 + BAC + SAL-injected groups, respectively ($P<0.05$, ANOVA + Bonferroni test). Veh = vehicle (ethanol 8% in saline).

The possibility that baclofen at a dose of 60 μg per paw produced antinociception by acting at sites outside the paw was excluded, since its administration into the left paw did not alter hyperalgesia in the contralateral paw. In these experiments, PGE_2 was administered in the left paw, so that this administration site would present similar conditions to that in the right paw, with an equal possibility that these agents would reach receptors outside the injected paw. Baclofen is known to induce muscle-relaxant effects when administered systemically or centrally (Malcangio and Bowery, 1996) but, in the present study this was shown not to be the case, since baclofen induced a local effect. Furthermore, data from the literature demonstrated that the doses of baclofen which produced antinociception did not induce motor incoordination (Bowery, 1997; Patel *et al*., 2001; Balerio and Rubio, 2002).

The existence of $GABA_A$ and $GABA_B$ receptors in the periphery has been previously described (Carlton *et al*., 1999; Calver *et al*., 2000). A source of endogenous GABA for these peripheral receptors might be glutamate-containing primary afferent fibres. This amino acid is present in more than 90% of afferent primary fibres (Battaglia and Rustioni, 1988) and is converted by glutamic acid decarboxylase (GAD) into GABA (Malcangio and Bowery, 1996). In 2004, Stoyanova (2004) demonstrated the presence of GABA in primary afferent neurons of feline sensory ganglia: trigeminal and dorsal root ganglia.

Further support for a role of GABA in peripheral antinociception came from behavioural studies. For example, gabapentin, a GABA-mimetic drug, induced peripheral antinociceptive effect in the formalin test (Carlton and Zhou, 1998) and in formalin-induced secondary hyperalgesia (Motta *et al*., 2004). Carlton *et al*. (1999) also demonstrated that muscimol, a $GABA_A$ receptor agonist, induced peripheral antinociception in the formalin test.

Saclofen was used in the present study, in order to confirm the involvement of the $GABA_B$ receptor in peripheral antinociception induced by baclofen. The results demonstrated that this antagonist was able to prevent the peripheral antinociceptive effect induced by baclofen in a dose-dependent manner. Other studies also demonstrated the involvement of the $GABA_B$ receptors in central antinociception induced by baclofen. In a variety of nociceptive tests, $GABA_B$ receptor antagonists such as phaclofen, 2-hydroxysaclofen (Aran and Hammond, 1991; Shafizadeh *et al*., 1997) and CGP 35348 (Malcangio *et al*., 1991; Dirig and Yaksh, 1995; Sabetkasai *et al*., 1999) reversed the baclofen-induced antinociception. In contrast, the present results show that neither the $GABA_A$ receptor antagonist bicuculline nor the $GABA_C$ receptor antagonist TPMPA reversed the antinociceptive effect induced by baclofen, results which further support the argument that the antinociceptive response induced by baclofen in the paw pressure test is mediated by a $GABA_B$ mechanism.

The present results demonstrated that the peripheral antinociception induced by baclofen was reversed, in a dose-dependent manner, by two K^+ channel blockers, 4-AP and TEA. According to Alexander *et al*. (2001), 4-AP and TEA, aside from blocking the voltage-dependent K^+ channels (K_v), also block G-protein-regulated inward rectifier K^+ channels (GIRK). Studies have shown that the antinociception induced by baclofen was reduced in GIRK2 subunit knockout mice (Blednov *et al*., 2003) and in mice pretreated for several days with an antisense that lowered $K_v1.1$ gene expression (Galeotti *et al*., 1997). In a pharmacological study, Ocaña and Baeyens (1993) demonstrated that 4-AP and TEA antagonized the central antinociceptive effect of baclofen. The current results also agree with those who described 4-AP as more potent in blocking the voltage-dependent K^+ channels than TEA (Cook and Quast, 1990).

In contrast, the sulphonylureas glibenclamide and tolbutamide, specific blockers of ATP-sensitive K^+ channels, exhibited no effect in the peripheral antinociception induced by baclofen. These data are supported by those of Ocaña and Baeyens (1993) who reported that the central antinociception induced by baclofen was not modified by intracerebroventricular administration of the sulphonylurea, gliquidone. It is important to emphasize that experiments from our group have already demonstrated that the sulphonylureas (at the same dose as that used for baclofen) reversed the peripheral antinociceptive effect of morphine (Rodrigues and Duarte, 2000), dibutyryl cyclic GMP (Soares and Duarte, 2001) and SNC80 (Pacheco and Duarte, 2005). Consequently, the present results suggest that ATP-sensitive K^+ channels are not involved in the peripheral antinociception induced by baclofen.

Dequalinium, a selective blocker of small conductance Ca^{2+}-activated K^+ channels (Castle *et al*., 1993; Dunn, 1994) and charybdotoxin, a blocker of large conductance Ca^{2+}-activated K^+ channels (Mackinnon and Miller, 1988) also failed to antagonize the peripheral antinociceptive effect induced by baclofen. Alves *et al*. (2004) demonstrated that NS1619, a specific opener of large conductance Ca^{2+}-activated K^+ channels, did not produce antinociceptive action in hyperalgesia induced by PGE_2, suggesting that these channels may not exist in peripheral tissues or, more probably, that they were not activated under the current experimental conditions. In contrast, Ortiz *et al*. (2002) studying the involvement of K^+ channels in the antinociceptive action of diclofenac in the formalin test demonstrated that charybdotoxin was effective.

In summary, the data of the present study suggested that activation of peripheral $GABA_B$ receptors by baclofen induces antinociception via the opening of the voltage-dependent K^+ channel or the G-protein-coupled inwardly rectifying K^+ channel. Other K^+ channel types such as large and small conductance Ca^{2+}-activated and ATP-sensitive K^+ channels appear not to be involved. This is the first demonstration of the peripheral antinociceptive effect of baclofen and of one of its possible mechanisms of action.

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Conflict of interest

The authors state no conflict of interest.

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