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Short communication

Involvement of $GABA_A$ receptor-associated chloride channels in the peripheral antinociceptive effect induced by $GABA_A$ receptor agonist muscimol

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Abstract

The effect of chloride and potassium channel blockers on the antinociception induced by $GABA_A$ receptor agonist muscimol was investigated using the paw pressure test. Muscimol (1, 2, 4, 8 ng/paw) elicited a peripheral antinociceptive effect that was antagonized by bicuculline (10, 20, 40, 80 ng/paw), suggesting a specific effect. The muscimol effect was reverted by the chloride channel coupled $GABA_A$ receptor blocker, picrotoxin (0.4, 0.6, 0.8, 2 μg/paw). Potassium channel blockers did not modify the peripheral antinociception induced by muscimol. This study provides evidence that the peripheral antinociceptive effect of muscimol results from the activation of $GABA_A$ receptor-associated chloride channels.

Keywords: Muscimol; Cl^- channel; Peripheral antinociception; Bicuculline; Picrotoxin; $GABA_A$ receptors

1. Introduction

γ-aminobutyric acid (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 an increase in K^+ membrane conductance (Sigel et al., 1983; Johnston, 1997; Bowery and Enna, 2000).

The function of GABA in the modulation of nociception is crucial and complex. Several reports have demonstrated the participation of the GABAergic system in the modulation of pain at the supraspinal (Millan, 2002) and spinal level (Malcangio and Bowery, 1996; Hammond, 2001). However, there are only a few studies associating GABA and peripheral antinociception. GABAergic neurons in the ventral periaqueductal grey (PAG) matter and ventrolateral orbital cortex (VLO) modulate the analgesic effects of morphine microinjected into these brain areas. The hypothesis is that morphine may directly inhibit the GABAergic inhibitory interneurons leading to indirect activation of the descending antinociceptive pathway through a disinhibitory effect on the PAG and VLO output neurons and depression of the nociceptive inputs at the spinal cord level (De Paulis et al., 1987; Qu et al., 2006). 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). The existence of $GABA_A$ and $GABA_B$ receptors in the periphery has been previously described by Carlton et al. (1999) and Calver et al. (2000). A source of endogenous GABA for these peripheral receptors might be glutamate-containing primary afferent fibers. This amino acid is present in more than 90% of afferent primary fibers (Battaglia and Rustioni, 1988) and is converted by glutamic acid decarboxylase (GAD) into GABA (Malcangio and Bowery, 1996). In 2004, Stoyanova demonstrated the presence of GABA in primary afferent neurons of feline sensory ganglia: trigeminal and dorsal root ganglia (Stoyanova, 2004). More recently, the participation of voltage-dependent K^+ channels or G-protein-coupled inwardly rectifying K^+ channels in the peripheral

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antinociception induced by baclofen was demonstrated (Reis and Duarte, 2006).

The present study was undertaken with the aim of clarifying the role of GABA in peripheral antinociception. For this purpose, the effects of peripheral administration of the $GABA_A$ agonist muscimol were tested using the paw pressure test. The specificity of muscimol in $GABA_A$ receptor was also tested through intraplantar injection of $GABA_A$ receptor antagonist bicuculline. Furthermore, the possible antinociceptive action mechanism was evaluated using picrotoxin, a chloride channel coupled $GABA_A$ receptor blocker. With the aim of excluding nonspecific mechanisms, specific potassium channel blockers were also used.

2. Materials and methods

2.1. Animals

The experiments were performed on 160–200 g male Wistar rats from CEBIO-UFMG (The Animal Centre of the Federal University of Minas Gerais). The rats were housed in a temperature-controlled room (23±1 °C) on an automatic 12-h light/dark cycle (light phase from 06:00 to 18:00 h). All testing was concluded during the light phase (8:00–15:00). 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 for Animal Experimentation (CETEA) of the Federal University of Minas Gerais.

2.2. Measurement of the hyperalgesia

Hyperalgesia was induced by a subcutaneous injection of prostaglandin E_2 (PGE_2, 2 μg) into the plantar surface of the rat hindpaw and measured by the paw pressure test described by Randall and Selitto (1957). An analgesimeter (Ugo-Basile, Italy), which possessed a cone-shaped paw-presser with a rounded tip, was used to apply a linearly increasing force to the rat right hindpaw. The weight in grams required to elicit nociceptive paw 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 measured in the right paw and 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.

2.3. Drug administration

The drug used as a hyperalgesic agent was PGE_2 (Sigma, USA), and muscimol (Tocris, USA) was used as the $GABA_A$ receptor agonist. Bicuculline methochloride (Sigma), saclofen (ToCris) and [1,2,5,6 tetrahydropyridin-4-yl] methylphosphinic acid (TPMPA; Sigma) were used as $GABA_A$, $GABA_B$ and $GABA_C$ receptor antagonists, respectively. The chloride channel coupled $GABA_A$ receptor blocker used was picrotoxin (Sigma) and the K^+ channel blockers were glibenclamide (Sigma) tolbutamide (ICN Biomedicals, USA), charybdotoxin (Sigma), dequalinium (Calbiochem, USA), tetraethylammonium (Sigma), 4-aminopyridine (Sigma), and cesium (Mitsuwa's Pure Chemical, Japan). Prostaglandin E_2 (8% ethanol in saline), muscimol, bicuculline, saclofen, TPMPA, picrotoxin, tetraethylammonium, 4-aminopyridine, charybdotoxin, cesium and dequalinium were dissolved in isotonic saline, while the sulfonylureas 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 50 μl per paw, with exception of PGE_2 which was injected in a volume of 100 μl/paw.

2.4. Experimental protocol

Muscimol was administered subcutaneously in the right hindpaw 2 h and 45 min after the local injection of PGE_2. In the protocol used to determine whether muscimol was acting outside the injected paw, PGE_2 was injected into both hindpaws, while muscimol was administered into the left or right paw. The nociceptive threshold was always measured in the right hindpaw. Bicuculline was administered by intraplantar *via* in the right paw 20 min before muscimol and picrotoxin was injected 40 min before muscimol. Saclofen and TPMPA were injected 5 min before muscimol. All the K^+ channel blockers were injected subcutaneously into the right hindpaw 30 min before muscimol. The above protocol was assessed in pilot experiments to determine the best moment of injection for each substance.

2.5. Statistical analysis

The data were analyzed statistically by one-way analysis of variance (ANOVA) using the Bonferroni *post-hoc* test for

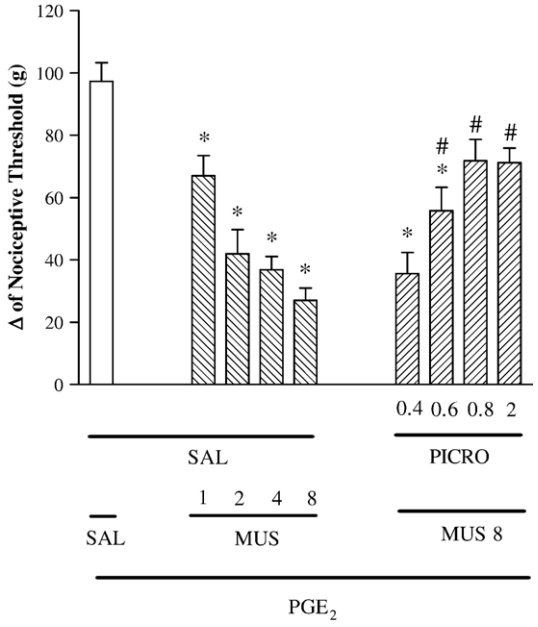

Fig. 1. Dose-dependent effect of muscimol on the nociceptive threshold of PGE_2-induced hyperalgesia in rats and its antagonism induced by the intraplantar administration of picrotoxin. Muscimol (ng/paw) was administered 2 h and 45 min after local administration of PGE_2 (2 μg). Picrotoxin (μg/paw) was injected 40 min before muscimol (8 ng/paw). Each column represents the mean±S.E.M. (n=5–10). *, # Indicate significant differences compared to PGE_2+SAL+SAL and PGE_2+MUS+SAL (P<0.05, ANOVA+Bonferroni test).