Spasmolytic Action of the Methanol Extract and Isojuripidine from *Solanum asterophorum* Mart. (Solanaceae) Leaves in Guinea-Pig Ileum

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Z. Naturforsch. **61 c**, 799–805 (2006); received March 20/May 16, 2006

Solanum asterophorum Mart. (Solanaceae) is a shrub popularly known as "jurubeba-de-fogo" in the northeast of Brazil. In the present work, the methanol extract (SA-MeOH, 3–750 μg/mL) and isojuripidine (10^{-7} – 3×10^{-4} M), a steroidal alkaloid obtained from *S. asterophorum* Mart. leaves, inhibited phasic contractions induced by both 1 μM histamine [IC_{50} = (225.8 ± 47.4) μg/mL and (3.5 ± 0.8) $\times 10^{-5}$ M] or 1 μM acetylcholine [IC_{50} = (112.5 ± 20.6) μg/mL and (2.3 ± 0.4) $\times 10^{-5}$ M] in guinea-pig ileum, respectively. The extract and isojuripidine also relaxed the ileum (SA-MeOH, 1–750 μg/mL, and isojuripidine, 10^{-9} – 3×10^{-4} M) pre-contracted with 1 μM histamine [EC_{50} = (101.1 ± 17.4) μg/mL and (1.2 ± 0.3) $\times 10^{-6}$ M] or 1 μM acetylcholine [EC_{50} = (136.8 ± 21.1) μg/mL and (1.9 ± 0.4) $\times 10^{-6}$ M] or 40 mM KCl [EC_{50} = (149.4 ± 19.5) μg/mL and (1.8 ± 0.7) $\times 10^{-6}$ M], respectively, in an equipotent and concentration-dependent manner. This effect is probably due to inhibition of calcium influx through voltage-operated calcium (Ca_v) channels. To confirm this hypothesis, we evaluated their effect on cumulative $CaCl_2$ curves in depolarizing medium nominally without Ca^{2+}. SA-MeOH (27, 243, 500, and 750 μg/mL) and isojuripidine (3×10^{-8}, 10^{-6}, 3×10^{-5}, and 3×10^{-4} M) inhibited the contractions induced by $CaCl_2$, in a concentration-dependent manner. The concentration-response curves to $CaCl_2$, in the presence of SA-MeOH and isojuripidine, were shifted downward in relation to a control curve in a non-parallel manner resulting in reduction of the maximum effect [E_{max} = (71.2 ± 9.2); (57.4 ± 9.2); (43.8 ± 3.4); (41.5 ± 2.4) and (90.6 ± 4.8); (74.7 ± 8.7); (66.4 ± 3.9); (31.3 ± 4.1)%, respectively]. SA-MeOH and isojuripidine present spasmolytic action in guinea-pig ileum due to a partially blockade of calcium influx through Ca_v channels.

Key words: Solanum asterophorum, Spasmolytic Action, Guinea-Pig Ileum

Introduction

The Solanaceae family, one of the biggest families distributed worldwide, comprises approx. 92 genera and 2300 species (Hunziker, 2001). The *Solanum* genus is considered as one of the largest among the angiosperms with approx. 1250 species (Nee, 2001). It is a rich source of active secondary metabolites. Many species of *Solanum* are popularly known as "jurubeba". Plants of this genus are known to produce a great variety of steroidal saponins and glycoalkaloids that are important in the plants natural resistance against several pests (Friedman *et al*., 1991).

In Brazil, several species of *Solanum* (*S. paniculatum* L., *S. melongena* L. and *S. stipulaceum* Roem & Schult.) were reported to induce hypotension in rats (Ribeiro *et al.*, 1986; Almeida *et al.*, 1984; Shum and Chiu, 1991; Ribeiro, 2001). Moreover other species also presented significant spasmolytic effects, for example, *S. indicum* L., *S. paludosum* Moric., *S. torvum* Sw., *S. melongena* L. and *S. dulcamara* L. (Shum and Chiu, 1991; Bha-

0939–5075/2006/1100–0799 $ 06.00

kuni *et al*., 1969; Silva *et al*., 2002; Ataíde, 1982; Abraham *et al*., 1986; Boyd, 1928).

Solanum asterophorum Mart. is a shrub popularly known in the northeast of Brazil as "jurubeba-de-fogo". This rare species, included in the *Leptostemonum* subgenus, presents a restricted distribution in the states of Bahia and Paraíba and it is used in folk medicine to treat hepatic symptoms (Agra and Bhattacharyya, 1999). This plant was chosen based on chemotaxonomic criteria since the *Solanum* genus is rich in secondary metabolites with pharmacological activity (Silva *et al*., 2005a, b).

A search in the NAPRALERT database (Natural Products ALERT) and Web of Science did not show any biological data published.

As part of our chemical and pharmacological studies on Brazilian *Solanum* (Silva *et al*., 2005a, b) we report the spasmolytic effect of isojuripidine and the *S. asterophorum* leaves methanol extract (SA-MeOH) in isolated guinea-pig ileum for the first time.

Material and Methods

Plant material

Leaves of *S. asterophorum* Mart. were collected in June 2003 near the city of Areia, state of Paraíba, Brazil. The plant was collected and determined by Dr. Maria de Fátima Agra. A voucher specimen (Agra 6002) has been deposited in the Herbarium Lauro Pires Xavier (JPB) and the reference collection from Laboratório de Tecnologia Farmacêutica (LTF) both from the Universidade Federal da Paraíba (UFPB), João Pessoa, Brazil.

Extraction and isolation

The powdered leaves of *S. asterophorum* (396.0 g) were extracted with MeOH in a Soxhlet apparatus. The extract was concentrated under vacuum in a rotaevaporator. The crude residue (37.5 g), after standing in the refrigerator, furnished a white precipitate that was separated from the extract and recrystallized from methanol to yield 205.0 mg of isojuripidine (25*R*-3β-amino-5-22α-*O*-spirostan-6α-ol, Fig. 1) (Silva *et al*., 2005b). Both the dry SA-MeOH extract and the isojuripidine crystals were solubilized in cremophor, and then diluted in distilled water in a concentration of 10 mg/mL and 10^{-2} M, respectively. These solutions were maintained at 0 °C, and diluted to their final concentrations in cubes during the experimental procedure.

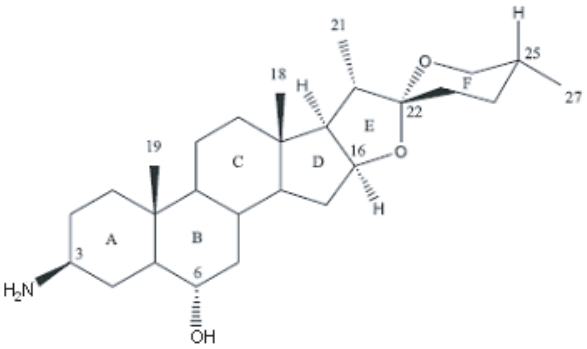

Fig. 1. Chemical structure of isojuripidine.

Animals

Adult guinea-pigs of both sexes (*Cavia porcellus*, 350–500 g) were obtained from the Thomas George Biotery, LTF/UFPB. The animals had free access to food and water and were kept in rooms maintained at (22 ± 1) °C with a 12-h light-dark cycle and fasting of 18 h before experiments. The experimental procedure was approved by the Animal Experimentation Committee of the Universidade Federal da Paraíba (Protocol 0512/2005).

Study of spasmolytic activity of SA-MeOH and isojuripidine

Tissue preparation

To perform *in vitro* studies, the guinea-pig ileum was prepared according to Daniel *et al.* (2001). Guinea-pigs were killed by cervical dislocation, exsanguinated and the ileum was immediately removed. The terminal portions, 3 cm in length, were used after discarding the 10 cm portion close to the ileocaecal junction. The tissues were placed vertically in 6 mL isolated organ baths containing modified Krebs solution with the following composition (mM): NaCl (117), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4$ (1.3), NaH_2PO_4 (1.2), $NaHCO_3$ (25), glucose (11), bubbled with a mixture of 95% O_2 and 5% CO_2 and maintained at 37 °C, pH 7.4. Tension changes were recorded through an isometric force transducer (7003) counterbalanced by 1 g loading, connected to a polygraph (Gemini 7070), both from Ugo Basile (Italy). Phasic contractions were recorded using isotonic levers coupled to kymographs and smoked drums (DTF, Brazil). The tissues were allowed to stabilize for 30 min at a rest-

ing tension of 1 g. During the stabilization period the modified Krebs solution was changed every 10 min to avoid accumulation of metabolites (Altura and Altura, 1970).

Effect of SA-MeOH or isojuripidine on histamine- or acetylcholine-induced contractions in guinea-pig ileum

At the beginning of each experiment, the reactivity of tissue preparations was tested with 40 mM KCl. After washout and 15 min recovery in modified Krebs solution, two simple concentration-response curves were obtained for either histamine or acetylcholine (1 μM). Various concentrations of the methanol extract (SA-MeOH) or isojuripidine were added and, after an incubation period of 15 min, a third concentration-response curve was constructed in the presence of SA-MeOH or isojuripidine. The tissue was washed when the agonist responses returned to the resting level. Inhibition was measured by comparing the response before (100%) and after addition of SA-MeOH or isojuripidine in the organ bath. IC_{50} values were obtained graphically from simple concentration-response curves.

Mechanism of action of SA-MeOH and isojuripidine on guinea-pig ileum

Effect of SA-MeOH or isojuripidine on tonic contractions induced by histamine, acetylcholine or KCl

After stabilization of the preparations, an isometric contraction was elicited with 1 μM histamine, 1 μM acetylcholine or 40 mM KCl. Histamine, acetylcholine or KCl remained in contact with the preparation until a plateau of contraction was reached (approx. 8 min). Thereafter the tissue was washed. After further 30 min, the process was repeated and SA-MeOH or isojuripidine was added cumulatively at the plateau phase. Relaxation was expressed as the reverse of initial contraction elicited by histamine, acetylcholine or KCl.

Effect of SA-MeOH or isojuripidine on Ca^{2+}-induced contractions in depolarizing medium nominally without Ca^{2+}

After a 30 min stabilization period, modified Krebs solution was changed by a depolarized solution nominally without Ca^{2+} during further 45 min. Two similar $CaCl_2$ cumulative response-concentration curves were then induced at 60 min intervals and recorded through isometric transducers coupled to a polygraph. After this procedure the organ baths were washed, and several concentrations of SA-MeOH or isojuripidine were incubated for 15 min in different preparations and then a third $CaCl_2$ cumulative curve was obtained. The maximal contraction obtained with the first concentration-response curve to $CaCl_2$ was considered as 100%, and all contractions were calculated proportionally to this value.

Statistical analysis

Values were expressed as means ± S.E.M. Statistical analysis was performed using the GraphPad Prism® 3.03 software (GraphPad Software Inc., San Diego, CA). The EC_{50} and IC_{50} values were determined by non-linear regression (Jenkinson *et al.*, 1995). Differences between means were statistically compared using Student's *t*-test and/or one-way ANOVA followed by Bonferroni's test, as appropriate, and were considered to differ significantly when $p < 0.05$. Schild plots were also analyzed by a linear regression. Antagonism was judged to be non-competitive when the slope of the Schild's plot was significantly different from unity (Arunlakshana and Schild, 1959) and depression of the maximum response was observed (Van Rossum, 1963).

Results

Effect of SA-MeOH and isojuripidine on histamine- or acetylcholine-induced contractions in guinea-pig ileum

SA-MeOH (3–750 μg/mL) and isojuripidine (10^{-7} – 3×10^{-4} M) inhibited phasic contractions induced by either histamine (Fig. 2) or acetylcholine (Fig. 3). The corresponding IC_{50} values obtained for histamine and acetylcholine, respectively, were (225.8 ± 47.4) and (112.5 ± 20.6) μg/mL for SA-MeOH, and (3.5 ± 0.8) and (2.3 ± 0.4) $\times 10^{-5}$ M for isojuripidine.

Effect of SA-MeOH and isojuripidine on tonic contractions induced by histamine, acetylcholine or KCl

SA-MeOH (1–750 μg/mL) or isojuripidine (10^{-9} – 3×10^{-4} M) relaxed histamine- [EC_{50} = (101.1 ± 17.4) μg/mL and (1.2 ± 0.3) $\times 10^{-6}$ M], acetylcholine- [EC_{50} = (136.8 ± 21.1) μg/mL and (1.9 ± 0.4) $\times 10^{-6}$ M] or KCl- [EC_{50} = (149.4 ± 19.5) μg/mL and (1.8 ± 0.7) $\times 10^{-6}$ M] pre-con-

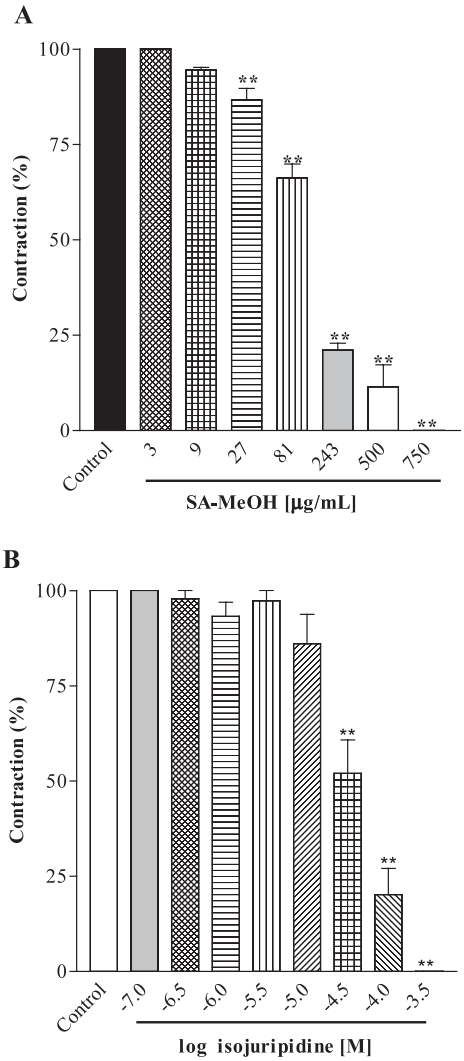

Fig. 2. Effect of SA-MeOH (A, $n = 5$) and isojuripidine (B, $n = 3$) on phasic contractions induced by 1 μM histamine in guinea-pig ileum. The columns and bars represent means ± S.E.M., respectively. Significant differences are indicated by $^{**}p < 0.001$ (control × SA-MeOH or isojuripidine; one-way ANOVA followed by Bonferroni's test).

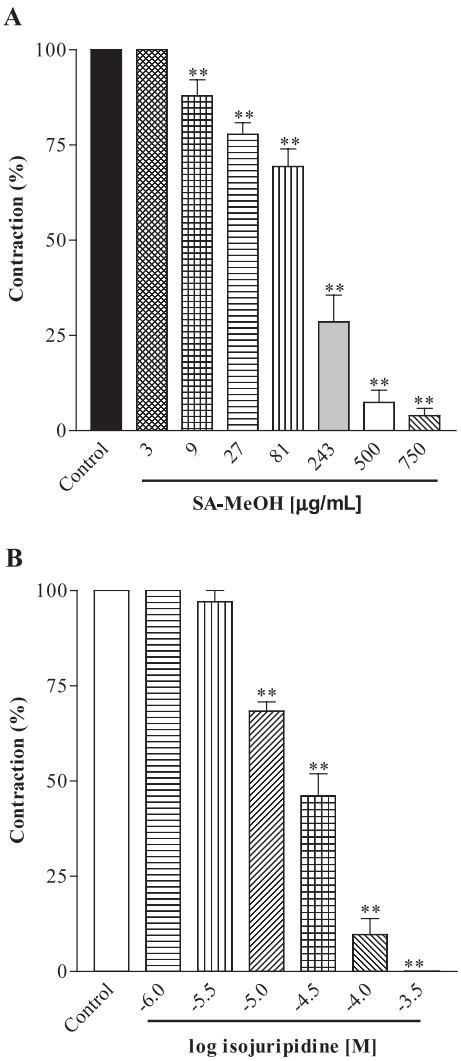

Fig. 3. Effect of SA-MeOH (A, $n = 5$) and isojuripidine (B, $n = 3$) on phasic contractions induced by 1 μM acetylcholine in guinea-pig ileum. The columns and bars represent means ± S.E.M., respectively. Significant differences are indicated by $^{**}p < 0.001$ (control × SA-MeOH or isojuripidine; one-way ANOVA followed by Bonferroni's test).

tracted guinea-pig ileum in an equipotent and concentration-dependent manner (Fig. 4).

Effect of SA-MeOH and isojuripidine on Ca^{2+}-induced contractions in depolarizing medium nominally without Ca^{2+}

SA-MeOH (27, 243, 500, and 750 μg/mL) or isojuripidine (3×10^{-8}, 10^{-6}, 3×10^{-5}, and 3×10^{-4} M) inhibited the contractions induced by $CaCl_2$ in a concentration-dependent manner. The cumulative concentration-response curves to $CaCl_2$, in the presence of SA-MeOH and isojuripidine, were shifted downward in relation to a control curve in a non-parallel manner resulting in reduction of the maximum effect [E_{max} = (71.2 ± 9.2); (57.4 ± 9.2); (43.8 ± 3.4); (41.5 ± 2.4) and (90.6 ±

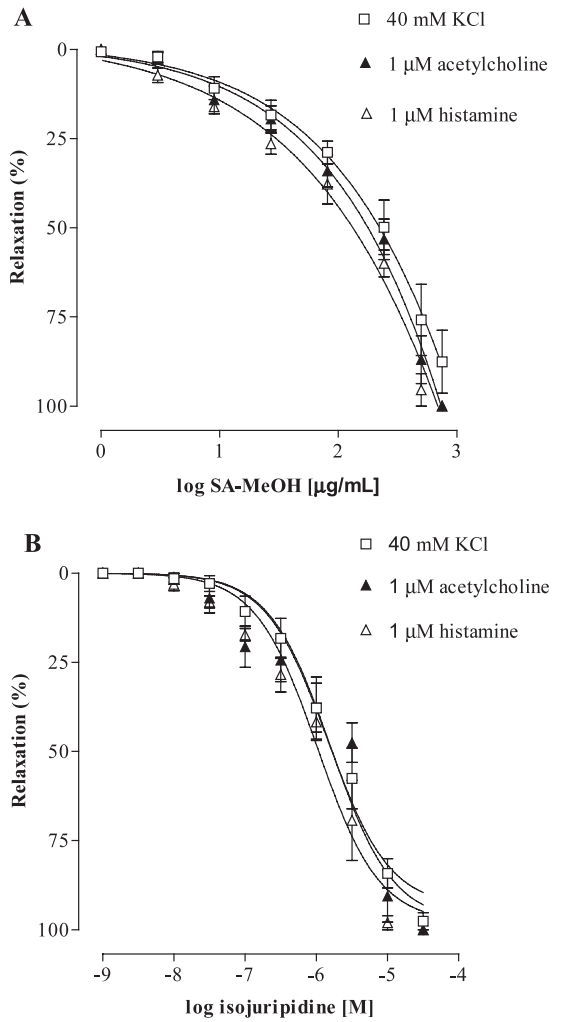

Fig. 4. Effect of several concentrations of SA-MeOH (A, $n = 5$) and isojuripidine (B, $n = 4$) on the 1 μM histamine-, 1 μM acetylcholine- or 40 mM KCl-induced tonic contractions in guinea-pig ileum. The symbols and vertical bars represent means ± S.E.M.

4.8); (74.7 ± 8.7); (66.4 ± 3.9); (31.3 ± 4.1)%] for isojuripidine. This data suggests a non-competitive antagonism (Fig. 5), that was corroborated by Schild's slope value (0.2537 ± 0.1073), which was statistically different from 1. Linear regression analysis of the data showed a low correlation coefficient value ($r^2 = 0.74$) and the mean value of PD'_2 was 6.5. The antagonism was reverted after 60 min in the absence of SA-MeOH and isojuripidine. SA-MeOH or isojuripidine present a spasmolytic effect in guinea-pig ileum due, in part, to a blockade of the calcium influx through voltage-operated calcium channels (Ca_v) channels.

Discussion

These results show that leaves of *S. asterophorum* Mart. present secondary metabolites with non-selective spasmolytic action in guinea-pig ileum, isojuripidine being one of them. In the present work, we have investigated the effects of the methanol extract (SA-MeOH) and isojuripidine on isolated guinea-pig ileum. The most important finding of this work is the demonstration, for the first time, that SA-MeOH and isojuripidine exert a non-selective spasmolytic action, and that this effect is due in part to the inhibition of Ca^{2+} influx probably through Ca_v channels.

The absence of the significant difference between the IC_{50} values of SA-MeOH and isojuripidine on acetylcholine- or histamine-induced contractions in guinea-pig ileum may be suggestive that SA-MeOH and isojuripidine are acting by a similar mechanism of action common to these agents.

Two general forms of excitation initiate the contraction of smooth muscles. The initiation of contraction may occur due to innervation and consequent depolarization of the membrane's resting potential, termed electromechanical coupling, whereas activation by ligands of cell surface receptors has been termed pharmacomechanical coupling (Somlyo and Somlyo, 2000). The electrical component of smooth muscle cell excitation is accounted by action potentials, triggering the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels. This rise in intracellular Ca^{2+} may be augmented by Ca^{2+}-induced Ca^{2+} release from intracellular stores [for a comprehensive review, see Bolton (2006)]. Many agonists that induce guinea-pig ileum contraction cause a biphasic contraction; in the first phase the muscle exhibits a fast and transient contraction followed by a long-lasting second phase, which is characterized by the maintained tonic contraction (Horie *et al.*, 2005). On the other hand, pharmacomechanical coupling involves activation of cell surface receptors to augment the increase in Ca^{2+}, either by the release of Ca^{2+} from intracellular stores or through cell signaling-mediated mechanisms that increase the Ca^{2+} sensitivity of the contractile apparatus (Stevens *et al.*, 2000).

To verify whether SA-MeOH and isojuripidine act on Ca^{2+} influx across the membrane, we evalu-

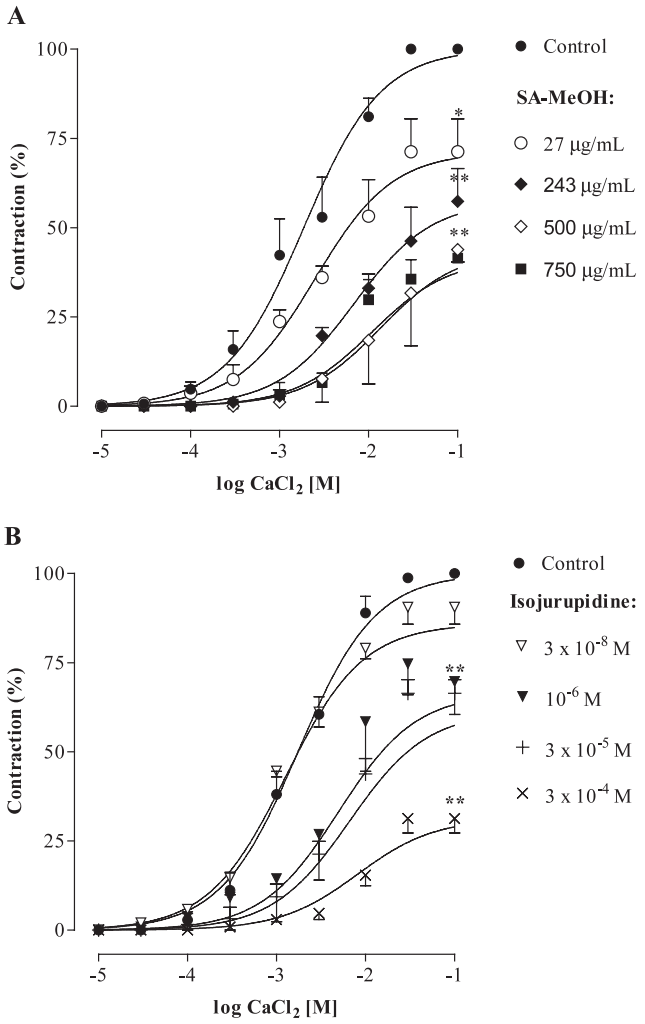

Fig. 5. Effect of SA-MeOH (A) and isojuripidine (B) on the cumulative concentration-response curves to $CaCl_2$ in depolarizing medium nominally without Ca^{2+} in guinea-pig isolated ileum ($n = 5$). Symbols and vertical bars represent means ± S.E.M. Significant differences are indicated by $^{*}p < 0.05$ and $^{**}p < 0.001$ (control × SA-MeOH or isojuripidine; one-way ANOVA followed by Bonferroni's test).

ated its effect on the tonic component of the contractile response induced by either acetylcholine or histamine (pharmacomechanical and electromechanical coupling) and KCl (electromechanical coupling) in the guinea-pig ileum. As shown in Fig. 4, SA-MeOH and isojuripidine relaxed in an equipotent and concentration-dependent manner the ileum pre-contracted with acetylcholine, histamine or KCl. Independently whether the contraction is evoked by either pharmacomechanical or electromechanical coupling, the maintenance of the tonic component involves activation of the Ca_v channels (Rembold, 1996). Therefore we can postulate that SA-MeOH and isojuripidine may inhibit the Ca^{2+} influx through these channels to produce non-selective spasmolytic effects.

We confirm the hypothesis that SA-MeOH and isojuripidine inhibit the Ca^{2+} influx through Ca_v channels, on cumulative curves to $CaCl_2$ in depolarizing medium nominally without Ca^{2+} through a non-competitive blockade of $CaCl_2$-induced curves, since SA-MeOH and isojuripidine produced a non-parallel and concentration-dependent downward displacement of the concentration-response to $CaCl_2$, significantly reducing the maximal response.

In conclusion, we have shown that SA-MeOH and isojuripidine produce spasmolytic effects in guinea-pig ileum and that this effect is due in part to the inhibition of the Ca^{2+} influx through Ca_v channels. However, we do not discard other possible mechanisms that have not been studied yet.

Acknowledgements

The authors thank José Crispim Duarte for providing technical assistance. This work was supported by CAPES and CNPq for grants and fellowships.

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