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CARDIOVASCULAR EFFECTS ON RATS INDUCED BY THE TOTAL ALKALOID FRACTION OF *SIDA CORDIFOLIA*

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ABSTRACT

Cardiovascular effects induced by the total alkaloid fraction of *Sida cordifolia* L. (TAF) were investigated in unanesthetized normotensive rats. In these animals, TAF (0.5, 1, 5 and 10 mg/kg, i.v.) induced hypotension, which was attenuated by atropine, L-NAME or hexamethonium at the 10 mg/kg dose, and bradycardia, which was eliminated by atropine at all doses, and attenuated by hexamethonium at 5 and 10 mg/kg, but was unchanged by L-NAME. These results demonstrate that TAF induces hypotension and bradycardia, which appear to be mediated by direct and indirect activation of muscarinic receptors. Nitric oxide also appears to be involved in the hypotensive response.

Keywords: *Sida cordifolia* , total alkaloid fraction, cardiovascular effects, hypotension, rat.

RESUMO

Os efeitos cardiovasculares induzidos pela fração de alcalóides totais da *Sida cordifolia* L. (TAF) foram avaliados em ratos normotensos não-anestesiados. Nestes animais, TAF (0,5; 1; 5 e 10 mg/kg, i.v.) induziu hipotensão, que foi atenuada pela atropina, L-NAME ou hexametônio apenas na dose de 10 mg/kg, e bradicardia que foi abolida pela atropina, em todas as doses, e atenuada pelo hexametônio nas doses de 5 e 10 mg/kg, mas não foi alterada pelo L-NAME. Estes resultados demonstram que TAF induz hipotensão e bradicardia, que parece envolver uma ativação direta e indireta de receptores muscarínicos. Além disso, o óxido nítrico parece também participar do efeito hipotensor induzido pelo TAF.

Palavras-chave: *Sida cordifolia* , fração de alcalóides totais, efeitos cardiovasculares, hipotensão, rato.

INTRODUCTION

The use of medicinal plants for the treatment of human diseases has increased considerably throughout the World. Evaluation of the effects of these plants on organs and systems contributes to the development of the scientific basis for therapeutic applications, and enriches alternatives for the treatment of a growing number of diseases (Elizabetsky, 1986).

Sida cordifolia L. (Malvaceae), a native species of the Brazilian Northeast, is known popularly as "Malva branca". It is used in folk medicine as an antirheumatic and antipyretic (Muzaffer *et al*., 1991), an antiinflammatory and analgesic (Franzotti *et al*., 2000), and an antiasthmatic and nasal anticongestant (Ghosh & Dutt, 1930; Mukerji, 1953). Previous studies have demonstrated that vasicine, an alkaloid isolated from this plant (Ghosal *et al*., 1975), produced hypotension and bradycardia in anaesthetized dogs (Gupta *et al*., 1977) and unanaesthetized rats (Silveira

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et al., 2003). In the pharmacological study reported here, we evaluated the effects of the total alkaloid fraction of *Sida cordifolia* L. on the arterial pressure and heart rate of unanesthetized, normotensive rats (*Rattus norvegicus*). We demonstrate that the hydroalcoholic extract of this plant also induces marked hypotension, associated with intense bradycardia, in rats.

MATERIALS AND METHODS

Preparation of the total alkaloid fraction: *Sida cordifolia* leaves (voucher specimen nº. 30171, deposited in the hebarium of the Department of Biology, Universidade Federal de Sergipe, Brazil) were dried, pulverized and immersed in 95% EtOH at room temperature for 72 h. After vacuum concentration, the extract obtained was submitted to conventional acid-base treatment to produce the total alkaloid fraction (TAF). To avoid decomposition, the TAF was dissolved in a cremophor-saline solution (0.1% v/v) at desired concentrations just before each experiment. Cremophor had no effect when tested under controlled conditions (data not shown).

Animals: Male Wistar rats (250-350 g) were used for all experiments. The animals were housed under controlled conditions of temperature (21 ± 1ºC) and lighting (lights on: 06:00-18:00 h), with free access to food and tap water.

Drugs: The drugs used were: heparin sodium salt (Ariston), sodium thiopental (Cristália), atropine sulfate, N^{w}-nitro-*L-arginine* methyl ester (L-NAME), hexamethonium. sodium nitroprusside and cremophor (all from Sigma). All drugs were dissolved freely in saline solution.

Blood pressure measurements: Under sodium thiopental anesthesia (45 mg/kg, i.v.), the lower abdominal aorta and inferior *vena cava* were canulated via the left femoral artery and vein using polyethylene catheters. The catheters were then filled with heparinized saline solution and slid under the skin to emerge between the scapulae. Arterial pressure was measured after 24 h by connecting the arterial catheter to a pre-calibrated pressure transducer (Edwards Lifescience, Irvine, CA, USA) and pressure outputs were recorded by an amplifier-recorder (BioData, Model BD-01, PB, Brazil) connected to a personal computer equipped with an analog-to-digital converter board (BioData, PB, Brazil). Data were collected at a frequency of 200 Hz. For each cardiac cycle, the computer calculated mean arterial pressure (MAP) and pulse interval, referred to here as the heart rate (HR). The venous catheter was inserted for drug administration. Sodium nitroprusside (10 µg/kg) was injected to check the efficacy of catheter insertion.

Experimental protocol: After stabilization of cardiovascular parameters, MAP and HR were recorded before (baseline values) and after randomized i.v. administration of different doses of TAF (0.5, 1, 5 and 10 mg/kg). These administrations were separated by a time interval sufficient to allow full recovery of cardiovascular parameters. A control dose-response curve was then obtained. Dose-response curve were also obtained separately in animals pre-treated with atropine (2 mg/kg; i.v.; 15 min.), a non-selective antagonist of muscarinic receptors (Mitchelson, 1984), L-NAME (20 mg/kg, i.v. 30 min.), a competitive inhibitor of NO-synthase (Moncada & Higgs, 1993) or hexamethonium, a ganglionic blockade (Takahashi & Owyang, 1997).

Statistics: Values are expressed as means ± SEM. The significance of differences among means was tested using one-way ANOVA with Dunnett's post test. All procedures were carried out on Graph Pad Prism™ version 3.02 software.

RESULTS

As expected from control animals, atropine and hexamethonium increased HR significantly, from 350 ± 7 bpm to 485 ± 11 and 416 ± 20 bpm ($p < 0.05$; $n = 6$), respectively. The administration of L-NAME increased MAP and HR from 110 ± 3 mmHg to 136 ± 4 mmHg and from 350 ± 7 bpm to 373 ± 9 bpm ($p < 0.05$; $n = 6$), respectively.

Figure 1 shows original traces of the effect induced by *S. cordifolia* TAF (10 mg/kg, i.v.) in one unanesthetized normotensive rat. In these animals, TAF (0.5, 1, 5 and 10 mg/kg; i.v., randomly) induced transitory hypotension (-1 ± 1; -5 ± 1; -21 ± 4 and -39 ± 3 %) associated with intense bradycardia (-0.7 ± 0.1; -5 ± 0.7; -44 ± 7 and -70 ± 2 %) (Figure 2).

In animals pre-treated with atropine (2 mg/kg), L-NAME (20 mg/kg) or hexamethonium (20 mg/kg), the hypotensive response induced by TAF was only attenuated at the highest dose (10 mg/kg). However, the bradycardic response was eliminated by atropine at all doses, and attenuated by hexamethonium at 5 and 10 mg/kg, although it was not altered by L-NAME (Figure 2).

DISCUSSION

We chose to evaluate the effects of the TAF of *S. cordifolia* on the cardiovascular parameters of unanesthetized rats in order to avoid the possible influence of anesthesia and surgical stress (Smith & Hutchins, 1980; Fluckiger *et al*., 1985). Baseline MAP and HR values recorded here were similar to those reported in previous studies (Lahlou *et al*., 2002; Silveira *et al*., 2003; Cunha *et al.*, 2004). Acute administration of TAF induced hypotension associated with bradycardia.

Peterson *et al*. (1984) established that the primary autonomic regulation of sinoatrial node function is carried out by vagal action via stimulation of cardiac muscarinic receptors. Stimulation of these receptors induces intense bradycardia followed by hypotension due to a decrease in cardiac output. It is also well known that activation of endothelial muscarinic receptors induces intense vasodilatation due to release of endothelium-derived relaxing factors (Moncada *et al*., 1991), mainly NO (Furchgott & Zawadzki, 1980; Moncada *et al.*, 1991). This activation can cause decrease in peripheral vascular resistance and, consequently, hypotension.

In order to evaluate the role of these receptors in TAF-induced responses, we performed experiments in the presence of atropine, a non-selective antagonist of muscarinic receptors (Mitchelson, 1984). Under these conditions, bradycardia was abolished at all doses, although hypotension was attenuated significantly at 10 mg/kg. This indicates that TAF induces bradycardia either through direct activation of receptors, or indirectly via vagal stimulation and release of acetylcholine into the sinoatrial node. However, hypotension does not appear to be due exclusively to the decrease in cardiac output related to bradycardia, given that it was only attenuated, but not abolished by atropine. This indicates that a decrease in peripheral vascular resistance may also contribute to TAF-induced hypotension.

To verify a possible indirect effect of TAF via the vagal nerve, we performed experiments with hexamethonium, a ganglionic blocker (Takahashi & Owyang, 1997). This drug attenuated bradycardia and hypotension significantly, but did not eliminate them altogether. This indicates that the bradycardic response is mediated by two distinct pathways, indirect and direct activation of cardiac muscarinic receptors.

Finally, we investigated the role of nitric oxide. In this condition, L-NAME, a competitive inhibitor of NO-synthase (Moncada & Higgs, 1993), changed the hypotensive response only at the 10 mg/kg dose, suggesting that NO is at least partly involved in the hypotensive effect.

These results agree with those of Silveira *et al.*

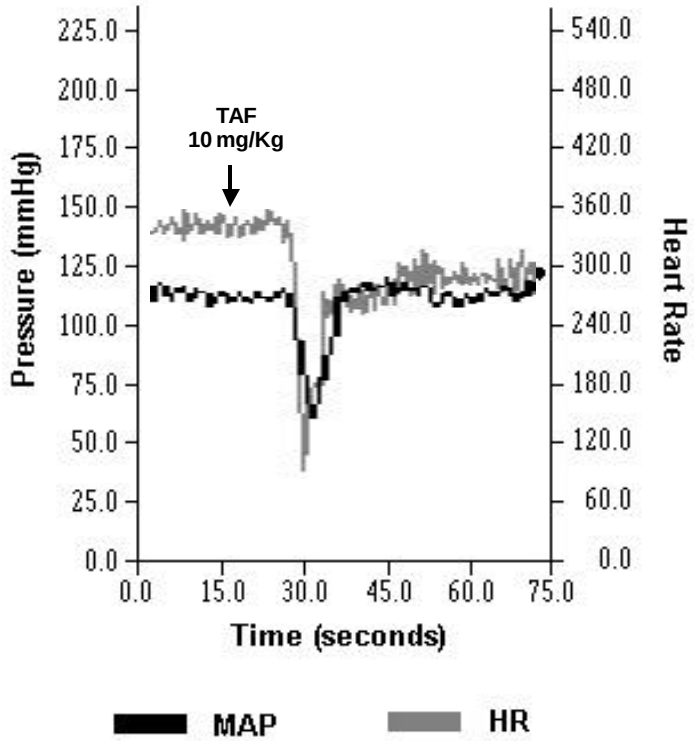

Figure 1. Original traces showing the effect of TAF (10 mg/kg; i.v.) on MAP and HR in one non-anaesthetized normotensive rat. The arrow indicates point of TAF administration.

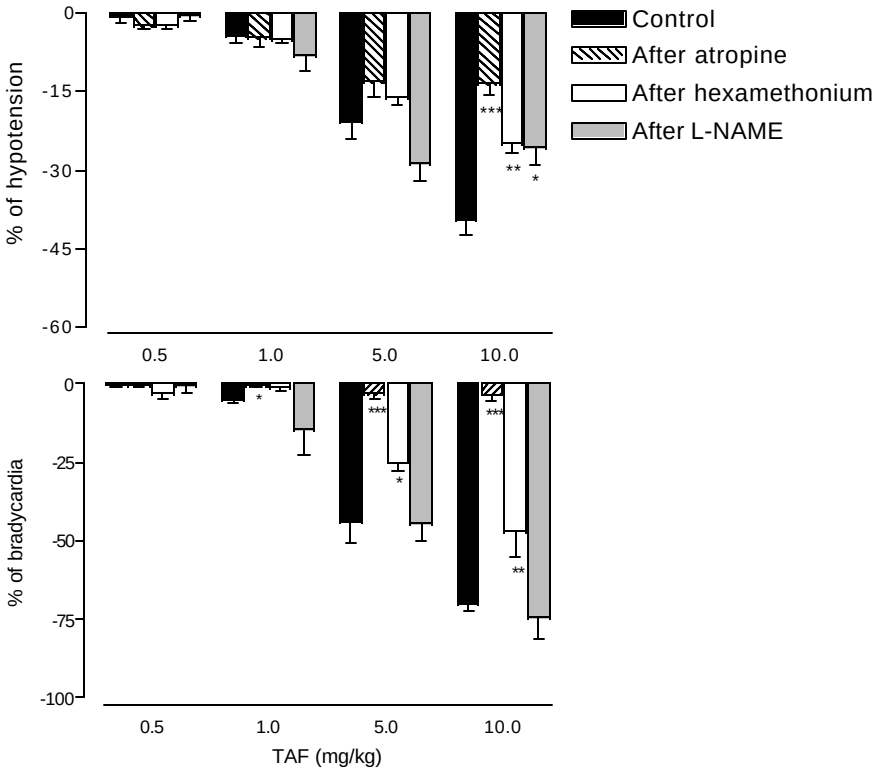

Figure 2. Hypotensive and bradycardic response induced by TAF (0.5, 1, 5 and 10 mg/Kg; i.v.) in non-anaesthetized normotensive rats before (Control) and after pre-treatment with atropine (2 mg/kg), hexamethonium (20 mg/kg) or L-NAME (20 mg/kg). Values are expressed as mean ± SEM of six experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs Control.

(2003), who demonstrated that vasicine, an alkaloid isolated from *S. cordifolia* (Ghosal *et al*., 1975), also produced marked hypotension associated with bradycardia in unanesthetized rats. The effects recorded in the present study may thus be due to the presence of vasicine in the TAF, although further data would be required to confirm this.

Overall. our results demonstrate that the TAF of *S. cordifolia* induces hypotension and bradycardia, apparently mediated by direct and indirect activation of muscarinic receptors. Nitric oxide also appears to be involved in the hypotensive response.

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