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SHORT COMMUNICATION

Effect of a Diterpenoid from *Salvia cinnabarina* on Arterial Blood Pressure in Rats

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The effect of a diterpenoid isolated from *Salvia cinnabarina*, 3,4-seicosopimar-4(18),7,15-triene-3-oic acid (SCB), on arterial blood pressure was evaluated in anaesthetized rats.

Male Wistar rats, anaesthetized with urethane (sol. 10% p/v; 10 mL/kg), underwent surgery for continuous monitoring of arterial blood pressure. After preliminary experiments to evaluate the dose response (3, 10 and 30 mg/kg i.v.) of SCB, a dose of 3 mg/kg was chosen for all successive experiments. On different groups of rats treated with the ganglion-blocking agent chlorisondamine (2.5 mg/kg i.p.) the effect of SCB (3 mg/kg i.v.) was evaluated before and following an infusion of the nitric oxide synthase inhibitor L-NAME (0.3 mg/kg/min i.v.). Intravenous administration of SCB at doses of 3, 10 and 30 mg/kg led to a fall in mean arterial blood pressure (MABP) of 14.75 ± 1.44 mmHg, 36.60 ± 31.40 mmHg and 31.40 ± 6.28 mmHg, respectively ($n = 4–5$), that was not modified by treatment of the rat with chlorisondamine nor with L-NAME. The results demonstrate a hypotensive effect of SCB – due to a peripheral mechanism but independent of endothelial nitric oxide release. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: Salvia cinnabarina; rat; blood pressure; hypotension; herbal medicine.

INTRODUCTION

The genus *Salvia* is known for its wide spectrum of pharmacological properties, such as antioxidant, antibacterial, antifungal, antinociceptive, antiinflammatory (Dobrynin *et al.*, 1976; Hohmann *et al.*, 1999; Baricevic *et al.*, 2001). *Salvia cinnabarina* is an American species in the subgenus Calosphace, section Incarnate (Epling, 1939) containing a high amount of oxygenated monoterpenes (particularly linalool) and oxygenated sesquiterpenes (Bisio *et al.*, 1998). A new secoisopimarane diterpenoid, namely 3,4-seicosopimar-4(18),7,15-triene-3-oic acid (SCB), has been recently isolated from the leaf exudates of aerial parts of *S. cinnabarina* and tested for its biological properties. SCB has been shown to have intestinal spasmolytic activity *in vitro*, with an aspecific mechanism (Romussi *et al.*, 2001). *In vivo*, SCB inhibits mouse intestinal motility with a mechanism involving L-type Ca^{2+} channels (Capasso *et al.*, 2004a). Furthermore, SCB inhibits rat bladder contractility *in vitro* with the partial involvement of nitric oxide (Capasso *et al.*, 2004b). To further characterize the pharmacological profile of SCB, in the present study investigated the effect of SCB on rat arterial blood pressure.

MATERIAL AND METHODS

Animals. Male Wistar rats (200–250 g; Harlan Nossan), housed under conditions of constant temperature (22–24 °C) and humidity (50 ± 10%) under a 24 h light–dark cycle with food and water freely available, were used for the experiments. All animal experiments complied with the Italian D.L. no. 116 of 27 January 1992 and Associated Guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

Drugs. Urethane, N^{ω}-nitro L-arginine methyl ester (L-NAME) and heparin were purchased from Sigma (Milan, Italy). Chlorisondamine was purchased from Tocris (UK). Extraction, isolation and structural characterization of SCB were performed as described previously (Romussi *et al.*, 2001). HPLC analysis indicated that SCB was 96% pure.

Measurement of arterial blood pressure. Male Wistar rats were anaesthetized with urethane (sol 10% w/v i.p.) and placed supine on an operating table. The right

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jugular vein was cannulated for drug administration, the left carotid artery was cannulated with a cannula containing heparinized saline (5 U/mL) and connected to a pressure transducer (Basile, Comerio (VA), Italy) for continuous monitoring of arterial blood pressure. Acquisition data were performed by a computerized system PowerLab (ADInstruments, v 3.4.3). After surgery, the arterial blood pressure was allowed to stabilize for about 30 min. The effect of three different doses (3, 10 and 30 mg/kg i.v.) of SCB was tested in different groups of animals. Each dose was administered on three consecutive times, for 20 min each, in the same animals. Subsequently, a single administration of SCB (3 mg/kg i.v.) was chosen, after the preliminary experiments in which each dose was administered for three consecutive times in the same animal.

Ganglion-blockade experiments. To eliminate any influence of autonomic nervous system activation on changes in mean arterial blood pressure (MABP) induced by SCB, different groups of animals were pretreated with the irreversible ganglion-blocking agent chlorisondamine at a dose of 2.5 mg/kg i.p., before administering SCB (3 mg/kg i.v.) and changes in blood pressure were evaluated.

Role of nitric oxide. The role of nitric oxide was assessed by infusing L-NAME (0.3 mg/kg/min i.v.), through a butterfly needle inserted into the caudal vein, in ganglion blocked rats and changes in blood pressure induced by SCB (3 mg/kg i.v.) were evaluated and compared with values obtained before the infusion. The dose of L-NAME chosen was able to restore blood pressure in ganglion-blocked rats to the normal value.

Statistical analysis. Changes in MABP have been evaluated as the difference from the basal value or as the percentage change from the baseline, when groups with different basal blood pressure were compared. The results obtained are expressed mean ± SEM and analysed by one-way analysis of variance (ANOVA) followed by Bonferroni's or Dunnett's test, as appropriate. When requested, Student's *t*-test was used. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Intravenous administration of SCB (3–10 mg/kg i.v.) into anaesthetized rats caused a transient dose-dependent fall in MABP. The dose of 3 mg/kg i.v. caused a fall of 14.75 ± 1.44 mmHg; there was no tachyphylaxis after repeated administrations of 20 min each into the same animal and so this dose was chosen for all successive experiments (Fig. 1). Hypotension induced by SCB (3 mg/kg i.v.) was associated with a fall in heart rate from 443 ± 24 to 264 ± 44 beats per minute (bpm) ($p < 0.01$; $n = 9$). To differentiate between a central and a peripheral effect of SCB, experiments were performed in groups of rats treated with the irreversible ganglion-blocking agent chlorisondamine (2.5 mg/kg i.p.). After treatment with chlorisondamine, the MABP value was 54.64 ± 1.59 mmHg ($n = 11$), significantly different from

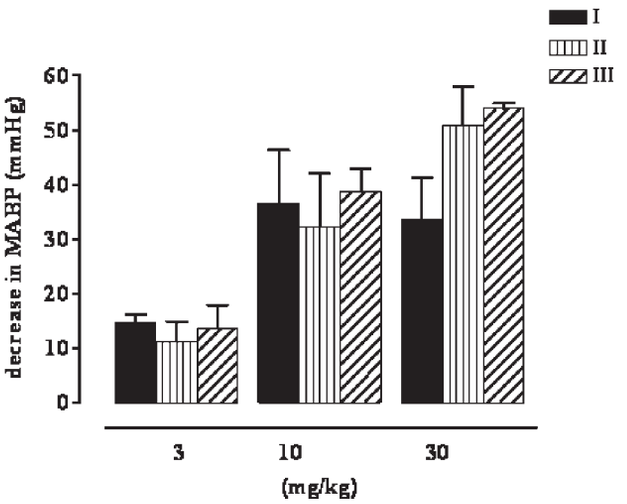

Figure 1. Decrease in MABP induced by SCB (3, 10 and 30 mg/kg i.v.) in anaesthetized rats. Each dose of SCB was administered in different groups of rats and repeated into the same animals three consecutive times at 20 min intervals ($n = 5$).

the control value ($p < 0.0001$); however, the hypotensive effect of SCB, evaluated as the percentage (%) change in MABP, was not different from the control animals (21.50 ± 6.76% vs 16.00 ± 2.38%), suggesting that it does not involve the autonomic nervous system activation deriving from a central effect of SCB. Interestingly, in ganglion-blocked rats the hypotensive effect of SCB was followed by a slight but significant hypertension (10.75 ± 2.14%; $n = 4$) that was not evident before ganglion-blockade. Several studies describing the effect of L-NAME on changes in MABP induced by drugs have been performed in normal animals, in which the substantial increase in MABP and vascular resistance above physiological values, due to nitric oxide inhibition, might activate a reflex response masking the real effect of drugs. For this reason, to investigate on the possible role of nitric oxide on changes in blood pressure induced by SCB, experiments were performed on ganglion-blocked rats, in which an infusion of L-NAME (0.3 mg/kg/min i.v.) restored blood pressure to the normal value. Under these conditions, no inhibition of SCB-induced hypotension was observed, ruling out the involvement of nitric oxide (Fig. 2A). This finding, together with the observation that in ganglion-blocked rats SCB-induced hypotension was not different from the value obtained in normal, non-ganglion-blocked rats, suggests that the vasodilator mechanism is due to a peripheral action but independent from nitric oxide release. Interestingly, following L-NAME infusion, hypertension that was evident only after chlorisondamine treatment was abolished (Fig. 2B). In conclusion, this is the first work demonstrating an *in vivo* hypotensive effect of SCB due to a direct peripheral action but independent of nitric oxide release. Our findings further contribute to delineate the pharmacological profile of this natural compound.

Acknowledgement

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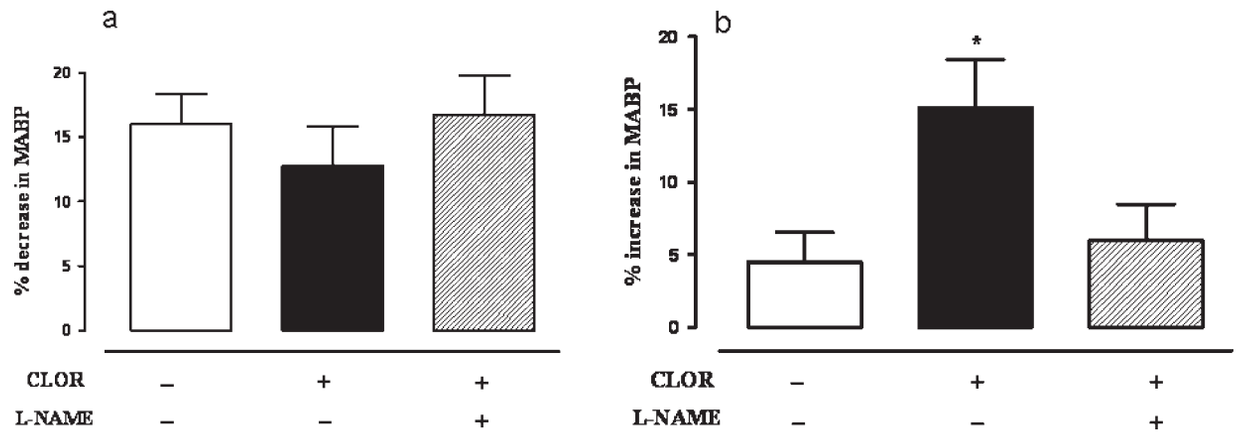

Figure 2. Change in MABP induced by SCB in ganglion-blocked rats and effect of L-NAME infusion. (a) Decrease in MABP induced by SCB (3 mg/kg i.v.) before (white bar), after treatment with the ganglion-blocking agent chlorisondamine (2.5 mg/kg i.p. solid bar) and in ganglion-blocked rats treated with an infusion of L-NAME (0.3 mg/kg/min i.v. hatched bar). (b) Increase in MABP induced by SCB (3 mg/kg i.v.) before (white bar), after treatment with ganglion-blocking agent chlorisondamine (2.5 mg/kg i.p. solid bar) and in ganglion-blocked rats treated with an infusion of L-NAME (0.3 mg/kg/min i.v. hatched bar) ($n = 4–8$, * $p < 0.01$ vs before treatment with chlorisondamine and vs after L-NAME infusion).

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