Vasorelaxant effect of labdane-type diterpene isolated from Copaifera langsdorffii in the isolated rat aorta.

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Introduction: Copaifera langsdorffii is a large tree that grows well in the north and northeastern part of Brazil. Phytochemical studies carried out on the oleo-resin from Copaifera langsdorffii revealed the presence of a mixture of diterpenes. Compounds diterpenoids are the main constituents of plant extracts that are used in folk medicine for the treatment of hypertension. Labdane-type diterpenes are described to exert antispasmodic and relaxant action in vascular tissues. Objectives: The present investigation aims to evaluate the mechanisms underlying the cardiovascular effects displayed by the labdane ent-3-acetoxylabda-8(17),13-dien-15-oic acid (labda-15-oico) isolated from Copaifera langsdorffii. Methods: Male Wistar rats (200 and 250 g) were used in accordance with the Ethical Animal Committee from the Campus of Ribeirão Preto—University of São Paulo (Protocol: 09.1.1007.53.0). The thoracic aorta was isolated for vascular reactivity experiments. cAMP and cGMP were measured by enzyme immunoassay (EIA). Nitrate was measured performed by chemiluminescence. Statistical analysis was performed using one-way analysis of variance (ANOVA). P values of less than 0.05 were considered significant. Results: No differences were found between the two periods of incubation (30 and 60 min), indicating that labda-15-oico achieve its maximal inhibitory action at 30 min. E\textsubscript{max} values for phenylephrine in endothelium-intact (E+) rings were reduced in the presence of labda-15-oic acid at 100 µmol/L. In endothelium-denuded (E-) rings, labda-15-oic acid reduced phenylephrine-induced contraction at 50 and 100 µmol/L. The E\textsubscript{max} values for serotonin in E+ and E- rings were reduced in the presence of the labdane at 10, 50 and 100 µmol/L. The Labda-15-oic acid inhibited the contraction induced CaCl\textsubscript{2} in E- rings in the concentration of 10, 50 and 100 µmol/L, after stimulation with phenylephrine (0.73±0.07g, n=6; 0.79±0.12g, n=5; 0.41±0.06g, n=6), compared to control (1.28±0.10g, n=6), or with KCl (0.91±0.13g, n=5; 0.78±0.14g, n=7; 0.67±0.05g, n=6), compared to control (1.42±0.06g, n=8). The labdane induced relaxation in E+ and E- rings pre-contracted with phenylephrine or KCl. The inhibitory effect elicited by lada-15-oico was totally abolished 60 and 120 min after the removal of the labdane from the medium bath in E- and E+ rings, respectively. In E+ rings pre-contracted with phenylephrine, the E\textsubscript{max} values for relaxation were reduced in the presence of L-NAME, ODQ, haemoglobin, RP-8-Br-Pet, 4-amynopiridine, Apamine and glibenclamide. There was a rightward displacement of the concentration–response curve for labda-15-oico in the presence of L-NAME, ODQ, haemoglobin, 7-nitroindazole, Rp-8-Br-Pet, thapsigargin, tetraethylammonium, 4- amynopiridine, Apamine, Charybdotoxin and glibenclamide. On the other hand, indomethacin, wortmannin, LY294002, atropine, propranolol, H89 and SQ22536 did not have a significant effect on labdane induced relaxation. The labdane increased the levels of cGMP and nitrate but not cAMP in E+ rings. Conclusion: The effect of labda-15-oico is reversible and involves different pathways. The labdane acts on vascular smooth muscle where it blocks Ca\textsuperscript{2+} influx through interference with both voltage and receptor-operated channels. The relaxant action of the labdane is also partly mediated by the activation of endothelial NO-cGMP pathway and opening of K\textsuperscript{+} channels.

Keywords: Diterpenes, Cardiovascular Effects, Relaxation, rat aorta.

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