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European Journal of Pharmacology 305 (1996) 169–171

ejp

Short communication

The effect of verbascoside on cyclic 3′,5′-adenosine monophosphate levels in isolated rat heart

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Received 13 February 1996; accepted 20 February 1996

Abstract

The effects mediated by the naturally occurring phenylethanoid glycoside verbascoside (1 mM) in isolated rat hearts (significant increases in heart rate (42%), contractile force (13%) and coronary perfusion rate (113%)) all coincided with a marked increase in intracellular levels of cyclic-3′,5′-adenosine monophosphate (1733% increase).

Keywords: Verbascoside; Cyclic adenosine monophosphate; Langendorff heart preparation; (*Eremophila alternifolia*)

1. Introduction

Two classes of cardiotonic agents exist: those that affect intracellular cyclic 3′,5′-adenosine monophosphate (cAMP) levels and those that act independently of this mechanism. Compounds in the cAMP-dependent class mediate cardiac contractility by increasing the levels of intracellular cAMP or by inhibiting its metabolism. These include the β-adrenergic agonists, forskolin from *Coleus forskohlii* (Metzger and Lindner, 1981) and the phosphodiesterase inhibitors. The cAMP-independent class of compounds, e.g. cardiac glycosides, inhibit the Na^+/K^+-ATPase enzyme (Packer, 1992).

It is generally accepted that, when stimulated, the adenylate cyclase enzyme converts adenosine triphosphate (ATP) into cAMP. This second messenger then initiates a series of phosphorylation reactions by protein kinases, which mediate the influx of extracellular Ca^{2+} through the slow calcium channels and by release of stored calcium ions by the sarcoplasmic reticulum. A phosphodiesterase III enzyme then inactivates cAMP by converting it into the inert 5′-adenosine monophosphate (5′-AMP).

Recently, we reported on the cardioactive effects of an aqueous extract from the leaves of *Eremophila alternifolia* R.Br. (Myoporaceae) on isolated rat heart (Pennacchio et al., 1995a). These effects were dose-dependent (unpublished data). *E. alternifolia* is a member of a genus endemic to Australia and a species highly valued in the pharmacopoeia of the Australian Aboriginal people (Ghisalberti, 1994). Subsequent chemical investigations revealed that the major active constituent of this extract was the phenylethanoid glycoside verbascoside (**1**) (Scheme 1) which significantly increased heart rate, contractile force and coronary perfusion rate in the Langendorff rat heart (Pennacchio et al., 1995b).

To explore the mode of action of verbascoside on the isolated rat heart, increases in intracellular levels of cAMP were investigated as the possible cause of cardioactivity.

2. Materials and methods

2.1. Plant material

Leaf samples of *E. alternifolia* were collected from a granite outcrop in the Mt. Dimer region, Jaurdi Nature Reserve, approximately 100 km NE of Southern Cross, Western Australia in November 1993. Sample identification was confirmed in the field by the Myoporaceae authority, Dr. R.J. Chinnock, Botanic Gardens of Adelaide and State Herbarium (voucher No. RJC 8653). The leaves were dried at 40°C for 48 h and were ground up to a fine powder using a vegetative grinder.

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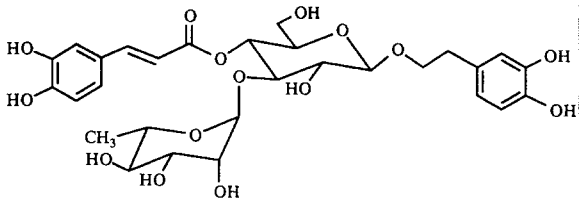
PII S0014-2999(96)00153-7



Verbascoside (1)

Scheme 1.

2.2. Extraction of plant material and fractionation of extracts

Powdered leaves (85 g) of the plant were successively extracted into four fractions with light petroleum (4.7 g, 5.5% of dry weight), dichloromethane (4.0 g, 4.7%), methanol (20 g, 23.5%) and water (11 g, 13%). A portion (3 g) of the aqueous extract was separated by chromatography on a polyamide column (60 g, 120–150 mesh, Koch-Light); and the fraction eluted with methanol was purified by radial plate chromatography. Elution with methanol : ethyl acetate (1.5 : 8.5) yielded pure verbascoside (**1**) (220 mg).

2.3. Analysis of chromatography fractions

^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were obtained for methanol or D_2O solutions using a Bruker AM 300 spectrometer. The identity of compound **1** was confirmed by comparison of its spectral parameters with those described in the literature (Andary et al., 1980) and by comparative thin layer chromatographic behaviour with that of a standard sample.

2.4. Isolated heart preparation

Isolated rat hearts were prepared from male and female albino Wistar rats of body mass between 400–600 g. The animals were fed and given water ad libitum until they were killed by spinal dislocation. The hearts were rapidly excised, freed of adhering tissue and immediately mounted intact on the Langendorff heart apparatus. This technique and all experiments were approved by the Animal Ethics and Experimentation Committee (approval Nos. R43/94 and R7/95).

2.5. Perfusate

The hearts were retrogradely perfused with a saline Krebs-Henseleit solution (pH 7.36). The solution was kept at a constant temperature of 37°C and constant pressure of 70 cm of H_2O. It was aerated with a 95% O_2 and 5% CO_2 gas mixture (Carbogen) prior to and during the experiment. The Krebs-Henseleit solution consisted of the following (mM): NaCl, 118.0; KCl, 4.7; $MgCl_2 \cdot 6H_2O$, 0.5; $NaHCO_3$, 25.0; NaH_2PO_4, 1.0; glucose, 10.0 and $CaCl_2 \cdot 2H_2O$, 2.2.

2.6. Data recording

Heart rates were monitored throughout each experiment by electrocardiograms recorded by micro-electrodes attached to the right atrial appendage and to the right and left ventricular walls of the isolated heart. The electrodes were directed through to a MacLab unit and recorded on a Macintosh computer. Contractile force was measured with a Nihon Kohden Kogyo Co. force-displacement transducer. The transducer was coupled with the MacLab and computer set-up and attached perpendicular to the heart by a small hook and thread. Coronary perfusion rate was measured with a drop counter constructed in our laboratory.

2.7. cAMP level analysis

A freshly prepared solution of verbascoside (1 mM) was administered through a polyethylene cannula in 1 ml/min retrograde perfusion. Each heart was allowed to reach a maximum response, after which it was immediately freeze clamped using liquid nitrogen (−196°C), according to Brown et al. (1971), and stored at −15°C until analysed for cAMP levels. The control hearts were allowed to reach a constant rate of contraction before being treated with liquid nitrogen. Frozen tissue was homogenised in cold 6% trichloroacetic acid at 2–8°C to give a 10% (w/v) homogenate which was centrifuged at $2000 \times g$ for 15 min. The resulting pellet was discarded and the supernatant washed five times with water-saturated diethyl ether. The aqueous extract was lyophilized. Just prior to the analysis, the dried extract was dissolved in assay buffer. Intracellular cAMP levels were measured using a Biotrak radioassay kit from Amersham International.

2.8. Data analysis

At least ten hearts were used for each group. The data were analysed by *t*-tests with probabilities of less than 0.05 considered statistically significant. All results are expressed as mean ± S.E.M.

3. Results

Following the mounting of hearts onto the Langendorff heart apparatus, they were allowed to reach equilibrium (usually 30 min) before any measurements were taken. After the equilibration period, the normal heart rate was 231 ± 12 beats/min, the contractile force was 1.05 ± 0.04 g and the coronary perfusion rate was 9.8 ± 1.5 ml/min. The intracellular level of cAMP was 3.0 ± 0.2 nM/g dry weight.

The introduction of verbascoside (0.6 mg/ml; 1 ml of 1 mM) into the perfusate increased the heart rate to 327 ± 14 beats/min (42%) and the coronary perfusion rate to 20.9 ± 2.2 ml/min (113%). Just prior to that, the contractile force increased to 1.21 ± 0.06 g (13%). All of these responses were statistically significant ($P < 0.05$) and occurred within a minute of the compound being entered into the system. During the increases, a significant ($P < 0.05$) rise in cAMP was measured. Cyclic adenosine monophosphate levels had risen to 55.2 ± 4.8 nM/g dry weight (1733%).

4. Discussion

The results presented here suggest that verbascoside increases heart rate, contractile force and coronary perfusion rate via a cAMP-dependent mechanism. Injection of verbascoside (1 mM) significantly increased intracellular levels of cAMP from 3.01 ± 0.12 nM/g dry weight to 55.17 ± 4.80 nM/g dry weight (1733%). By comparison, forskolin (0.2 μM) increased intracellular cAMP levels from 3.0 ± 0.1 to 14.1 ± 0.9 nM/g dry weight (370%) and epinephrine (1 μM) by 257% (Manning et al., 1985).

Although our results show a significant increase in intracellular cAMP levels due to 1 mM verbascoside, they do not reveal whether the observed increases were the result of stimulation of the adenylate cyclase enzyme or by inhibition of the phosphodiesterase III enzyme. There is, however, some evidence to rule out the involvement of the latter mechanism. Kitagawa et al. (1984) reported that verbascoside had no inhibitory effect on the phosphodiesterase III enzyme in vitro. It is tempting to suggest that the rise in intracellular cAMP mediated by verbascoside may be the result of increases in levels of prostacyclin. In 1977, Moncada and co-workers showed that prostacyclin was both a potent vasodilator and stimulator of cAMP production (Moncada et al., 1977). This prostanoid, which is synthesised via the arachidonic acid pathway, is spontaneously transformed into 6-keto-$PGF_{1\alpha}$.

Significant quantities of 6-keto-$PGF_{1\alpha}$ were observed when 1 mM verbascoside was administered to rat peritoneal cell preparations (Kimura et al., 1987). Also, the vasodilatory effects of prostacyclin would explain the massive increases in coronary perfusion rate (113%), which were mediated by verbascoside in the isolated rat heart. We are currently investigating if the effect of verbascoside is mediated by an increase in prostacyclin production.

Verbascoside (syn: acteoside), a compound widely distributed in the plant kingdom, has been shown to have a range of biological activities (Jiménez and Riguera, 1994). In previous experiments, verbascoside did not seem to affect blood pressure when injected intravenously into rats nor did it appear to mediate any chronotropic or inotropic effect on the artificially stimulated isolated heart auricle of a rat (Andary et al., 1980). Our results show for the first time that verbascoside (1 mM) induces massive increases in cAMP levels in the isolated rat heart.

Acknowledgements

The WA Department of Conservation and Land Management (CALM) is thanked for giving permission to carry out field work on Jaurdi Nature Reserve and for allowing sample collection. Dr. R.J. Chinnock (Botanic Gardens of Adelaide and State Herbarium) is thanked for providing *Eremophila* taxonomy. Steve Dawson of the School of Biomedical Sciences, Curtin University of Technology, is thanked for assistance with the cAMP analysis.

References

Andary, C., G. Privat, P. Chevallet, H. Orzalesi, J.J. Serrano and M. Bouchard, 1980, Chemical and pharmacological study of heterodisic esters of caffeic acid isolated from *Orobanche rapum-genistae*, Farm. Ed. Sci. 35, 3.

Brown, B.L, J.D.M. Albano, R.P. Ekins and A.M. Sgherzi, 1971, A simple and sensitive assay method for the measurement of adenosine 3′:5′-cyclic monophosphate, Biochem. J. 121, 561.

Ghisalberti, E.L., 1994, The ethnopharmacology and phytochemistry of *Eremophila* species (Myoporaceae), J. Ethnopharmacol. 44, 1.

Jiménez, C. and R. Riguera, 1994, Phenylethanoid glycosides in plants: Structure and biological activity, Nat. Prod. Rep. 12, 591.

Kimura, Y., H. Okuda, S. Nishibe and S. Arichi, 1987, Effects of caffeoylglycosides on arachidonate metabolism in leukocytes, Planta Med. 53, 148.

Kitagawa, S., H. Tsukamoto, S. Hisada and S. Nishibe, 1984, Studies on the Chinese drug 'Forsythiae Fructus' VII. A new caffeoyl glycoside from *Forsythia viridissima*, Chem. Pharm. Bull. 32, 1209.

Manning, A.S., K. Kinoshita, E. Buschmans, D.J. Coltart and D.J. Hearse, 1985, The genesis of arrhythmias during myocardial ischaemia. Dissociation between changes in cyclic adenosine monophosphate and electrical instability in the rat, Circ. Res. 57, 668.

Metzger, H. and E. Lindner, 1981, The positive inotropic-acting forskolin, a potent adenylate cyclase activator, Arzneim.-Forsch. Drug Res. 31, 1248.

Moncada, S., E.A. Higgs and J.R. Vane, 1977, Human arterial and venous tissues generate prostacyclin (Prostaglandin X), a potent inhibitor of platelet aggregation, Lancet 1, 18.

Packer, M., 1992, Pathophysiology of chronic heart failure, Lancet 340, 88.

Pennacchio, M., E. Alexander, E.L. Ghisalberti and G.S. Richmond, 1995a, Cardioactive effects of *Eremophila alternifolia* extracts, J. Ethnopharmacol. 47, 91.

Pennacchio, M., Y.M. Syah, E.L. Ghisalberti and E. Alexander, 1995b, Cardioactive compounds from *Eremophila* species, J. Ethnopharmacol., in press.