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RESEARCH ARTICLE

Cardiovascular effects of the alkaloid hippadine on the isolated perfused rat heart

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Abstract: *Crinum macowanii* has been used extensively in traditional medicines for treatment of various illnesses such as oedema and 'heart disease'. Previous studies of the crude bulb extracts on Langendorff perfused isolated rat hearts indicated a positive inotropic effect. The aim of this study was to isolate and characterize compound(s) from *C. macowanii* with cardiovascular effects similar to that observed with the crude extracts of the plant. The methanol extract of dried bulbs was extracted for alkaloids, and structural elucidation of the isolated alkaloid identified it as hippadine. The cardiovascular effects of hippadine was evaluated *in vitro* in isolated perfused rat hearts using the "double sided" working heart system. Perfusion with 0.5 µg/ml and 5.0 µg/ml hippadine in Krebs-Hanseleit buffer led to significant decreases in coronary flow, aortic output, cardiac output, systolic pressure, and heart rate, accompanied by increases in diastolic pressure. Hippadine exhibited a negative chronotropic and inotropic effect on the isolated rat heart and is responsible either partly or fully for the cardiovascular effects of *C. macowanii*.

Keywords: Alkaloid; Cardiovascular; *Crinum macowanii*; Hippadine; Rats.

Introduction

Plants in the Amaryllidaceae family have been used quite extensively in traditional medicines for the treatment of various illnesses. *Crinum macowanii*, a member of this family of plants has found extensive use in traditional medicines for the treatment of various illnesses such as oedema, 'heart disease', rheumatic fever, cancer and skin diseases (Duncan et al. 1999; Van Wyk et al. 2000; Elgorashi et al. 2001; Elgorashi et al. 2002; Elgorashi et al. 2003). In a previous study to examine the cardiovascular effect of the plant, Mugabo et al. (2001) reported a positive inotropic effect and no effect on heart rate in isolated male Wistar rat hearts perfused with the crude extract of the bulbs of *Crinum macowanii* via a Langendorff perfusion system. Plants in the Amaryllidaceae family are rich in alkaloids and many alkaloids extracted from plants have been shown to exhibit cardiovascular activity (Andraws et al. 2005; Osorio et al. 2010; Rostoff et al. 2010; Wang et al. 2010; Jayakumar and Sheu 2011; Jensen et al. 2011; Nair et al. 2011). As such it was hypothesized that the cardiovascular effects produced by the plant extracts were partly or wholly due to alkaloids present in the plant. The aim of the present study was to isolate and characterise a cardioactive alkaloid from extracts of the bulbs of *C macowanii*.

Materials and Methods

Isolation of hippadine

Crinum macowanii was obtained from Kirstenbosch botanical gardens (Cape Town, South Africa), identified and a voucher specimen was deposited at the herbarium at the University of the Western Cape (UWC). Fresh bulbs were diced, dried at 30˚C to a constant mass and milled to a fine powder. The powdered bulbs were extracted with methanol, and the methanol extract extracted for alkaloids according to method described by Nair et al.

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(2000). The alkaloid-containing extract was fractionated by column chromatography (CC) using a silica gel (70-230 mesh) column and ethyl acetate: hexane (1:4); (2:3); (3:2); and (4:1) as sequential eluents. Fraction G from the ethyl acetate: hexane (1:4) elution showed significant cardiovascular effects *in vivo* (results not shown) and was subjected to further CC separation using the eluents mentioned above. A pure compound C045 was isolated from fraction E of the ethyl acetate: hexane (2:3) eluent and purified by crystallization from chloroform. Identification tests revealed that C045 was hippadine, an alkaloid previously isolated from the Amaryllidaceae family (Hutchings and Meyers 1996; Torres et al. 2004).

Animals

Normotensive male Wistar rats weighing 250 - 350 g and less than four months old were used for the experiments. The animals were obtained from the Physiology department, UWC and were allowed free access to food and water. Ethical approval for the study was obtained and all animals were treated according to ethical regulations as required by the University of Western Cape ethics committee.

Drugs and Chemicals

Adrenaline (Bodene; Cape Town, South Africa) and hippadine were dissolved in normal saline and further diluted with Krebs-Hanseleit buffer (KHB) solution. Fresh KHB solution containing (in mM); 118.5 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$ and 10.0 g glucose (all Sigma; Cape Town, South Africa) was prepared daily (Sutherland and Hearse 2000a).

Measurement of blood pressure and heart rate in vitro

A modified version of the isolated perfused working heart model was used to evaluate the *in vitro* effects of hippadine (Depre 1998; Sutherland and Hearse 2000a,b). The modification as described by Mugabo et al. (2001) was to enable the perfusion of the working heart from two distinct working heart systems via a system of taps to reduce the risk of cross-contamination of the perfused drug/extracts. The rats were anaesthetized with sodium pentobarbitone (30 mg/kg IP), and the beating hearts were rapidly excised and immersed in cold (<4^{o}C) Krebs-Hanseleit buffer. The excised hearts were then mounted and cannulated for working heart perfusion according to the method described by Sutherland and Hearse (2000a,b), and then perfused retrogradely (Langendorff perfusion) for ten minutes with Krebs-Hanseleit buffer, following which a ten minute working heart perfusion with either control (Krebs-Hanseleit buffer or adrenaline) or test substance (hippadine) occurred. Blood pressure (BP) and heart rate (HR) were measured via a pressure transducer, connected to a side arm of the aortic cannula and linked to a computer system running the chart recorder (version 2.0 Gentronics, SA). Coronary flow (Qe) was measured as runoff from the organ chamber per minute, aortic output (Qa) as runoff from the cardiac output compliance chamber per minute, and cardiac output (CO) measured as the sum or aortic output (Qa) and coronary flow (Qe). The effects of the standard drug (adrenaline - 0.01 µg/ml and 1.0 µg/ml) and test substance (hippadine - 0.5 µg/ml and 5.0 µg/ml) on these cardiovascular parameters following working heart perfusion with the following perfusion protocol were evaluated:

i) 0 – 10 minutes (reperfusion period): Langendorff perfusion with KHB.

ii) 10 – 20 minutes (stabilization period): Working heart perfusion with KHB.

iii) 20 – 30 minutes (drug perfusion period): Working heart perfusion with drug in KHB.

iv) 30 – 40 minutes (stabilization period): Same as ii above.

v) 40-50 minutes (drug perfusion period): Same as iii above.

vi) 50-60 minutes (stabilization period): Same as ii above.

Statistical analysis

The data is expressed as mean ± SD of HR, SP, DP, Qe, CO and Qa for five (5) replicates.

Statistical significance between means for control and test substances was calculated using the unpaired Student's t-test ($p<0.05$).

Results

Re-crystallization yielded white crystals with the chemical formula $C_{16}H_9NO_3$. Mp 217–218 ^oC. HREIMS m/z value 263.247289. A search of chemical database Chembase® and other literature identified the isolated compound as hippadine (Hutchings and Meyers 1996; Torres et al. 2004).

In isolated perfused working hearts, perfusion with hippadine (0.5 µg/ml and 5.0 µg/ml) produced significant dose dependent decreases in systolic pressure (10.03% ± 0.012 and 20.56% ± 0.021 for 0.5 µg/ml and 5.0 µg/ml doses respectively). This effect was the opposite of the significant dose dependent increases in systolic pressure produced by adrenaline (0.01 µg/ml and 1.0 µg/ml) (Figure 1). Diastolic pressure increased in a dose-dependent manner with hippadine perfusion, although statistically significant only with the 0.5 µg/ml dose. This effect was opposite to that obtained for adrenaline (Figure 1).

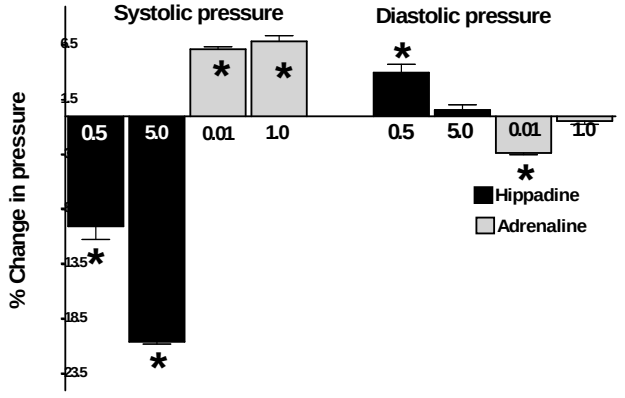

Figure 1: Effect of hippadine (0.5 µg/ml and 5.0 µg/ml) and adrenaline (0.01 µg/ml and 1.0 µg/ml) on systolic pressure and diastolic pressure in isolated perfused working rat hearts.
* *P* <0.05 with respect to control (Krebs-Hanseleit buffer).

Hippadine perfusion also led to significant dose-dependent decreases in heart rate (7.59% ± 0.112 and 36.01% ± 0.266 for 0.5 µg/ml and 5.0 µg/ml dose respectively). This effect was opposite to that observed with adrenaline perfusion (Figure 2).

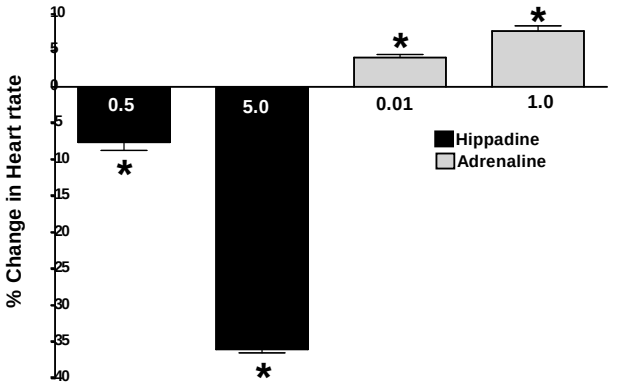

Figure 2: Effect of hippadine (0.5 µg/ml and 5.0 µg/ml) and adrenaline (0.01 µg/ml and 1.0 µg/ml) on heart rate in isolated perfused working rat hearts.
* *P* <0.05 with respect to control (Krebs-Hanseleit buffer).

Hippadine perfusion led to dose-dependent statistically significant decreases in coronary flow (15.82% ± 0.109 and 48.83% ± 0.172 for 0.5 µg/ml and 5.0 µg/ml doses respectively) and cardiac output (14.35% ± 0.670 and 89.13% ± 0.390 for 0.5 µg/ml and 5.0 µg/ml doses respectively) (Figures 3-4). Aortic output also decreased in a dose dependent manner (1.38% ± 0.180 and 100.00% ± 2.010 for 0.5 µg/ml and 5.0 µg/ml doses respectively) though only significantly with the 5.0 µg/ml dose (Figure 3). These effects were opposite to the increases observed with adrenaline perfusion (Figures 3- 4).

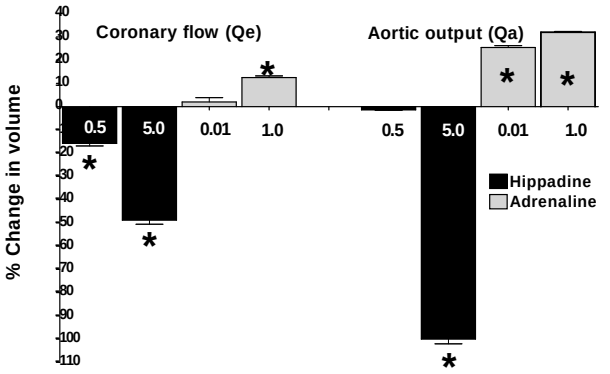

Figure 3: Effect of hippadine (0.5 µg/ml and 5.0 µg/ml) and adrenaline (0.01 µg/ml and 1.0 µg/ml) on coronary flow and aortic output in isolated perfused working rat hearts.
* *P* <0.05 with respect to control (Krebs-Hanseleit buffer).

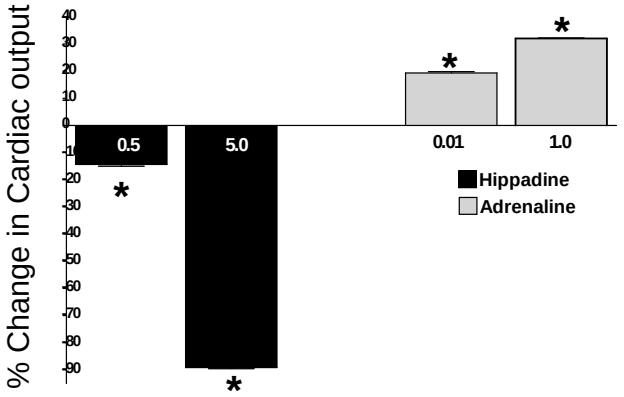

Figure 4: Effect of hippadine (0.5 µg/ml and 5.0 µg/ml) and adrenaline (0.01 µg/ml and 1.0 µg/ml) on cardiac output in isolated perfused working rat hearts.
* *P* <0.05 with respect to control (Krebs-Hanseleit buffer).

Discussion

In this study hippadine was extracted from the bulbs of *Crinum macowanii* and evaluated for cardiovascular effects using isolated perfused working rat hearts. It is interesting to note that although the alkaloid had been previously isolated from other plants within the Amaryllidaceae family, this was the first time it has been isolated from the genus *Crinum*, despite extensive isolation of alkaloids from *C. latifolium*, *C. bulbispermum* and *C. macowanii* (Koboyashi et al. 1984; Nair et al. 2000).

Both doses (0.5 µg/ml and 5.0 µg/ml) of hippadine perfusion produced decreases in systolic pressure, heart rate, coronary flow, aortic output and cardiac output, while increasing diastolic pressure in the isolated working rat heart. The effects on these parameters were contrary to those observed with the adrenaline perfusion.

In the intact animal, blood pressure is determined by cardiac output and peripheral vascular resistance (Laurence and Bennett 1980). In the absence of vascular resistance in isolated perfused hearts, systolic blood pressure is entirely dependent on cardiac output. The decrease in systolic pressure produced by hippadine was the result of the decrease in cardiac output, coronary flow and heart rate by the compound. Any change in heart rate affects coronary flow since myocardial contraction is one of the factors that determine the rate of coronary flow. The reduction in coronary flow with hippadine could be due to the reduction in heart rate and cardiac output, or may be due to a direct vasodilatory effect by the alkaloid as has been reported with lepadiformine, an alkaloid isolated from *Clavelina lepadiformis* (Alloatti et al. 1995). Hippadine produced decreases in systolic pressure, opposite to the effect of adrenaline, thus suggesting that the effect of hippadine on cardiac contractility is the opposite of the effect of adrenaline, possibly via antagonistic activity on the sympathetic system. The increases in diastolic blood pressure observed with hippadine were due to the slowing down of the cardiac cycle probably due to a prolongation of the action potential that would lead to incomplete contraction of the myocardium. A similar effect has been reported with the plant alkaloid lepadiformine (Juge et al. 2001). The reduction in myocardial contractility, coronary flow and cardiac output observed with hippadine is similar to those observed in other alkaloids and their corresponding cinnamates isolated from other plants (Alloatti et al. 1995).

The reduction in heart rate suggests possible β adrenergic receptor blockade or prolongation of the action potential (Laurence and Bennett 1980). Considering that the effect of hippadine on the cardiovascular system seems to mimic those of another plant-based alkaloid, lepadiformine, it is possible that hippadine produces its effect on the cardiovascular system by vasodilatation and elongation of the action potential.

In conclusion, hippadine, an alkaloid isolated from the bulbs of *Crinum macowanii* exhibited a negative chronotropic and inotropic effect in isolated perfused working rat hearts. Further investigation would be required to conclusively confirm the mechanism for the cardiovascular effects of hippadine.

References

Alloatti, G., Penna, C., Levi, R.C., Gallo, M.P., Appendino, G., Fenoglio, I. 1995. Effects of yew alkaloids and related compounds on guinea-pig isolated perfused heart and papillary muscle. *Life Sciences*, **58(10)**: 845-854.

Andraws, R., Chawla, P., Brown, D.L. 2005. Cardiovascular effects of ephedra alkaloids: A comprehensive review. *Progress in Cardiovascular Diseases*, **47(4)**: 217 – 225.

Depre, C. 1998. Isolated working heart: Description of models relevant to radioisotopic and pharmacological assessments. *Nuclear Medicine and Biology*, **25**: 711-713.

Duncan, A.C., Jäger, A.K., Van Staden, J. 1999. Screening of Zulu medicinal plants for angiotensin converting enzyme (ACE) inhibitors**.** *Journal of Ethnopharmacology*, **68(1)**: 63-70.

Elgorashi, E.E., Drewes, S.E., Morris, C., Van Staden, J. 2003. Variation among three *Crinum* species in alkaloid content. *Biochemical Systematics and Ecology*, **31(6)**: 601-615.

Elgorashi, E.E., Drewes, S.E., Van Staden, J. 2001. Alkaloids from *Crinum macowanii*. *Biochemical Systematics and Ecology*, **29(7)**: 749-750.

Elgorashi, E.E., Drewes, S.E., Van Staden, J. 2002. Organ-to-organ and seasonal variation in alkaloids from *Crinum macowanii*. *Fitoterapia*, **73(6)**: 490-495.

Hutchings, R.H., Meyers, A.I. 1996. An oxazoline-mediated synthesis of the pyrrolophenanthridine alkaloids and some novel derivatives. *Journal of Organic Chemistry*, **61(3)**: 1004-1013.

Jayakumar, T., Sheu, J. R. 2011. Cardiovascular pharmacological actions of rutaecarpine, a quinazolinocarboline alkaloid isolated from *Evodia rutaecarpa*. *Journal of Experimental and Clinical Medicine*, **3(2)**: 63 – 69.

Jensen, B.S., Christensen, S.B., Jäger, A.K., Rønsted, N. 2011. Amaryllidaceae alkaloids from the Australasian tribe Calostemmateae with acetylcholinesterase inhibitory activity. *Biochemical Systematics and Ecology*, **39(2)**: 153-155.

Juge, M., Grimaud, N., Biard, J., Sauviat, M., Nabil, M., Verbist, J., Petit, J. 2001. Cardiovascular effects of lepadiformine, an alkaloid isolated from the ascidians *Clavelina lepadiformis* (Muller) and C. *moluccensis (Sluiter). Toxicon*, **39**: 1231-1237.

Kobayashi, S., Tokumoto, T., Kihara, M., Imakura, Y., Shingu, T., Taira, Z. 1984. Alkaloidal constituents of *Crinum latifolium* and *Crinum bulbispermum* (Amaryllidaceae). *Chemistry Pharmacy Bulletin*, **32(8)**: 3015-3022.

Laurence, D.R., Bennett, P.N. 1980. Sympathomimetics, Asthma, Shock, Hypotension. C1inical Pharmacology. The Pitman Press, Bath, pp. 585-607.

Mugabo, P., Davids, F., Mohamed, F., Burger, A.P., Cyster, L. 2001. Cardiovascular effects of *Crinum macowanii* on the isolated perfused rat heart. Conference proceedings: International Immunopharmacology Congress. 17 - 21 September. Sun City, South Africa.

Nair, J., Machocho, A., Campbell, W., Brun, R., Vildomat, F., Codina, C., Bastida, J. 2000. Alkloids in *Crinum macowanii. Journal of Phytochemistry*, **54(5)**: 945-950.

Nair, J.J., Aremu, A.O., van Staden, J. 2011. Isolation of narciprimine from *Cyrtanthus contractus* (Amaryllidaceae) and evaluation of its acetylcholinesterase inhibitory activity. *Journal of Ethnopharmacology*, **137(3)**: 1102-1106.

Osorio, E.J., Berkov, S., Brun, R., Codina, C., Viladomat, F., Cabezas, F., Bastida, J. 2010. *In vitro* antiprotozoal activity of alkaloids from *Phaedranassa dubia* (Amaryllidacea). *Phytochemistry Letters*, **3(3)**: 161 – 163.

Rostoff, P., Gajos, G., Latacz, P., Wozniczko, M., Matysek, J., Piwowarska, W. 2010. Ergotamine-induced cardiovascular toxicity: mechanisms and clinical significance. *International Journal of Cardiology*, **141(1)**: 111 – 114.

Sutherland, F., Hearse, J. 2000a. Experimental models for the study of cardiovascular function and disease. *Pharmacological Research*, **41**: 597-603.

Sutherland, F., Hearse, J. 2000b. The isolated blood and perfusion fluid perfused heart. *Pharmacological Research*, **41**: 613-647.

Torres, C.J., Pinto, A.C., Garden, S.J. 2004. Application of a catalytic palladium biaryl synthesis reaction, via C–H functionalization, to the total synthesis of Amaryllidaceae alkaloids. *Tetrahedron*, **60(44)**: 9889-9900.

Van Wyk, B., Van Oudtshoorn, B., Gericke, N. 2000. Medicinal Plants of South Africa. Briza Publications, Cape Town.

Wang, L., Yin, Z. Q., Cai, Y., Zhang, X.Q., Yao, X.S., Ye, W.C. 2010. Amaryllidaceae alkaloids from the bulbs of Lycoris radiate. Biochemical Systematics and Ecology, 38(3): 444 -446.