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Research Paper

Effects of hippadine on the blood pressure and heart rate in male spontaneously hypertensive Wistar rats

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Pentobarbitone (PubChem CID: 4737)
Heparin (PubChem CID: 25244225)

ABSTRACT

Ethnopharmacological relevance: Hippadine is an alkaloid isolated from *Crinum macowanii*. *Crinum macowanii* is used in South Africa to treat oedema, 'heart disease', rheumatic fever, cancer and skin diseases, and belongs to the plant family Amaryllidaceae, assumed to have originated in the South African region. The aim of this study was to evaluate the effect of hippadine, an alkaloid extracted from *Crinum macowanii*, on the blood pressure (BP) and heart rate (HR) in anaesthetized male spontaneously hypertensive Wistar rats (SHR); and to find out if α_1 and/or β_1 adrenoceptors contribute to its effects.

Materials and methods: Hippadine (2.5–12.5 mg/kg), adrenaline (0.05–0.20 mg/kg), atenolol (0.5–40 mg/kg) and prazosin hydrochloride (100–500 μg/kg) were infused intravenously, and the BP and HR measured via a pressure transducer connecting the femoral artery and the PowerLab.

Adrenaline increased the systolic, diastolic and mean arterial BP, while hippadine, atenolol and prazosin respectively decreased the systolic, diastolic and mean arterial BP. Increases in HR were observed with both adrenaline and prazosin, while reductions in HR were observed with atenolol and hippadine. Infusion of adrenaline in rats pre-treated with atenolol (30 mg/kg), prazosin (400 μg/kg), and hippadine (10 mg/kg) led to similar increases in BP and HR in all groups. All changes in HR or BP were significant ($p < 0.05$) and dose dependent.

Conclusion: Hippadine decreases the BP and HR in SHR, and these effects may be due to α_1 and β_1 adrenoceptor inhibition.

1. Introduction

Hippadine is one of the alkaloids that have been isolated from *Crinum macowanii* (Boger and Wolkenberg, 2000; Nkanwen et al., 2009; Mugabo et al., 2012; Jin, 2013; Nair and van Staden, 2013) so far. *Crinum macowanii* has found extensive use in traditional medicines for the treatment of various illnesses such as oedema, gynaecological conditions, psychosis, wounds, rheumatic fever, cancer, skin diseases, and 'heart disease' (Duncan et al., 1999; Van Wyk et al., 2000; Van Wyk and Gericke, 2000; Elgorashi et al., 2001, 2002, 2003b; Van Wyk, 2011a, 2011b). *Crinum macowanii* belongs to the large plant family Amaryllidaceae which originates in the Southern African region, and has naturally been used extensively in the local traditional medicines in the region (Chattopadhyay et al., 1983; Boger and Wolkenberg, 2000; Koorbanally et al., 2000; Nair et al., 2000; Hiroya et al., 2004; Ganton and Kerr, 2005; Kissling et al., 2005; Mentzel et al., 2006; Nkanwen et al., 2009; Cheesman et al., 2012; Jin, 2013; Nair and van Staden, 2013, 2014). The medicinal properties of the family Amaryllidaceae are largely associated with the vast number of alkaloids that are produced by the plants in the family (Elgorashi et al., 2003a; Nkanwen et al., 2009; Cheesman et al., 2012; Refaat et al., 2012a, 2012b, 2012c, 2013a, 2013b; Nair and van Staden, 2013, 2014).

Recent scientific research has established the potency and selective inhibitory activity of galanthamine, an alkaloid obtained from the family Amaryllidaceae, against the enzyme acetylcholinesterase, an activity beneficial in the management of Alzheimer's disease. Pancratistatin is another alkaloid obtained from this family that has shown potent and cell line specific antiproliferative properties (Jensen et al., 2011; Nair and van Staden, 2013). Some other plants in this family, such as *Boophone disticha* and *Cyclamen purpurascens* have displayed novel, broad spectrum antibacterial activity (Cheesman et al., 2012) and may contain antibacterial agents which could be used in the treatment of typhoid fevers and urogenital infections respectively (Nkanwen et al., 2009). Some

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of the abundant alkaloids obtained from the family Amaryllidaceae are hypothesized to be responsible for various medicinal properties of the family (Chattopadhyay et al., 1983; Boger and Wolkenberg, 2000; Koorbanally et al., 2000; Nair et al., 2000; Elgorashi et al., 2003a; ; Osorio et al., 2010; Cheesman et al., 2012; Refaat et al., 2012a, 2012b, 2012c, 2013a, 2013bJin, 2013) and have shown cardiovascular activity (Mugabo et al., 2001; Andraws et al., 2005; Burger et al., 2009; Rostoff et al., 2010; Jayakumar and Sheu, 2011; Nair et al., 2011; Mugabo et al., 2012). Previously, we have reported positive inotropic effect, with no chronotropic effect (Mugabo et al., 2001) and negative inotropic and chronotropic effects (Mugabo et al., 2012) with crude extracts of the bulbs of *Crinum macowanii* and hippadine respectively, in isolated perfused rat hearts. Therefore, in this study we intend to determine the effects of hippadine on the blood pressure (BP) and heart rate (HR) in anaesthetized spontaneously hypertensive male Wistar rats (SHR) and to find out whether adrenoceptors contribute to this.

2. Materials and methods

2.1. Study design

The study was designed as an experimental model investigating the effects of the unknown drug (hippadine) and that of the standard drugs (atenolol or prazosin) on the BP and HR in male SHR.

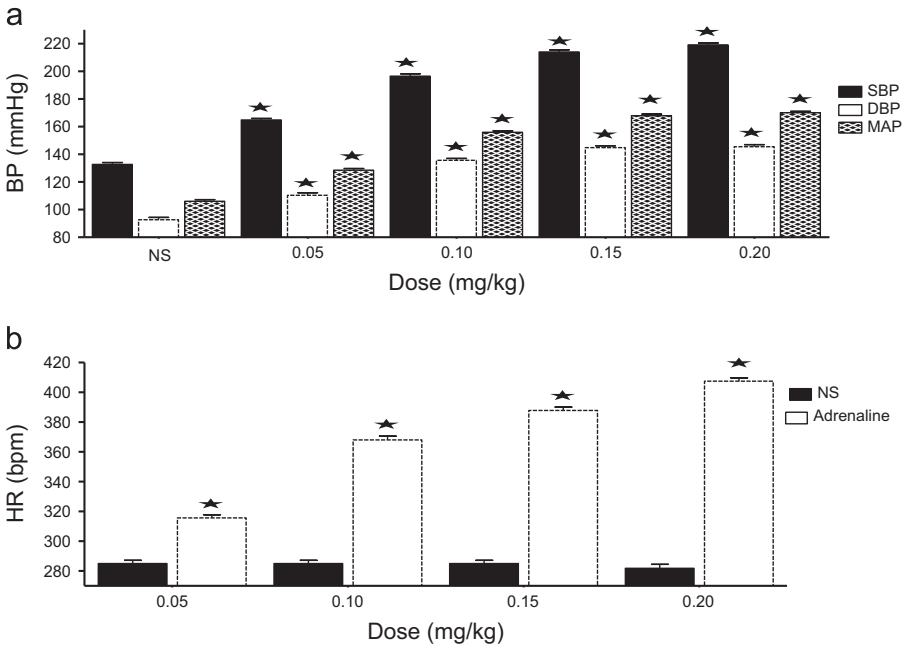

Fig. 1. Effect of adrenaline (0.05–0.20 mg/kg) on BP (a) and HR (b). Values are presented as mean ± SEM. * indicates statistical significance.

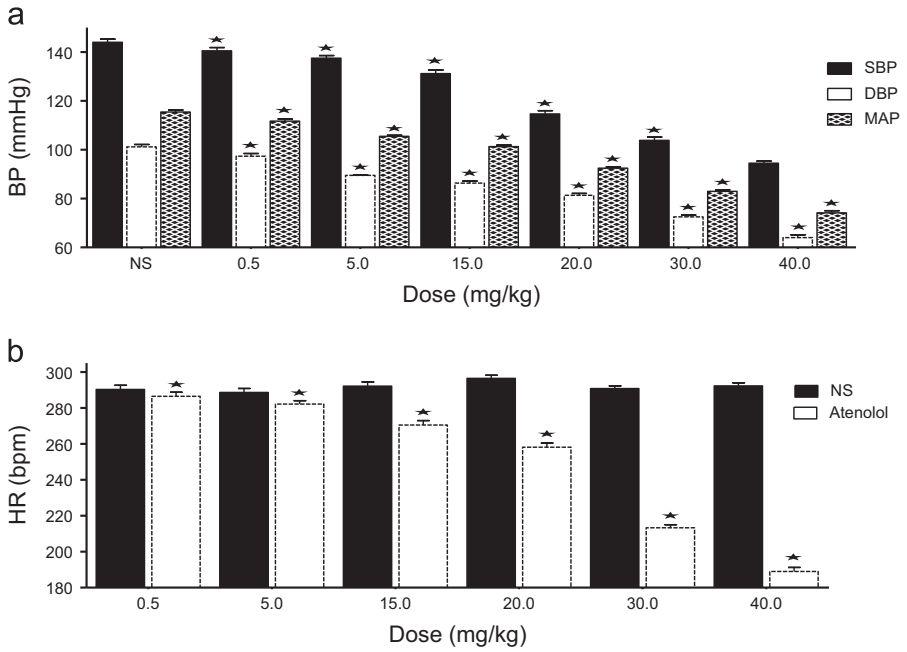

Fig. 2. Effect of atenolol (0.5–40 mg/kg) on BP (a) and HR (b). Values are presented as mean ± SEM. * indicates statistical significance.

2.2. Plant collection and identification

Plants were collected at the New plant Nursery, George, (South Africa) during the summer. A taxonomist at the University of Western Cape (UWC) Herbarium authenticated the plants as *Crinum macowanii* and a voucher specimen 3864 is held at the herbarium.

2.3. Extraction and isolation of hippadine

Hippadine extraction and isolation from *Crinum macowanii* bulbs were conducted using the methods previously described by Mugabo et al. (2012).

2.4. Experimental animals

Spontaneously hypertensive male Wistar rats weighing 250–350 g and aged less than 6 months old were used. Rats were obtained from the Animal Unit of the University of Cape Town and housed indoors in groups of 3 per cage with 12 h light:12 h dark cycle in the animal house, and received water and feed (commercial food pellets) ad libitum.

2.5. Animal preparation and cannulation

Rats were weighed and anaesthetized with sodium pentobarbitone (40 mg/kg body weight) administered intraperitoneally. They were then placed on a heated operating table in order to

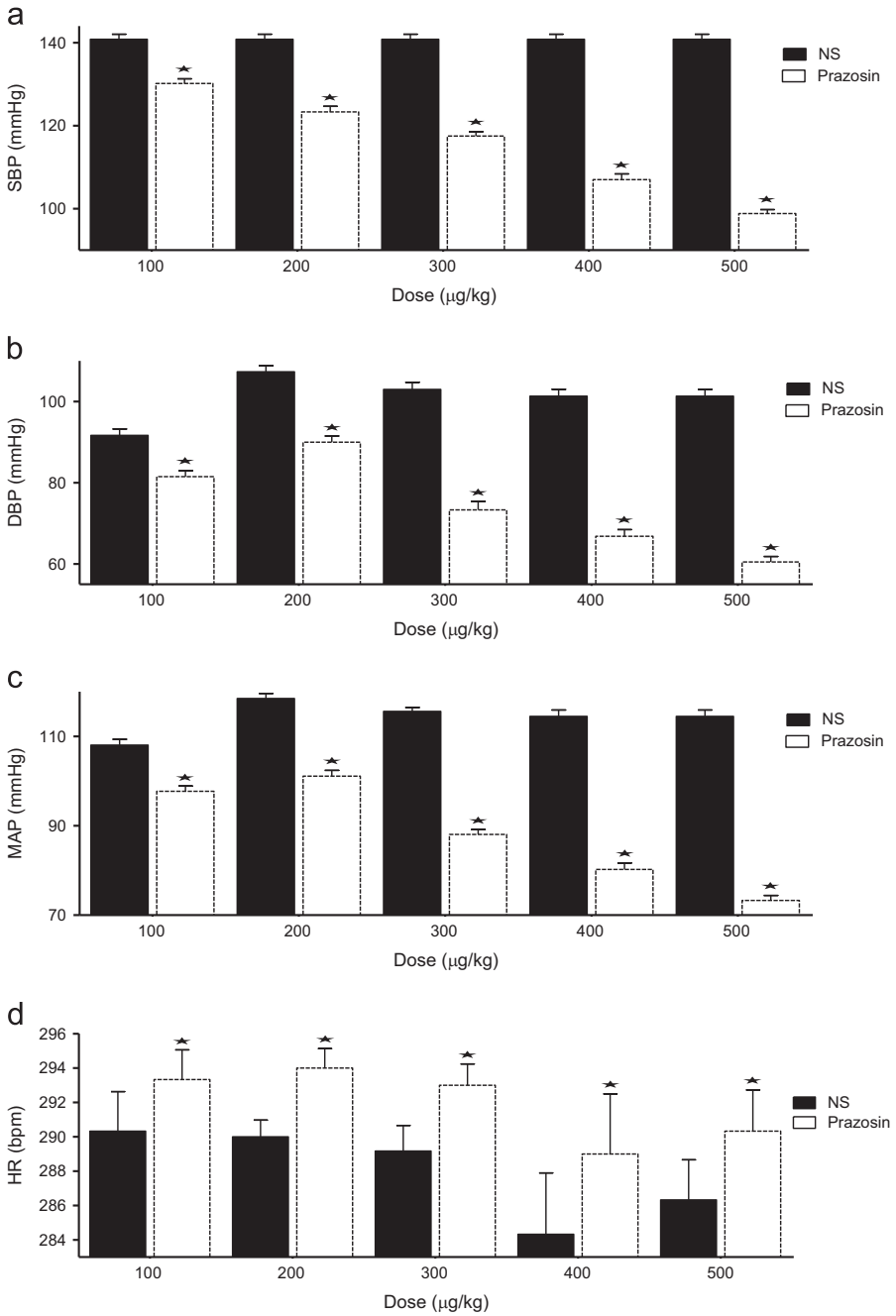

Fig. 3. Effect of prazosin (100–500 μg/kg) on SBP (a), DBP (b), MAP (c) and HR (d). Values are presented as mean ± SEM. * indicates statistical significance.

keep the animal body temperature at 37 °C. The trachea was exposed via a midline incision, and cannulated to facilitate breathing. Oxygen was supplied via a mask placed over the head of the animal. The external jugular vein was exposed and excess tissue carefully cleaned off it. A small cut into the vein was made and a thin lubricated catheter, containing 10% heparin-normal saline to prevent blood coagulation from occurring, was placed inside the vein and tied off with a thread. This catheter was used for administration of drugs and other substances to be tested. Another incision was made in the abdominal region and the femoral artery cannulated with a catheter containing a 10% solution of normal saline and heparin. The catheter was connected to a BP transducer and to a computer via a PowerLab. Blood pressure calibrations were done on the Chart 5 software before the start of BP and HR recording. The incisions were covered with normal saline wet gauze for the duration of the experiment to prevent the surgical incision area from drying. After surgery, animals were allowed a 30-min recovery period before the start of experiments. All drugs and substances used were diluted with normal saline and administered in a volume of 0.1 ml/min for a maximum of 3 min. After drug and substances administration, the jugular catheter was flushed with 1 ml normal saline (Hearse and Sutherland, 2000; Raji et al., 2012). Six rats were used for each set of experiments.

2.6. Drugs and chemical used

Adrenaline was used as a standard non-selective sympathomimetic drug. Atenolol was used a standard selective beta-1 antagonist and prazosin as a standard selective alpha-1 antagonist. (Khwanchuea et al., 2008; Poirier and Lacourciere, 2012).

2.7. Experimental protocol

Time zero: Surgical procedure (20 min). Recording of cardiovascular parameters right through the experiment
Time 20–50 min: Stabilization of animal.
Time 51–53 min: Drug administration.
Time 56–76 min: Recovery period. No normal saline administration during this period.
Time 77–80 min: Second drug administration.
Time 81–100 min: Stabilization of animal. No normal saline administration during this period.

2.8. Parameters assessed

The following parameters were assessed throughout the experiments: HR, systolic BP (SBP), diastolic BP (DBP) and mean arterial pressure (MAP).

2.9. Experimental conditions of the animals

Haemodynamic, respiratory and metabolic conditions of the rats were monitored according to the method described by Mugabo and Raji (2013).

2.10. Statistical analysis

The results obtained are presented as mean values (± SEM). Statistical significance between means was calculated using the Student's *t*-test and p value < 0.05 was considered significant.

2.11. Ethical considerations

The study was approved by the ethics committee of the UWC and was conducted according to the UWC rules and regulations in terms of animal experiments, and the European (Economic) Community guidelines (EEC Directive of 1986; 86/609/EEC).

3. Results

3.1. Effect of adrenaline on the blood pressure and heart rate

Adrenaline (0.05–0.20 mg/kg) significantly increased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 1).

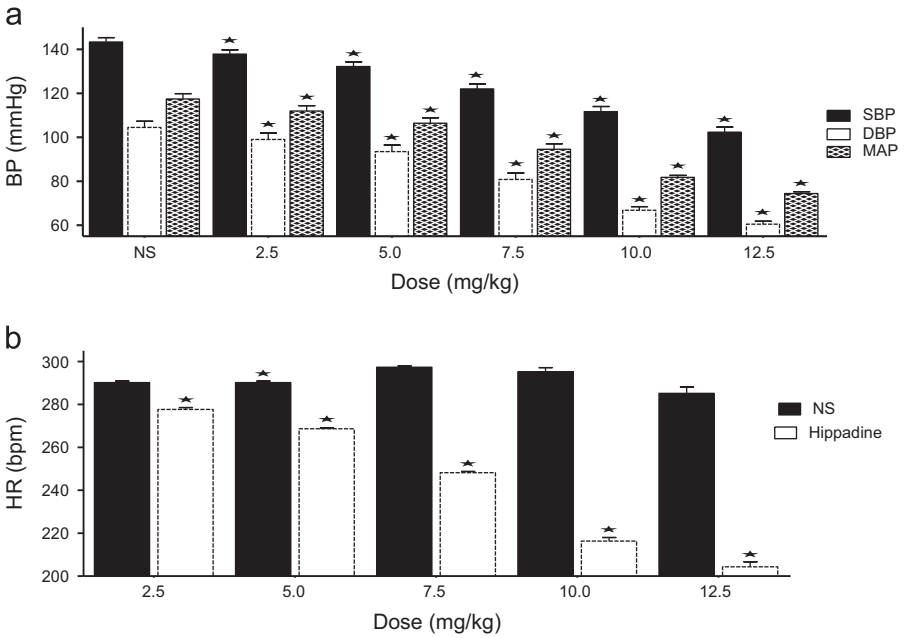

Fig. 4. Effect of hippadine (2.5–12.5 mg/kg) on BP (a) and HR (b). Values are presented as mean ± SEM. * indicates statistical significance.

3.2. Effect of atenolol on the blood pressure and heart rate

Atenolol (0.5–40 mg/kg) significantly decreased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 2).

3.3. Effect of prazosin on the blood pressure and heart rate

Prazosin (100–500 μg/kg) significantly decreased the SBP, MAP and DBP, while increasing HR in a dose-dependent fashion (Fig. 3).

3.4. Effect of hippadine on the blood pressure and heart rate

Hippadine (2.5–12.5 mg/kg) significantly decreased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 4).

3.5. Effect of adrenaline on the blood pressure and heart rate in rats pre-treated with atenolol

In rats pre-treated with atenolol (30 mg/kg), adrenaline (0.05–0.20 mg/kg) significantly increased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 5).

3.6. Effect of adrenaline on the blood pressure and heart rate in rats pre-treated with prazosin

In rats pre-treated with prazosin (400 μg/kg), adrenaline (0.05–0.20 mg/kg) significantly increased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 6).

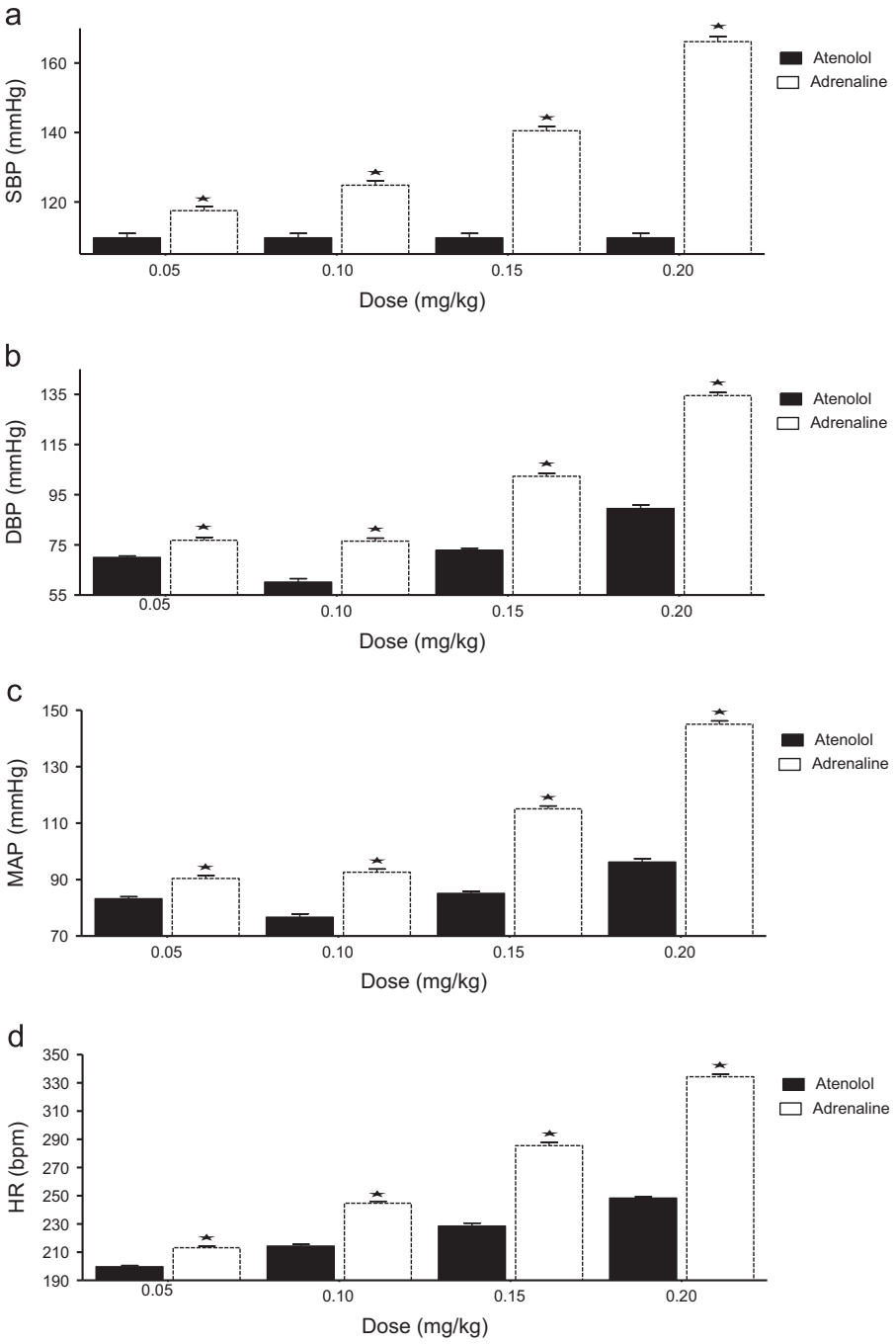

Fig. 5. Effect of adrenaline (0.05–0.20 mg/kg) on SBP (a), DBP (b), MAP (c) and HR (d) in rats pre-treated with atenolol (30 mg/kg). Values are presented as mean ± SEM. * indicates statistical significance.

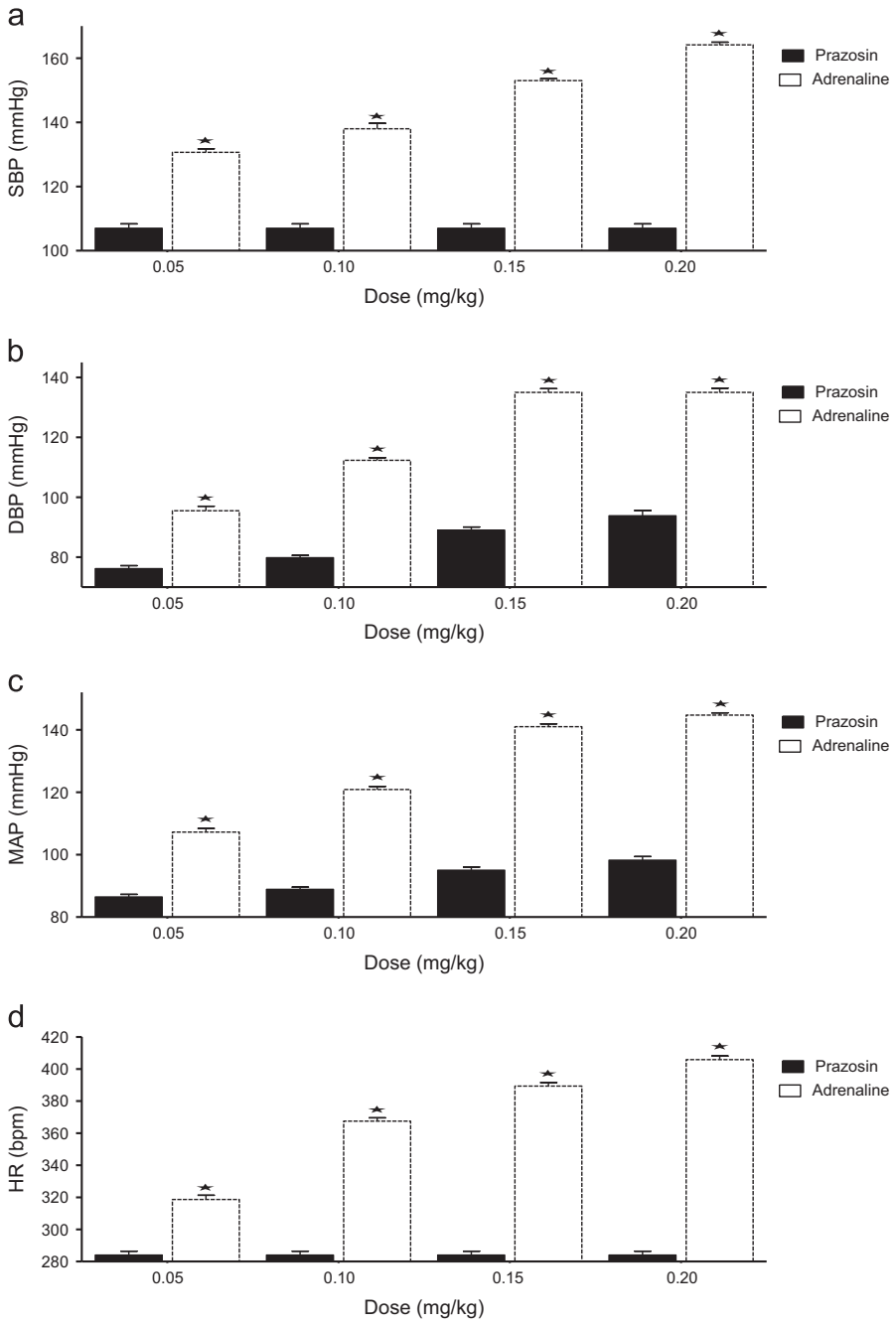

Fig. 6. Effect of adrenaline (0.05–0.20 mg/kg) on SBP (a), DBP (b), MAP (c) and HR (d) in rats pre-treated with prazosin (400 μg/kg). Values are presented as mean ± SEM. * indicates statistical significance.

3.7. Effect of adrenaline on the blood pressure and heart rate in rats pre-treated with hippadine

In rats pre-treated with hippadine (10 mg/kg), adrenaline (0.05–0.20 mg/kg) significantly increased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 7).

4. Discussion

4.1. Effect of the standard drugs on the blood pressure and heart rate

Adrenaline, a non-selective alpha and beta-adrenoceptor agonist, significantly increased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 1). The increase in BP can be explained by an increase in peripheral vascular resistances mediated by the direct stimulation of α_1 adrenoceptors in blood vessels coupled with a positive inotropic effect mediated by the stimulation of β_1 adrenoceptors in the heart. The increase in HR is due to the positive chronotropic effect exhibited by adrenaline via β_1 adrenoceptor stimulation (Dabire, 2004; Khwanchuea et al., 2008).

Atenolol, a selective β_1 adrenoceptor antagonist (Daskalopoulou et al., 2012; Poirier and Lacourciere, 2012), significantly decreased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 2). These effects could be explained by the negative inotropic and negative chronotropic effects of atenolol.

Prazosin, a selective α_1 adrenoceptors antagonist (Dabire, 2004; Khwanchuea et al., 2008), significantly decreased the

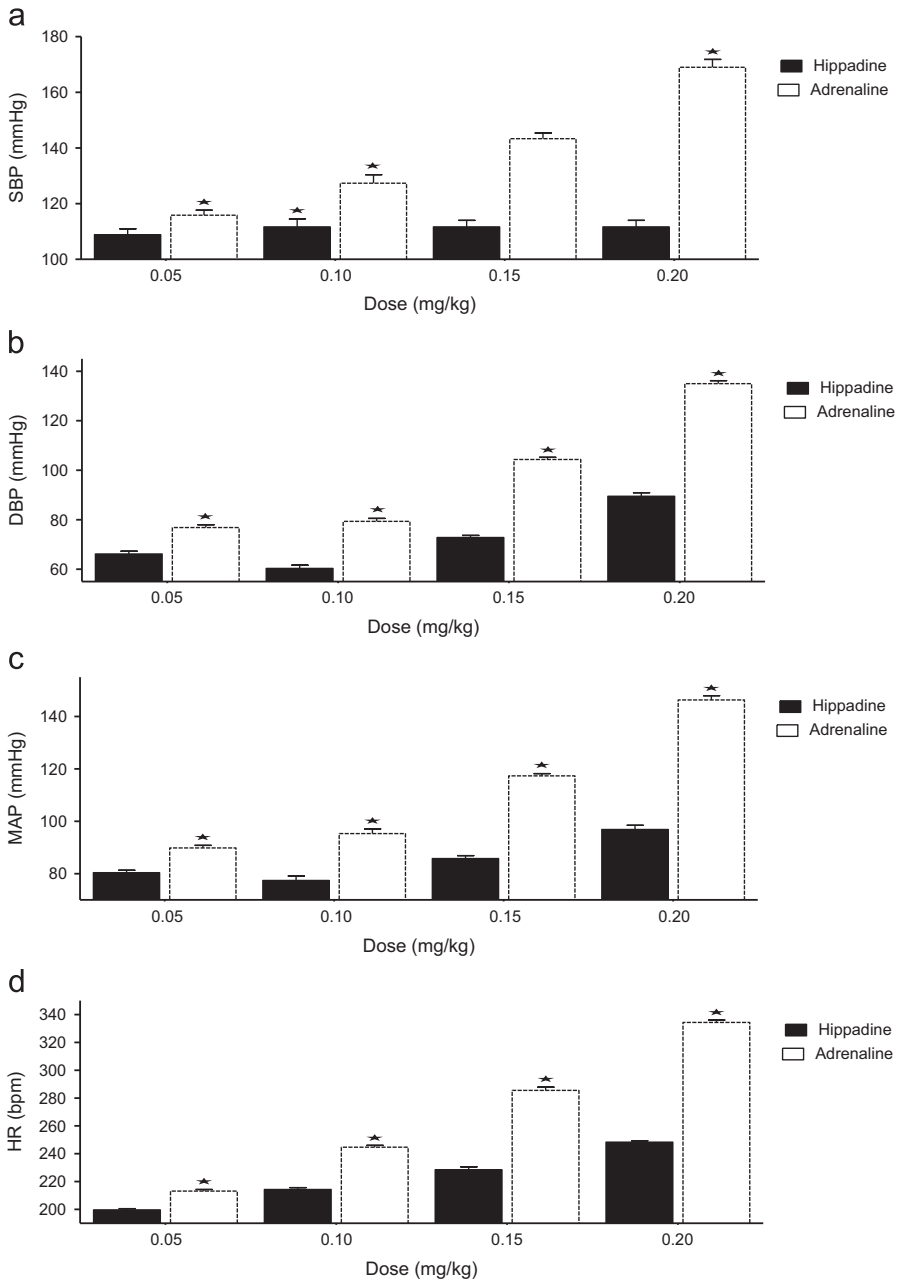

Fig. 7. Effect of adrenaline (0.05–0.20 mg/kg) on SBP (a), DBP (b), MAP (c) and HR (d) in rats pre-treated with hippadine (10 mg/kg). Values are presented as mean ± SEM. * indicates statistical significance.

SBP, MAP and DBP, while increasing HR in a dose-dependent fashion (Fig. 3). Prazosin relaxes smooth muscles in blood vessels by blocking postsynaptic alpha-1 adrenoceptors. The tachycardia observed is a reflex response to the decrease in BP (Grassi et al., 2012; Iriki and Simon, 2012; Lohmeier and Iliescu, 2012; Tsyrlin, 2013; Wehrwein and Joyner, 2013).

4.2. Effect of hippadine on the blood pressure and heart rate

Hippadine, the unknown drug, significantly decreased the SBP, MAP, DBP and HR in a dose-dependent fashion (Fig. 4). These effects are concordant with previous findings of Mugabo et al. (2012) who reported that hippadine produced significant and dose-dependent decrease in HR, coronary flow, cardiac output and aortic output in isolated perfused working rat heart.

4.3. Do adrenoceptors mediate hippadine effects on the blood pressure and heart rate?

Adrenaline reversed the negative inotropic and negative chronotropic effects due to atenolol (Fig. 5). Adrenaline also reversed the hypotension produced by prazosin (Fig. 6). In rats pre-treated with hippadine, adrenaline significantly reversed the negative inotropic and chronotropic effects of hippadine (Fig. 7). The similarities in the BP and HR effects of adrenaline in rats after pretreatment with hippadine (Fig. 7), with those obtained with

pretreatment with atenolol (Fig. 5) or prazosin (Fig. 6), suggest that the decrease in SBP, DBP, MAP and HR exhibited by hippadine may be due to a competitive inhibition of the sympathetic effect on α_1 and β_1 adrenoceptors.

5. Limitations of the study

Possible pharmacological effects of hippadine on the BP via other mechanisms such as the stimulation of presynaptic α_2 adrenoceptors, inhibitions of the calcium channels, the cholinoceptors, the angiotensin converting enzyme and the angiotensin II receptors also need to be investigated.

6. Conclusions

Hippadine decreases the BP and HR in SHR. These actions may be secondary to an inhibition of the postsynaptic α_1 adrenoceptors in blood vessels and/or an inhibition of the postsynaptic β_1 adrenoceptors in the heart.

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