Br. J. Pharmac. (1986), **89**, 389–394

The effects of noradrenaline, B-HT 920, methoxamine, angiotensin II and vasopressin on mean circulatory filling pressure in conscious rats

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1 The effects of vasoactive substances on mean circulatory filling pressure (MCFP), an index of total body venous tone, were determined in conscious rats.

2 Cumulative doses of saline (0.9% w/v NaCl solution), methoxamine (α_1-adrenoceptor agonist), B-HT 920 (α_2-adrenoceptor agonist) noradrenaline and vasopressin, and individual doses of angiotensin II (AII), were infused into the rats. Mean arterial pressure (MAP), MCFP and heart rate (HR) were determined before and during the plateau responses to infusions of the vasoactive substances.

3 The infusions of all the agonists caused a dose-dependent increase in MAP and a decrease in HR. The infusion of saline affected neither MAP nor HR.

4 The infusions of saline and methoxamine did not affect MCFP while the infusions of B-HT 920, noradrenaline and AII increased MCFP. MCFP was slightly increased during the infusion of high doses of vasopressin.

5 It was concluded that receptors for the α_2-adrenoceptor agonist and AII are involved in the control of venous tone. Receptors for the α_1-adrenoceptor agonist and vasopressin are not important for the control of venous tone.

Introduction

It has been shown that α_2-adrenoceptors are present in peripheral postjunctional sites, and various agonists and antagonists exert similar selectivities and activities on postjunctional α_2-adrenoceptors as on prejunctional α_2-adrenoceptors (Kobinger & Pichler, 1980; Docherty & Hyland, 1985). The postjunctional α_2-adrenoceptors have been shown to be involved in the mediation of vasoconstriction (Flavahan & McGrath, 1980; Timmermans & van Zwieten, 1980; Kobinger & Pichler, 1982; Elliot & Reid, 1983; van Meel *et al.*, 1983) and venoconstriction (Shoji *et al.*, 1983; Steen *et al.*, 1984) responses following sympathetic nerve stimulation or noradrenaline infusion. Recent studies have shown that postjunctional α_1-adrenoceptors predominate in human mesenteric arteries while postjunctional α_2-adrenoceptors predominate in human mesenteric veins (Törnebrandt *et al.*, 1985). This study investigates the effects of stimulation of either α_1-, α_2- or both α_1- and α_2-adrenoceptors by methoxamine (α_1-agonist), B-HT 920 (α_2-agonist) and noradrenaline, respectively, on mean circulatory filling pressure (MCFP). MCFP has been shown to be an index of total body venous tone (Grodins, 1959). Since vasopressin and angiotensin II (AII) have been shown to be involved in the maintenance of arterial pressure under various physiological and pathophysiological conditions (e.g., Share, 1976; Keeton & Campbell, 1981; Burnier *et al.*, 1983; Pang, 1983; Share & Crofton, 1984), we also investigated the effects of these vasoactive peptides on MCFP. MCFP is the pressure that would occur throughout the circulation if one could instantaneously bring all pressures in the circulation to an equilibrium. It has been shown that venous return is proportional to MCFP (Guyton, 1955). In addition, the difference between MCFP and right atrial pressure has been shown experimentally to be proportional to cardiac output (Guyton, 1955; Guyton *et al.*, 1973).

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Methods

Surgical preparations

MCFP was determined by the method of Yamamoto *et al.* (1980). A normal saline-filled balloon tipped catheter was inserted into the right atrium through the right external jugular vein of halothane anaesthetized Sprague-Dawley rats (350–450 g). The proper location of the balloon was tested by inflation of the balloon to stop the circulation completely. This was shown by a simultaneous increase in venous pressure and a decrease in mean arterial pressure (MAP) to less than 25 mmHg. Cannulae were also inserted into the iliac artery for the measurement of arterial pressure, into the femoral vein for the infusion of drugs, and into the inferior vena cava via the femoral vein for the measurement of central venous pressure by a pressure transducer (P23DB, Gould Statham, CA, U.S.A.). All cannulae were filled with heparinized normal saline ($25\ u\ ml^{-1}$) and tunnelled subcutaneously to the back of the neck, exteriorized and secured. The rats were allowed to recover for 2 h before measurements of pressures were made.

Effects of methoxamine, B-HT 920, noradrenaline, vasopressin and angiotensin II on mean circulatory filling pressure

MCFP was determined in conscious rats. This was accomplished by stopping the circulation of the rats by the injection of a small volume of fluid into the balloon that was previously inserted into the right atrium. Within 5 s following the inflation of the balloon, MAP decreased and central venous pressure increased simultaneously to a plateau. Central venous pressure measured within 5 s following the cessation of circulation was referred to as venous plateau pressure (VPP). MAP, heart rate (HR) and VPP were measured in rats ($n = 8$ for each drug, $n = 6$ for saline (0.9% NaCl)) before, and after, a 10 min infusion of various doses of saline (7 to $26 \times 10^{-3}\ ml\ min^{-1}$), methoxamine ($1.6 \times 10^{-10}$ to $4.8 \times 10^{-9}\ mol\ kg^{-1}\ min^{-1}$), B-HT 920 ($3.5 \times 10^{-9}$ to $11.2 \times 10^{-8}\ mol\ kg^{-1}\ min^{-1}$), noradrenaline ($3.0 \times 10^{-10}$ to $8 \times 10^{-9}\ mol\ kg^{-1}\ min^{-1}$), AII ($9.7 \times 10^{-11}$ to $2.8 \times 10^{-9}\ mol\ kg^{-1}\ min^{-1}$) or vasopressin ($4.5 \times 10^{-11}$ to $1.4 \times 10^{-9}\ mol\ kg^{-1}\ min^{-1}$). Dose-response curves were obtained for all agonists. The rats subjected to noradrenaline infusion were first pretreated with propranolol ($8 \times 10^{-7}\ mol\ kg^{-1}$ i.v. bolus injection followed by $3.4 \times 10^{-9}\ mol\ kg^{-1}\ min^{-1}$ continuous i.v. infusion) to prevent the stimulation of β-adrenoceptors by noradrenaline. In the determination of dose-response curves for AII, each dose of AII was infused for 5 min followed by a recovery period of 12 min to avoid the development of tachyphylaxis. The maximum volume of fluid infused during the 2 h infusion period varied between 0.9 ml for saline and noradrenaline groups, 0.3 ml for the AII group and 0.6 ml for the rest of the groups.

Calculations

MCFP was calculated using the equation of Samar & Coleman (1978) and a value of 1/60 for arterial-to-venous compliance ratio (Yamamoto *et al.*, 1980).

$$MCFP = VPP + \frac{1}{60}(FAP - VPP)$$

FAP represents the final arterial pressure (mmHg) obtained within 5 s following circulatory arrest.

Statistical analysis

All data were analysed by use of analysis of variance with repeated measures. For multiple comparisons of data, Duncan's multiple range test was used to compare group means. In all cases, a probability of error of less than 0.05 was preselected as the criterion for statistical significance.

Drugs

All drugs were made up fresh daily and dissolved in normal saline. The following drugs were used: B-HT 920 HCl (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-d]azepin-dihydrochloride; Boehringer Ingelheim Canada Ltd., Ontario), methoxamine HCl (Burroughs Wellcome, London), noradrenaline bitartrate (Sigma Chemical Co. MO, U.S.A.), vasopressin (Calbiochem, CA, U.S.A.), AII (CIBA-Geigy, Ontario, Canada) and propranolol HCl (Sigma Chemical Co. MO, U.S.A.).

Results

Table 1 shows the control values of MAP before the infusions of saline and the various vasoconstrictor agents. There were no differences in the control values of MAP, MCFP and HR between any of the groups prior to the infusion of drugs or saline. The infusion of saline did not alter MAP (Figure 1). The infusions of B-HT 920, methoxamine, noradrenaline, vasopressin and AII all caused dose-dependent increases in MAP (Figure 2) compared to the corresponding MAP obtained before the drug infusion (Table 1). Vasopressin, methoxamine and B-HT 920 caused significant increases of MAP at doses equal to and higher than the third infused dose. Noradrenaline and AII caused significant increases of MAP at doses equal to and

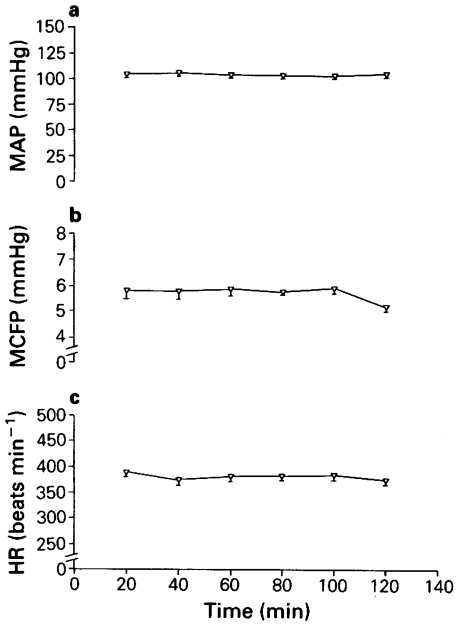

Figure 1 (a) Mean arterial pressure (MAP), (b) mean circulatory filling pressure (MCFP) and (c) heart rate (HR) during the infusion of saline at different rates ($7-26 \times 10^{-3}$ ml min^{-1} per rat) over a 120 min period. Each point represents the mean ($n = 6$) and vertical lines show s.e.

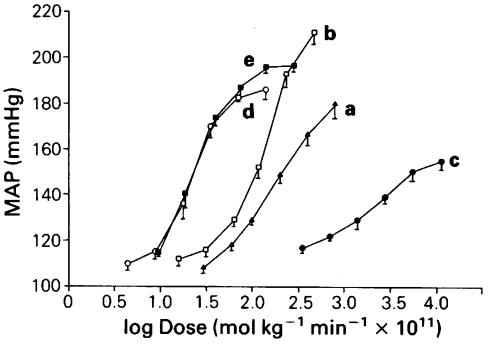

Figure 2 Dose-response curves for the effects of (a) noradrenaline, (b) methoxamine, (c) B-HT 920, (d) vasopressin and (e) angiotensin II (AII) on mean arterial pressure (MAP). The rats subjected to noradrenaline infusion were first pretreated with propranolol to prevent the stimulation of β-adrenoceptors by noradrenaline. In the determination of a dose-response curve for AII, each dose of AII was infused for 5 min followed by a recovery period of 12 min to avoid the development of tachyphylaxis. Maximum responses to AII were recorded. Each point represents the mean ($n = 8$ for each group) and vertical lines show s.e.

Table 1 Control values of mean arterial pressure (MAP), mean circulatory filling pressure (MCFP) and heart rate (HR)

	Methoxamine	*B-HT 920*	*Vasopressin*	*Angiotensin II*	*Noradrenaline*	*Saline*
MAP	115 ± 3	116 ± 2	108 ± 2	111 ± 3	106 ± 2	111 ± 4
MCFP	5.9 ± 0.1	5.8 ± 0.1	6.2 ± 0.2	6.0 ± 0.02	5.7 ± 0.2	6.0 ± 0.02
HR	413 ± 9	386 ± 14	404 ± 7	402 ± 10	380 ± 15	377 ± 8

All values shown are mean ± s.e. $n = 8$ in each group except for saline where $n = 6$. Values of MAP (mmHg), MCFP (mmHg) for HR (beats min^{-1}) were obtained prior to the administration of drugs or normal saline and are given as mean ± s.e.

higher than the second infused dose.

HR was not changed during the infusion of saline (Figure 1). Infusions of all the vasoactive agents produced decreases in HR (Figure 3) compared to control HR obtained before the infusions of these drugs (Table 1).

MCFP readings were not changed during the infusion of saline (Figure 1). Values for MCFP obtained during the infusion of various doses of methoxamine (Figure 4) were not significantly different from the control MCFP value prior to drug infusion (Table 1). MCFP was slightly, but significantly increased during an infusion of the three highest doses of vasopressin. B-HT 920 caused a significant increase of MCFP at doses equal to, and higher than the second infused dose, while noradrenaline and AII significantly increased MCFP at doses equal to, and higher than the third infused dose (Figure 4).

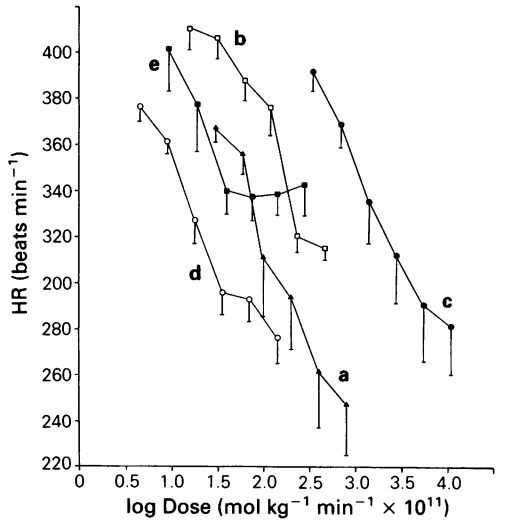

Figure 3 Dose-response curves for the effects of (a) noradrenaline, (b) methoxamine, (c) B-HT 920, (d) vasopressin and (e) angiotensin II (AII) on heart rate (HR). The rats subjected to noradrenaline infusion were first pretreated with propranolol to prevent the stimulation of β-adrenoceptors by noradrenaline. In the determination of a dose-response curve for AII, each dose of AII was infused for 5 min followed by a recovery period of 12 min to avoid the development of tachyphylaxis to the drug. The HR obtained at the time of maximum pressor response to AII is given. Each point represents the mean ($n = 8$ for each group) and vertical lines show s.e.

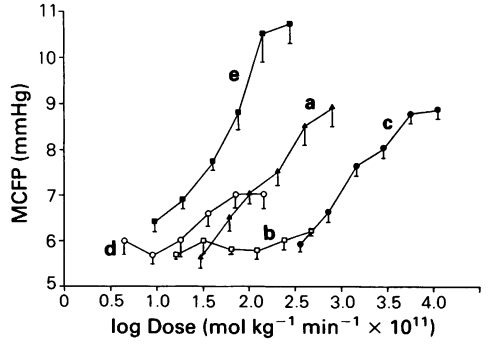

Figure 4 Dose-response curves for the effects of (a) noradrenaline, (b) methoxamine, (c) B-HT 920, (d) vasopressin and (e) angiotensin II (AII) on mean circulatory filling pressure (MCFP). The rats subjected to noradrenaline infusion were first pretreated with propranolol to prevent the stimulation of β-adrenoceptors by noradrenaline. In the determination of a dose-response curve for AII, each dose of AII was infused for 5 min followed by a recovery period of 12 min to avoid the development of tachyphylaxis to the drug. MCFP obtained at the time of maximum pressor response to AII was recorded. Each point represents the mean ($n = 8$ for each group) and vertical lines show s.e.

Discussion

It is well recognized that cardiac output is controlled by cardiac as well as vascular factors (Guyton *et al.*, 1973; Sagawa, 1973; Levy, 1979; Greenway, 1982). These factors include heart rate, cardiac contractility, blood volume, vascular compliances and vascular resistances (Greenway, 1982). In 1959, Grodins formulated mathematical equations to describe cardiac and vascular factors which influence the systemic circulation. Mathematically, it can be shown that when the circulation is stopped, an equilibrium pressure is obtained throughout the circulation (Grodins, 1959). This equilibrium pressure is proportional to total blood volume and inversely proportional to the overall compliance of the systemic circulation. Guyton (1955) called the equilibrium pressure whereby no circulation occurred 'mean circulatory filling pressure' and he measured this pressure in dogs by suddenly stopping the heart and quickly pumping blood from the arterial to the venous side until an equilibrium pressure was obtained. MCFP is independent of cardiac contractility, vascular resistance and HR since it is measured at a time when the circulation is stopped. Since venous compliance is many times greater than arterial compliance (Guyton *et al.*, 1973; Samar & Coleman, 1978; Yamamoto *et al.*, 1980), MCFP predominantly reflects venous tone and it is inversely dependent on venous compliance. An increase in MCFP represents an increase in total body venous tone. Samar & Coleman (1978) developed a method for measuring MCFP in rats by implanting an externally operated hydraulic occluder around the pulmonary artery which could be used to stop briefly the systemic circulation. Yamamoto *et al.* (1980), modified the method by the insertion of an inflatable balloon into the right atrium of rats. Yamamoto's method eliminated the requirement of open-chest surgery and prolonged recovery of the rats.

Using the method of Yamamoto *et al.* (1980), experiments were conducted to determine the influences of various vasoactive agents on the control of MCFP in rats. Since this methodology involves the cessation of circulation and the inflation of a balloon in the right atrium, it is expected that physiological mechanisms will be altered. For example, there may be activation of the sympathetic nervous system, release of atrial natriuretic peptides, etc. To avoid interferences from various cardiovascular reflex mechanisms, measurements of pressures were made within 5 s following the inflation of a balloon, before the appearance of secondary changes of venous pressure (Samar & Coleman, 1978). We were able to obtain, in conscious rats, reproducible readings of MCFP that were similar to those reported from other laboratories (Samar & Coleman, 1978; Yamamoto *et al.*, 1980). We have found that the infusion of methoxamine, a

specific α_1-agonist, increased MAP but did not change MCFP. The infusions of B-HT 920 (α_2-agonist) and noradrenaline increased MAP as well as MCFP. Therefore, our results show that α_2- are more important than α_1-adrenoceptors in the control of venous tone in the rat.

We have previously determined the effects of α-adrenoceptor antagonists on cardiac output and its distribution by the microsphere technique in anaesthetized Sprague-Dawley rats. Injections of rauwolscine and phentolamine, respectively α_2-selective and non-selective α-blockers, were found to cause a reduction of cardiac output (Tabrizchi & Pang, 1985). In contrast, the administration of prazosin, a specific α_1-blocker, did not change cardiac output. It is well known that veins play an important role in the control of cardiac output (Greenway, 1982). Under steady-state conditions, venous return is equal to cardiac output since the cardiovascular system is a closed circuit (Levy, 1979; Greenway, 1982). Therefore, the results from the microsphere studies are consistent with results from this study and together they suggest that α_2-adrenoceptors are more important than α_1-adrenoceptors in the control of venous return and cardiac output in rats.

It has been shown that intravenous administration of phenylephrine increased MCFP in conscious and anaesthetized dogs (Hirakawa *et al.*, 1984; Appleton *et al.*, 1985). The intravenous injection of noradrenaline was shown to increase MCFP in conscious rats (Yamamoto *et al.*, 1980). Using a thermodilution technique, Kalkman *et al.* (1984) have shown that injections of B-HT 920 and methoxamine in pithed rats cause an increase in cardiac output. This is in contrast to our results which show that post-junctional α_2- but not α_1-adrenoceptors are involved in the control of venous tone and cardiac output in rats. Since pithed Wistar rats were used in the study conducted by Kalkman and his coworkers while conscious Sprague-Dawley rats were used in ours, it is possible that the discrepancy was due to the use of a different strain of rats or different experimental preparations. Kalkman's study was conducted in hexobarbitone-anaesthetized pithed rats. We used halothane-anaesthetized rats in a previous study and conscious rats in this one. Pithed rats have high serum levels of potassium ion (Curtis *et al.*, 1985), very low blood pressures due to the absence of sympathetic nervous activity, low cardiac output and supranormal responsiveness to catecholamines (Curtis & Walker, personal communication).

It has been shown that a continuous infusion of AII caused an increase in MAP (by about 20–30 mmHg) and an increase in MCFP (2–3 mmHg) of anaesthetized dogs (Hirakawa *et al.*, 1984). In our study, the infusion of AII caused a dose-dependent increase in MAP as well as MCFP. On the other hand, the infusion of high doses of vasopressin caused a marked increase of MAP but only a very small increase in MCFP. Our results show that receptors for AII but not vasopressin are important in the control of venous pressure.

In summary, the stimulation of α_1- and α_2-adrenoceptors by methoxamine and B-HT 920, respectively, caused an increase in MAP. This indicates that both α_1- and α_2-adrenoceptors are present for the control of arterial resistance. The infusion of B-HT 920 but not methoxamine was found to increase MCFP. These results show that in the conscious rat, α_2-adrenoceptors are responsible for the control of venous tone and cardiac output. In addition, it was found that an infusion of AII caused a dose-dependent increase in MAP and MCFP suggesting that AII may also be important in the control of venous tone.

This work was supported by the British Columbia Heart Foundation. Reza Tabrizchi was a recipient of a British Columbia Heart Foundation traineeship. The authors would like to thank Miss Janelle Harris for secretarial assistance and Mr Glenn A. Collins for statistical analysis. We would also like to thank CIBA-Geigy Canada Ltd for the supply of AII and Boehringer Ingelheim Canada Ltd for the supply of B-HT 920.

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(Received April 7, 1986.
Revised June 17, 1986.
Accepted June 23, 1986.)