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European Journal of Pharmacology 586 (2008) 266–274
www.elsevier.com/locate/ejphar

Haemodynamic effects of vasoactive agents following chronic state of high cardiac output in anaesthetized rats

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Received 3 October 2007; received in revised form 29 January 2008; accepted 14 February 2008
Available online 26 February 2008

Abstract

The arteriovenous fistula model of circulation can produce a high output and low peripheral resistance situation. Here, we have examined the effects of noradrenaline, vasopressin and sodium nitroprusside on cardiac index, mean arterial blood pressure, venous tone, resistance to venous return, arterial resistance, and blood volume in chronically shunted anaesthetized rats. The cardiac index of rats with chronic arteriovenous fistula (AVF) was significantly higher (36.65±2.28 ml/min per 100 g; (mean±S.E.M.; $n=24$) in comparison to sham-operated rats (20.04±0.86 ml/min per 100 g; mean±S.E.M.; $n=8$). Cardiac index did not significantly change during the infusion of noradrenaline (1.0, 3.0 and 10 μg/kg per min), vasopressin (10, 30, 100 ng/kg per min) or sodium nitroprusside (0.1, 0.3 and 1.0 μg/kg per min) compared to saline infusion in AVF animals. Infusion of noradrenaline significantly increased heart rate, dP/dt, mean circulatory filling pressure (P_{mcf}) and resistance to venous return without affecting mean arterial blood pressure when compared to saline infusion. Administration of vasopressin significantly increased dP/dt, mean arterial blood pressure, and P_{mcf} without affecting heart rate, resistance to venous return or arterial resistance compared to saline infusion. Infusion of sodium nitroprusside did not significantly affect any haemodynamic parameter measured when compared to saline infusion. The results indicate that the presence of chronic AVF alters responsiveness of the various segments of the circulatory system to vasoactive agents. Moreover, it produces a major impediment to overall changes that can normally be induced following the infusion of such agents.

Keywords: Resistance to venous return; Mean circulatory filling pressure; Arterial resistance; Vasoconstriction; Vasodilation; Blood volume

1. Introduction

Disruption of the circulatory system can occur in a number of pathophysiological states. While hyperdynamic circulation is commonly associated with sepsis (Hollenberg et al., 2004), high cardiac output and a low systemic resistive state also occurs in many other conditions such as in ductus arteriosus (Linder et al., 1990; Evans and Iyer, 1994), hyperthyroidism (Diekman et al. 2001; Faber et al., 2001; Palmieri et al., 2004), cirrhotic edema (Norris et al., 1987), anemia (Anand et al., 1995), multiple small arteriovenous shunts as observed in Paget's bone disease (Arnalich et al., 1984; Morales-Piga et al., 2000), as well as in patients with thermal injury (Holm et al., 2000), and individuals undergoing haemodialysis (Meeus et al., 2000).

Interestingly, vascular steal has been suggested as a cause of very low vascular resistive state and high cardiac output in clinical situations where circulation becomes hyperdynamic (Ince and Sinaasppel, 1999; Buwalda and Ince, 2002). It is well recognized that control of blood pressure and cardiac output is an important factor during conditions such as septic shock. Moreover, shunting in microvasculature can have a detrimental effect on haemodynamics with suboptimal perfusion of organs causing reduced oxygen delivery to tissues (Ince and Sinaasppel, 1999; Spronk et al., 2004).

Evidently, vasopressor agents such as noradrenaline, dopamine and vasopressin have been used in an attempt to increase and maintain an adequate blood pressure level and stabilize haemodynamics in a situation of high cardiac output and low systemic vascular resistive state (Hollenberg et al., 2004; Dellinger et al., 2004). However, the view that shunting may

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doi:10.1016/j.ejphar.2008.02.045

lead to a reduction in the perfusion of blood to tissues has prompted the hypothesis that vasodilation rather than vasoconstriction may be a more appropriate approach in the treatment of such conditions as noted by low resistive and high output state (Buwalda and Ince 2002). Of interest is the suggestion that in vasodilatory shock, there is a lack of benefit in increasing mean arterial blood pressure from 65 to 85 mmHg (Holmes, 2005).

In general, the impact of vasoconstrictors and vasodilators on global haemodynamics remains to be fully defined in a high cardiac output and low resistive state. The arteriovenous shunted model of the circulation can produce a high output and low peripheral resistance situation (Flaim et al., 1979, 1980; Liu et al., 1991). Cardiac output can become elevated by as much as 2 fold (Flaim et al., 1979; Huang et al., 1992). We have attempted to assess how the overall haemodynamics changes in a chronically shunted animal model in response to an acute administration of a number of vasoactive agents. To this end, we have examined the effects of noradrenaline, vasopressin and sodium nitroprusside on cardiac output, heart rate, dP/dt, blood pressure, venous tone, resistance to venous return, arterial resistance, and blood volume in chronically shunted anaesthetized rats.

2. Methods

Experiments conducted for this investigation conform to the Canadian Institute of Health Research Guidelines. The Protocols for these experiments was approved by the Memorial University of Newfoundland Animal Care Committee.

2.1. Preparation of animals with arteriovenous fistula

The method described by Garcia and Diebold (1990) was used for the introduction of a shunt between the abdominal aorta and inferior vena cava by inserting a 20-gauge needle from the abdominal aorta into the inferior vena cava. Briefly, 24 male Sprague–Dawley rats (230–250 g) were anaesthetized with halothane (induction with 7.5% halothane in 100% oxygen; maintenance with 1.5% halothane in 100% oxygen). Before surgery, the animals were heparinized (100 IU/kg) by an injection of heparin subcutaneously. A laparotomy was performed and both the vena cava and abdominal aorta were exposed. At positions caudal to the renal arteries and cephalic to the aortic bifurcation, two vascular clamps, Scharwtz micro serrefine 26 mm straight clamps (Fine Sciences Tools, Vancouver, BC, Canada) were placed on both the vena cava and abdominal aorta. Subsequently, the aorta was punctured with a 20 gauge disposable needle (Becton–Dickinson, Franklin Lakes, NJ, USA) at the place two thirds caudal to the renal artery and one third cephalic to the aortic bifurcation. Then the needle was forwarded into the aorta, perforating its adjacent wall and penetrating the vena cava. The needle was shaken mildly to make sure the hole was big enough for a patent fistula. Thereafter, the needle was fully withdrawn and the hole on the aorta was repaired with 6-0 Prolene vascular suture (Prolene, Johnson and Johnson, Somerville, NJ, USA). The vascular clamps were removed 30 s after completion of the anastomosis. The whole process of circulation cessation was less than 5 min. The patency of the fistula was verified visually by (a) swelling of the vena cava and (b) mixing of the bright arterial blood with dark venous blood. For the sham-operated rats, the needle was inserted into the abdominal aorta but not pushed into the inferior vena cava. Subsequently, the internal abdominal wall was closed with absorbable suture (Surgigut, Norwalk, CT, USA). The skin was closed by single stitches with 3-0 silk. A local anaesthetic, bupivacaine (1.0%), was topically applied to the skin and muscle following the completion of suturing. Immediately after the surgical procedure, each animal was treated with an analgesic, buprenorphine, (0.5 mg/kg s.c), and thereafter each animal received buprenorphine (0.5 mg/kg, s.c.) every 12 h for 48 h. The animals were allowed to recover for 5 weeks.

2.2. Surgical preparation

Under halothane anesthesia (induction with 7.5% halothane in 100% oxygen; maintenance with 1.5% halothane in 100% oxygen), catheters (polyethylene tubing; I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the left iliac artery and vein. The left venous tube was advanced into the inferior vena cava for the measurement of central venous pressure. The arterial line was for continuously recording the mean arterial pressure. Subsequently, sodium thiobarbital (up to 80–100 mg/kg) was injected slowly through the venous line with close monitoring of the arterial pressure as halothane was reduced and ceased. Thereafter, two catheters were put into the right iliac artery and vein for blood sample withdrawal and drug/vehicle administration respectively. In addition, a catheter was advanced into the left ventricle via the right carotid artery for measurement of left ventricular pressure and injection of radioactive-labeled microspheres. A saline-filled balloon-tipped catheter was advanced into the right atrium through the right external jugular vein (Pang and Tabrizchi, 1986). A laparotomy was performed and the abdominal aorta and inferior vena cava were exposed. A Transonic ultrasonic volume flow probe (3SB Series, Transonic System Ithaca, NY, USA) was placed on the aorta cephalic to the fistula. The blood flow in the aorta at this region was regarded as an apparent fistula flow. Finally, the abdominal cavity was closed. All catheters were filled with heparinized (25 IU/ml) normal saline (0.9% NaCl). The animals were tracheotomized and allowed to stabilize for 1 h when arterial pressure, central venous pressure, ventricular pressure, apparent fistula flow and heart rate were monitored continuously. Body temperature was maintained at 37±1 °C using a heating lamp and monitored with a rectal thermometer. Arterial blood pressure, ventricular pressure and P_{mcf} were recorded with a pressure transducer (Gould Statham, USA; Model PD23B). The pressure transducers were connected to an amplifier (DA 100A) that in turn was linked to a universal interface module (UIM 100) which then interfaced with an acquisition unit (MP 100). Apparent fistula flow was monitored using a flowmeter (Model T106, Transonic Systems, Ithaca, NY, USA) coupled to the universal interface module (UIM 100) (Tabrizchi and Pugsley, 2000). The data was collected using AcqKnowledge III system. Heart rate and dP/dt were calculated from the blood pressure and left ventricular pressure signal, respectively, with the aid of AcqKnowledge III system. Cardiac output was measured using the reference sample microsphere method, and P_{mcf} was

measured after circulation was transiently stopped by inflating the balloon in the right atrium. Final arterial pressure and venous plateau pressure were recorded at 5–7 s after the circulatory stop (Pang and Tabrizchi, 1986; Tabrizchi and Ford, 2003).

2.3. Measurement of cardiac output

This technique has been described in detail elsewhere (Pang, 1983). Briefly, suspensions of microspheres (Mandel, Ontario, Canada; 15 μm diameter) labelled with ^{57}Co (20,000–22,000 in 150 μl) were injected into the left ventricle over a period of 10 s. Blood was withdrawn from the right femoral artery at the rate of 0.35 ml/min starting 15 s before microsphere injection using an infusion/withdrawal pump (Kd Scientific, Holliston, MA, USA; Model 120) for 1 min. The blood sample and syringes used for injection of microspheres or withdrawal of blood were counted for radioactivity at 80–160 keV using a dual channel automatic gamma counter (Clinic Gamma Counter, LKB Wallac, Gaithersburg, MD, USA; Model 1272). The withdrawn blood sample was slowly injected back into the animals immediately after counting radioactivity.

2.4. Measurement of blood volume

Plasma volume (PLV) and total blood volume (TBV) were determined according to Migita et al. (1997) using Evans blue. A 100 μl blood sample was collected in two heparinized capillary tubes, before and after the administration of Evans blue dye (5 mg/kg). The samples were centrifuged for 3 min to obtain haematocrit values. A 50 μl sample of plasma was collected from haematocrit tubes and diluted 1:20 in normal saline. The absorbance was recorded using a 4050 UV/Visible spectrophotometer (LKB Biochrom, Cambridge, UK) at 620 nm (A_{620}) and was corrected first for the presence of haemoglobin at A_{620} (blank), and then for turbidity at A_{740}. PLV was determined by the equation $PLV=(C_i\times V_i)/C_P$; where C_i and V_i are the concentration and volume, respectively, of Evans blue dye that was injected and C_p is the plasma concentration of Evans blue dye. A plasma trapping factor (tp) of 0.96 and total body-to-venous haematocrit (Hct) ratio (F_{cells}) of 0.74 were employed (Migita et al., 1997) to estimate TBV using the equation, $TBV=PLV/1-(Hct\times tp\times F_{cells})$.

2.5. Experimental designs and protocols

The existence of AVF was noted by a higher flow in the abdominal aorta cephalic to the fistula and high cardiac output compared to sham-operated animals. Five weeks after the surgery shunted rats were randomly assigned to four groups ($n=5$–7), with the sham-operated group ($n=8$) as the control group. After surgical preparation, animals were stabilized for 1 h when arterial pressure, central venous pressure, ventricular pressure, fistula flow index and heart rate were monitored continuously. Five blood samples (100 μl into heparinized tubes from femoral artery) were taken for blood volume measurements. Haematocrit (for blood volume), cardiac output and P_{mcf} measurements were made at baseline (control) prior to the administration of saline or vasoactive agents, and thereafter during the infusion of each dose of vasoactive agent or infusion rate of saline. Shunted rats in each group were infused with one of the following solutions: saline (0.9% NaCl; 0.002, 0.006 and 0.02 ml/kg per min), noradrenaline (1, 3, 10 μg/kg per min), vasopressin (10, 30, 100 ng/kg per min), or sodium nitroprusside (100, 300, 1000 ng/kg per min), respectively. Over the course of the experiment five measurements of blood volume, cardiac output and P_{mcf} were made at first prior to the administration of saline or vasoactive agents and subsequently during (at 18–20 min) infusion of the three doses of saline or vasoactive agents. The sham-operated animals were infused with saline (0.002, 0.006 and 0.02 ml/kg per min) with protocol being parallel to shunted animals.

After completion of each experiment, heart and lungs were excised and removed. Right ventricle and left ventricle plus septum were separated. Fat tissue and fascia were removed carefully. The weights of the organs were recorded. The lungs were put into the oven (50 °C) for 6 h and the dry weight was also recorded.

2.6. Chemicals

All drugs were made up fresh daily and dissolved in normal saline (0.9% NaCl). Noradrenaline, vasopressin, sodium

Table 1
Cardiac index (CI; ml/min per 100 g), heart rate (HR; beat/min), contractility (dP/dt; mmHg/s), mean arterial blood pressure (MAP; mmHg), mean circulatory filling pressure ($P_{mcf;}$ mmHg), apparent arterial resistance (AR_{AP}; mmHg × min per ml), resistance to venous return (RVR; mmHg × min per ml), apparent fistula flow index (AFFI; ml/min per 100 g) at baseline control prior to the infusion of saline and at different infusion rates of saline (ml/kg per min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula ($n=6$) (5 weeks post shunt) or sham-operated ($n=8$)

		CI	HR	dP/dt	MAP	P_{mcf}	AR	RVR	AFFI
Fistula	Control	36.65±2.28[a]	339±11	6277±362	97.2±5.4	7.04±0.50[a]	0.58±0.04[a]	0.027±0.002[a]	24.97±3.44[a]
	0.002	36.96±3.33[a]	342±16	6169±483	96.0±6.2	6.88±0.49[a]	0.57±0.08[a]	0.027±0.003[a]	25.28±3.38[a]
	0.006	36.11±2.46[a]	345±9	6117±414	94.2±6.0	6.66±0.36[a]	0.55±0.05[a]	0.026±0.001[a]	23.85±3.24[a]
	0.02	36.81±3.58[a]	346±12	6099±348	94.8±5.7	7.09±0.38[a]	0.57±0.08[a]	0.028±0.002[a]	22.89±3.55[a]
Sham	Control	20.04±0.86	333±11	6373±351	104.9±50	5.05±0.14	1.16±0.06	0.042±0.003	2.03±0.40
	0.002	21.32±1.05	318±21	6445±510	105.9±7.9	5.09±0.21	1.12±0.12	0.040±0.003	1.87±0.37
	0.006	20.09±0.75	318±16	6556±507	110.0±8.6	4.96±0.18	1.64±0.08	0.041±0.002	1.85±0.33
	0.02	20.21±0.95	309±14	6282±430	109.2±8.7	4.92±0.18	1.21±0.12	0.041±0.002	1.87±0.32

Each value represents mean±S.E.M.
[a] Significantly different from respective values in sham-operated group; $P<0.05$.

nitroprusside and thiobutabarbital were purchased from Sigma Chemical Company (St Louis, MO, U.S.A.).

2.7. Calculations and statistical analysis

Cardiac output (ml/min) was calculated as the rate of withdrawal of blood multiplied by the total injected cpm divided by cpm in withdrawn blood. Resistance to venous return (mmHg × min per ml) was calculated as the difference of P_{mcf} and central venous pressure divided by cardiac output, and arterial resistance (mmHg × min per ml) was obtained by dividing mean arterial blood pressure by cardiac output (Tabrizchi, 1998). Cardiac index is cardiac output per 100 g body weight.

A correction was applied in calculating plasma volume and total blood volume over time using Evans blue dye. Evans blue dye is known to be eliminated from systemic circulation over time (Tabrizchi and Ford, 2004). In two groups of experiments using sham-operated rats and fistula rats, we determined the elimination rate constant (k) for Evans blue. Thiobutabarbital anaesthetized rats were injected with Evans blue dye (5 mg/kg) and plasma concentration of the dye was determined over a course of 2 h (at 2, 60, 90, 120 min). A plot of Log [Evans blue dye] in plasma against time indicated first order elimination kinetics ($C = C_o e^{-kt}$), and the information from the plot was employed to calculate k and half life ($t_{1/2}$) of Evans blue in plasma in rats. Assuming a two-compartment model, the calculated k for Evans blue dye were 0.3429±0.0168 and 0.192±0.0135 (mean±S.E.M.) for α and β respectively in sham-operated rats and 0.2912±0.0209 and 0.1791±0.0132 (mean±S.E.M.) for α and β respectively in fistula rats. The mentioned k values were used to correct for concentration of the dye in plasma over time in our study, and thus in calculating plasma volume and total blood volume.

All data are presented as mean±standard error mean. Analysis of variance with repeated measures was used for comparison between haemodynamic values. Newman–Keuls multiple range test was used for comparison between means. In all cases, a probability of error less than 0.05 was selected as the criterion for statistical significance.

Table 2
Baseline values of cardiac index (CI; ml/min per 100 g), heart rate (HR; beat/min), contractility (dP/dt; mmHg/s), mean arterial blood pressure (BP; mmHg), mean circulatory filling pressure ($P_{mcf;}$ mmHg), systemic arterial resistance (AR_S; mmHg × min per ml), resistance to venous return (RVR; mmHg × min per ml), apparent arterial resistance (AR_{AP}; mmHg × min per ml) apparent fistula flow index (AFFI; ml/min per 100 g), and apparent resistance of fistula ($R_{fistula}$; mmHg × min per ml) before administration of vasoactive agent and saline in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt)

Groups	Noradrenaline (n=7)	Vasopressin (n=5)	Na nitroprusside (n=6)	Saline (n=6)
CI	38.00±2.37	41.60±3.32	39.4±1.90	36.65±2.28
HR	340±11	292±5	332±12	339±11
dP/dt	6407±315	6443±252	6651±302	6277±362
MAP	101±6	97±3	102±4	97±5.4
P_{mcf}	6.28±0.36	6.64±0.45	6.72±0.35	7.04±0.50
AR_S	1.20±.09	1.48±0.23	1.10±0.74	1.42±0.11
RVR	0.025±0.002	0.037±0.004	0.023±0.003	0.027±0.002
AFFI	21.74±2.13	21.48±3.00	23.13±2.80	24.97±13.9
AR_{AP}	0.58±0.07	0.66±0.07	0.50±0.04	0.58±0.04
$R_{fistula}$	1.06±0.2	1.08±0.2	0.93±0.1	0.84±0.1

Each value represents mean±S.E.M.

3. Results

The cardiac index of rats with chronic AVF (36.65±2.28 ml/min per 100 g; mean±S.E.M.; n=24) in comparison to sham-operated rats (20.04±0.86 ml/min per 100 g; mean±S.E.M.; n=8) was significantly higher (+82.9%) while no differences were noted in heart rate, dP/dt or mean arterial blood pressure between the two groups (Table 1). P_{mcf} was significantly higher in the AVF group than in the sham-operated group while arterial resistance and resistance to venous return were significantly lower in rats with AVF compared to sham-operated rats (Table 1). Blood flow in abdominal aorta just below the renal artery was significantly higher in rats with chronic AVF when compared to sham-operated rats (Table 1).

3.1. Effects of noradrenaline on haemodynamics in AVF rats

Baseline haemodynamic values in various groups of animals are shown in Table 2. Cardiac index did not significantly change during the infusion of noradrenaline (1, 3 and 10 μg/kg per min) in rats with chronic AVF compared to baseline level or the respective values in saline treated rats (Fig. 1A). Infusion of noradrenaline significantly increased heart rate at the two higher doses when compared to the baseline or respective values in saline-treated rats (Fig. 1B), while there were significant increases in dP/dt at the lowest and highest doses of noradrenaline infusion compared to the baseline and respective values in saline treated group (Fig. 1C). Mean arterial blood pressure significantly increased by all three doses compared to the baseline level and respective values in saline treated rats (Fig. 2A). Administration of noradrenaline also significantly increased P_{mcf} compared to the baseline level at all the three doses (Fig. 2B). Moreover, administration of noradrenaline at the last two doses significantly increased P_{mcf} when compared to the respective values in saline treated group (Fig. 2B). Noradrenaline infusion significantly increased arterial resistance compared to the baseline at the lowest and highest doses as well as elevated the resistance to venous return at all three doses (Fig. 3A). Infusion of noradrenaline also significantly increased resistance to venous return when compared to respective values in the saline infused group at all the doses (Fig. 3B). While administration of noradrenaline significantly increased apparent fistula flow at all the three dose levels compared to the baseline or saline-treated groups, it did not change resistance of the fistula when compared to either the baseline level or respective values in saline treated rats (Fig. 4AB).

3.2. Effects of vasopressin on haemodynamics in AVF rats

Infusion with vasopressin (10, 30 and 100 ng/kg per min) had no significant effect on the cardiac index or heart rate (Fig. 1AB). However, administration of vasopressin at the highest dose significantly increased dP/dt when compared to the respective value in the saline treated group (Fig. 1C). Infusion

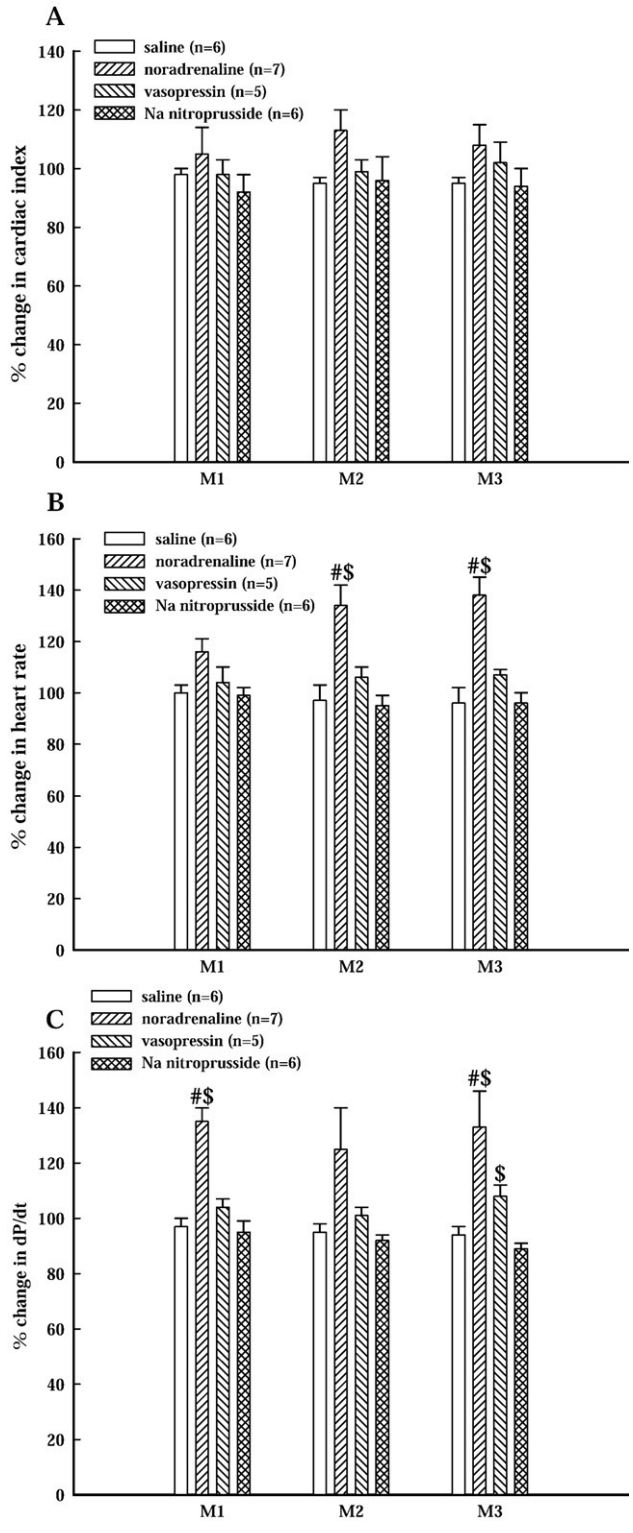

Fig. 1. Percent change from baseline control in cardiac index (A), heart rate (B) and contractility (dP/dt) (C) during administration of vasopressin (18–20 min) (10, 30 and 100 ng/kg per min), sodium nitroprusside (100, 300 and 1000 ng/kg per min), noradrenaline (1, 3 and 10 μg/kg per min), and saline (0.002, 0.006 and 0.02 ml/kg per min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt). M1, dose 1; M2, dose 2; M3, dose 3. Data represents mean value±S.E.M. #Significantly different from the baseline control level; $P<0.05$. $Significantly different from the respective value in saline treated group; $P<0.05$.

of vasopressin at the highest two doses significantly increased mean arterial blood pressure when compared to the respective values in the saline treated group (Fig. 2A). Moreover, AVP infusion at the highest dose significantly increased mean arterial blood pressure when compared to the baseline value (Fig. 2A). The highest dose of vasopressin significantly increased P_{mcf} compared to the baseline value and the respective values in saline treated group (Fig. 2B). Vasopressin infused at the highest dose did not significantly change arterial resistance compared to the baseline levels and respective values in the saline treated rats (Fig. 3A) while at the highest administered dose, it significantly increased resistance to venous return in comparison to the baseline level (Fig. 3B). Apparent flow in fistula was significantly increased at the two highest doses of vasopressin (Fig. 4A). In contrast, the apparent resistance of fistula did not change significantly compared to the baseline level and respective values in saline treated rats (Fig. 4B).

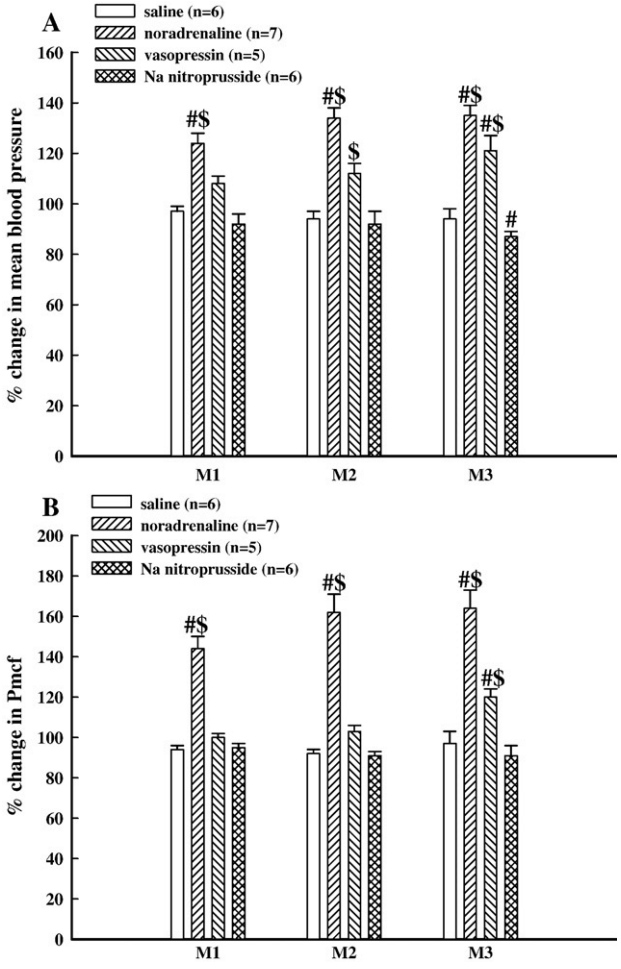

Fig. 2. Percent change from baseline control in mean arterial blood pressure (A) and mean circulatory filling pressure (Pmcf) (B) during administration of vasopressin (18–20 min) (10, 30 and 100 ng/kg per min), sodium nitroprusside (100, 300 and 1000 ng/kg per min), noradrenaline (1, 3 and 10 μg/kg per min), and saline (0.002, 0.006 and 0.02 ml/kg per min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt). M1, dose 1; M2, dose 2; M3, dose 3. Data represents mean value±S.E.M. #Significantly different from the baseline control level; $P<0.05$. $Significantly different from the respective value in saline treated group; $P<0.05$.

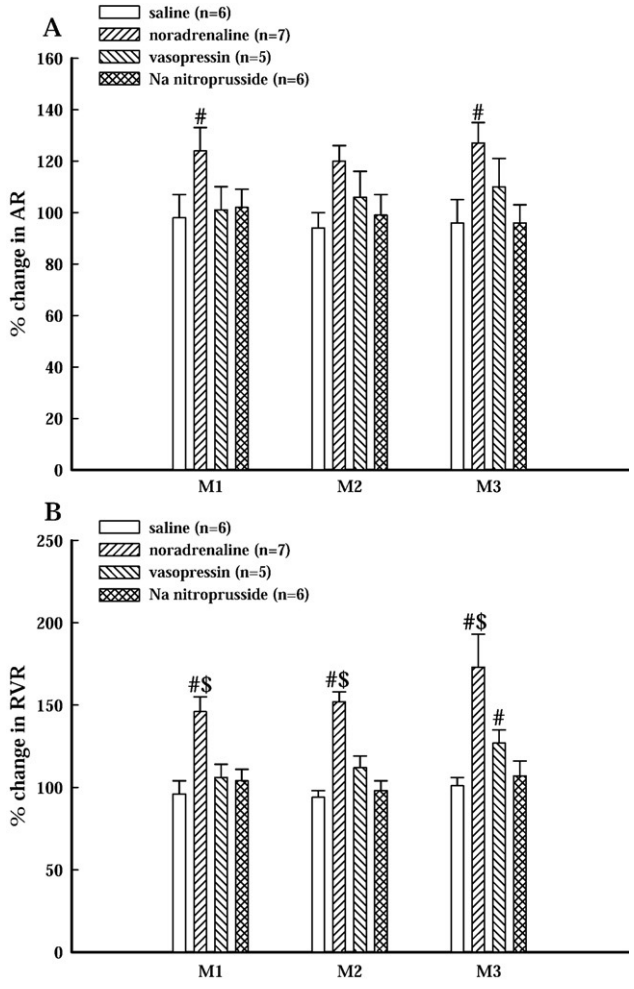

Fig. 3. Percent change from baseline control in apparent arterial resistance (AR) (A) and resistance to venous return (RVR) (B) during administration of vasopressin (18–20 min) (10, 30 and 100 ng/kg per min), sodium nitroprusside (100, 300 and 1000 ng/kg per min), noradrenaline (1, 3 and 10 μg/kg per min), and saline (0.002, 0.006 and 0.02 ml/kg per min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt). M1, dose 1; M2, dose 2; M3, dose 3. Data represents mean value±S.E.M. #Significantly different from the baseline control level; $P<0.05$. $Significantly different from the respective value in saline treated group; $P<0.05$.

3.3. Effects of sodium nitroprusside on haemodynamics in AVF rats

Administration of sodium nitroprusside (0.1, 0.3 and 1.0 μg/kg per min) did not change the cardiac index or heart rate (Fig. 1AB), while at the highest administered dose dP/dt was significantly reduced when compared to the baseline level (Fig. 1C). The highest dose of sodium nitroprusside also significantly decreased mean arterial blood pressure compared to the baseline level (Fig. 2A). Sodium nitroprusside infusion had a very limited effect on P_{mcf} (Fig. 2B). As well, infusion of sodium nitroprusside did not change arterial resistance or resistance to venous return (Fig. 3AB). While administration of sodium nitroprusside significantly decreased the apparent fistula flow when compared to the baseline level, it had no effect on the apparent fistula resistance (Fig. 4AB).

3.4. Blood and plasma volumes

The plasma volume and the total blood volume were significantly lower in the sham-operated group compared to the respective values in the fistula saline treated group (Table 3). However, the administration of saline did not affect plasma or total blood volumes over-time. In contrast, administration of vasopressin increased plasma volume significantly compared to the baseline level, while infusion of vasopressin at the second dose increased total blood volume significantly when compared to the baseline level (Table 3). Infusion of sodium nitroprusside increased plasma volume significantly compared to the baseline level at the two highest doses but it did not have a significant impact on total blood volume (Table 3). Administration of noradrenaline had very limited effects on plasma volume and the total blood volume (Table 3).

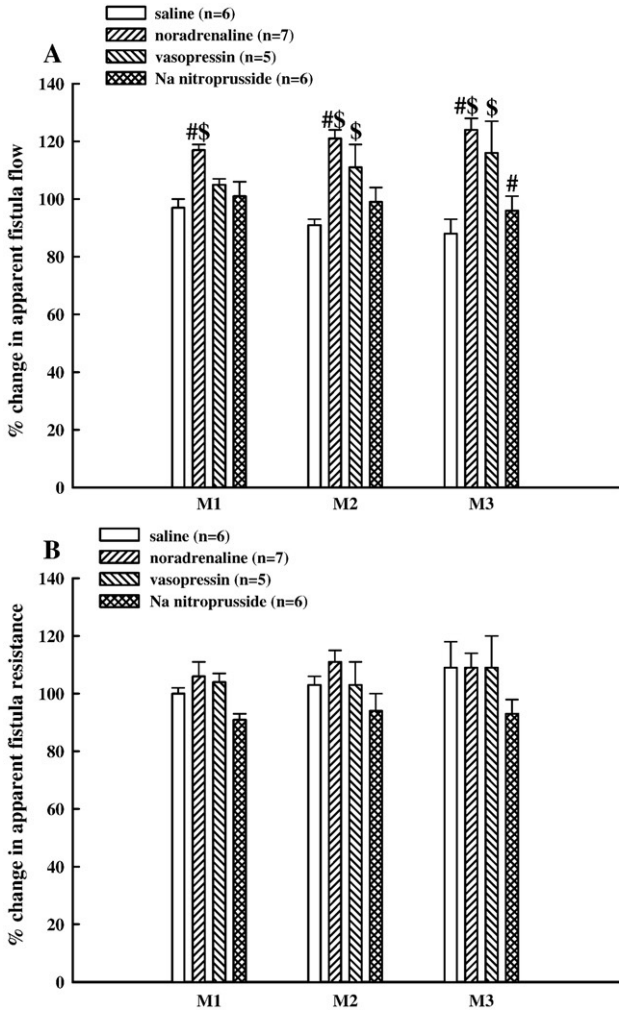

Fig. 4. Percent change from baseline control in apparent fistula flow (A) and apparent fistula resistance (B) during administration of (18–20 min) vasopressin (10, 30 and 100 ng/kg per min), sodium nitroprusside (100, 300 and 1000 ng/kg per min), noradrenaline (1, 3 and 10 μg/kg per min), and saline (0.002, 0.006 and 0.02 ml/kg per min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt). M1, dose 1; M2, dose 2; M3, dose 3. Data represents mean value±S.E.M. #Significantly different from the baseline control level; $P<0.05$. $Significantly different from the respective value in saline treated group; $P<0.05$.

Table 3
Plasma volume and total blood volume prior to the infusion of drugs/saline (control) and during the administration of different doses noradrenaline (μg/kg per min; $n=7$), vasopressin (ng/kg per min; $n=5$), sodium nitroprusside (ng/kg per min; $n=6$) and different infusion rates of saline (ml/kg per min; $n=6$) in anaesthetized rats with chronic (5 weeks post shunt) and saline (ml/kg per min; $n=8$) in anaesthetized sham rats

Groups	Plasma volume	Total blood volume
Noradrenaline		
Control	23.2±0.99	37.1±1.54
1.0	24.0±1.59	38.6±2.43
3.0	23.5±1.63	37.9±2.52
10	23.2±1.78	37.8±2.79
Vasopressin		
Control	21.0±1.21	33.0±2.00
10	24.3±1.48[a]	37.0±2.17
30	24.8±1.77[a]	37.3±2.63[a]
100	24.8±1.71[a]	36.8±2.68
Na nitroprusside		
Control	23.2±1.22	37.7±1.63
100	23.8±1.46	37.8±1.95
300	25.5±1.25[a]	40.4±1.55
1000	26.5±2.37[a]	41.5±3.23
Saline		
Control	22.2±1.54	35.7±2.25
0.002	20.8±1.00	33.1±1.46
0.006	21.5±0.90	33.6±1.33
0.02	22.0±1.12	34.3±1.53
Sham		
Control	19.0±1.00[b]	31.0±1.87[b]
0.002	20.0±1.26[b]	31.6±1.88[b]
0.006	19.7±1.25[b]	30.8±1.92[b]
0.02	19.2±1.16[b]	29.8±1.81[b]

Each value represents mean±S.E.M.
[a] Significantly different from baseline control value; $P<0.05$.
[b] Significantly different from the respective value in saline-treated animals; $P<0.05$.

3.5. Body, lung and heart weights

There were no differences between the body weights of rats with AVF and sham-operated animals (Table 4). The weight of the right ventricle and left ventricle plus septum in rats with chronic AVF was significantly higher than the respective values in the sham-operated group (Table 4). Furthermore, the ratio of right ventricle to left ventricle plus septum weights increased significantly in rats with chronic AVF when compared to the sham-operated group (Table 4). However, there were no significant changes between wet and dry lungs or the ratio of dry/wet lung weights in the various groups of animals (Table 4).

4. Discussion

The cardiovascular features of animals with arteriovenous fistula are high cardiac output and low arterial resistance as previously reported in such a model (Flaim et al., 1979, 1980; Liu et al., 1991). In addition, we have found that P_{mcf} was elevated while resistance to venous return was reduced in this model. Furthermore, blood volume expansion occurred and this can, in part, account for the elevation that was observed in P_{mcf} in the present investigation. Essentially, two parameters have a profound effect on P_{mcf}, one being venous tone and the other blood volume (Pang, 1994). Evidently, a combination of a low vascular resistance and an increase in blood volume are responsible for the increase in cardiac output in chronic experimental models of arteriovenous fistula. Furthermore, a lack of significant left ventricular failure in a high output situation is evident from an absence of significant increase in the ratio of wet/dry lung weights on sham compared to animals with fistula which is noted to occur in chronic heart failure in rats (Nekooeian and Tabrizchi, 1998). It seems that pulmonary oedema is not a major contributor to a dysfunctional circulatory system in this state.

The most significant finding of the present investigation is the inability of vasoactive agents to produce an impact on the cardiac output in animals with chronic arteriovenous shunt. It is clear that an infusion of a vasoconstrictor or dilator has a modest effect on the cardiac output. An absence of sufficient increase in arterial resistance leading to substantial impedance to flow seems to underlie the lack of reduction in cardiac output which would have occurred in a normal animal following infusion of a vasoconstrictor (Tabrizchi, 2001; Tabrizchi and Ford, 2004).

It is possible that in a low arterial resistive state, a significant increase in resistance to venous return will not translate into significant reduction in cardiac output. Normally, reduction in cardiac output occurs when resistance to venous return is significantly increased (Wang et al., 1995). In contrast, a reduction in resistance to venous return can result in significant increases in cardiac output (Tabrizchi, 1998). In the present investigation, it is

Table 4
Weight in grams of various organs and body weight (BW) of shunted animals treated with noradrenaline, vasopressin, sodium nitroprusside, saline and sham animals

Groups	Noradrenaline	Vasopressin	Na nitroprusside	Saline	Sham
BW	481±14	457±18	480±8.0	480±21	457±16
LV+S	2.88±0.07	2.82±0.09	2.59±0.13	2.63±0.14	2.17±0.06[a]
RV	0.87±0.11	1.05±0.06	1.05±0.06	0.98±0.06	0.66±0.04[a]
RV/LVS	0.32±0.02	0.37±0.03	0.33±0.02	0.37±0.02	0.3±0.01[a]
WL	3.94±0.44	4.62±0.44	3.54±0.12	3.50±0.30	3.07±0.31
DL	1.20±0.01	1.02±0.20	0.84±0.05	1.06±0.22	0.82±0.04
DL/WL	0.24±0.01	0.26±0.04	0.23±0.03	0.28±0.07	0.28±0.03

Left Ventricle+Septum (LV+S); Right Ventricle (RV); Wet Lung (WL); Dry Lung (DR).
[a] Significantly different from corresponding values in shunted groups; $P<0.05$.

evident that the infusion of neither noradrenaline nor vasopressin despite significantly increasing resistance to venous return were not able to reduce cardiac output. Furthermore, noradrenaline also significantly increased arterial resistance yet it failed to significantly reduce cardiac output. This would essentially suggest that an increase in impedance to flow and/or an increase in resistance to venous return was not sufficient to significantly reduce cardiac output in animals with chronic arteriovenous fistula. Not surprisingly, we have previously demonstrated that infusion of vasopressin can significantly reduce cardiac output by increasing arterial resistance and resistance to venous return in anaesthetized rats with normal circulation and in an absence of an arteriovenous shunt (Tabrizchi and Ford, 2004).

Based on the dynamic nature of the circulatory system, it can be predicted that an increase in venous tone together with an increase in arterial blood pressure could result in an increase in arterial resistance and resistance to venous return. Moreover, a substantial increase in the arterial resistance without any change in venous tone can also manifest as an increase in resistance to venous return due to significant increases in impedance to flow. There is evidence to indicate that an increase in vascular impedance can greatly affect resistance to venous return (Bower and Law, 1993). Furthermore, Wang et al. (1995) reported that a significant increase in arterial resistance resulting in impedance to flow was able to significantly reduce resistance to venous return.

Vasoconstrictors such as noradrenaline, dopamine and vasopressin can increase blood pressure with modest effects on cardiac output in a hyperdynamic state (Dellinger et al., 2004; Hollenberg et al., 2004). However, it is also well recognised that the infusion of vasoconstrictors in a condition of vasodilatory shock does not always lead to an increase in blood pressure (Holmes and Walley, 2004). The lack of increase in blood pressure could be due to shunting that occurs in a hyperdynamic circulatory situation (Ince and Sinaasppel, 1999; Spronk et al., 2004). Based on our data in this model, infusion of vasoconstrictors will not aid in reduction in the cardiac output even when systemic resistance is partially elevated by the infusion of the vasoconstrictor. Furthermore, the model employed in our current investigation further predicts that in a state where multiple shunting occurs in the circulatory system, it may be more difficult to significantly elevate blood pressure by the infusion of vasoconstrictors, and this appears to be the case in certain clinical conditions (Holmes and Walley, 2004).

While the infusion of noradrenaline was capable of significantly increasing heart rate and dP/dt (index cardiac contractility), it failed to increase cardiac output. It would appear that once the heart has reached a certain level of output, it becomes difficult to further elevate its capacity so as to increase its output. It seems also that in a chronic arteriovenous fistula model a dynamic equilibrium can be reached where vasoactive agents are not able to significantly disturb the cardiac output.

Surprisingly, infusion of vasopressin significantly increased P_{mcf}. In our previous studies, we have found vasopressin to have no impact on P_{mcf} in rats (Pang and Tabrizchi, 1986; Tabrizchi and Ford, 2004). It is possible that the presence of the fistula resulted in the expression of vasopressin receptors in veins and their stimulation was responsible for the increase in P_{mcf}. However, this issue cannot be resolved until further studies are conducted to delineate the basis for this effect of vasopressin on the venous circulation.

As well, in our present investigation, the infusion of sodium nitroprusside, a powerful arterial and venous dilator (D'Oyley et al., 1989), failed to substantially affect the overall haemodynamics while causing a modest reduction in arterial blood pressure. The basis for the lack of any major haemodynamic changes as a result of the infusion of sodium nitroprusside in this model could be because of substantial peripheral dilation within the circulatory system, thus reducing the immediate impact of this vasoactive agent on both the arterial and venous tree. We have previously reported that the infusion of sodium nitroprusside can reduce venous tone and P_{mcf} (D'Oyley et al., 1989). Certainly, our current observation that sodium nitroprusside did not reduce P_{mcf} may support the view that the elevation in P_{mcf} in animals with chronic arteriovenous fistula is predominantly due to an expansion of blood volume rather than an increase in venous tone due to extensive venoconstriction.

The effect of infusion of vasoactive agents on plasma and blood volume were modest in the present study. While noradrenaline did not have any significant impact on blood volume, both vasopressin and sodium nitroprusside significantly increased plasma volume.

The current studies indicate that the presence of chronic arteriovenous shunt produces a major impediment to overall changes that can normally be induced following the infusion of vasoactive agents. Cardiac output cannot be affected by the infusion of either vasoconstrictor or vasodilator agents, and blood pressure and peripheral resistance is also modestly affected by infusion of such agents.

Acknowledgement

This work was supported by a grant-in-aid from the Heart and Stroke Foundation of New Brunswick.

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