HAEMODYNAMICS AND VASOACTIVE AGENTS
THE HAEMODYNAMIC EFFECTS OF VASOACTIVE AGENTS IN RATS WITH CHRONIC ARTERIOVENOUS FISTULA

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The haemodynamic effects of vasoactive agents in rats with chronic arteriovenous fistula

by

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ABSTRACT

An arteriovenous fistula is an abnormal direct passage between an artery and a vein. The existence of an arteriovenous fistula generates a high output low total peripheral resistance state that resembles the hyper-haemodynamic condition of several diseases such as vascular injury, congenital malformation, chronic severe anemia, severe hepatic or renal disorders, and septic shock. In the present study, we investigated the haemodynamic changes in rats with chronic arteriovenous fistulae and the haemodynamic effects of noradrenaline, arginine vasopressin, and sodium nitroprusside in this model.

Creation of a chronic fistula between abdominal aorta and inferior vena cava increased cardiac output compared to sham-operated rats. The haemodynamic factors when compared to respective values in sham-operated rats changed in the following ways: 1) total peripheral resistance and resistance to venous return decreased; 2) heart rate and cardiac contractility based on dP/dt values recorded remained stable at the baseline level; and 3) blood volume and mean circulatory filling pressure increased. The augmentation of total blood volume, mean circulatory filling pressure and reduction in vascular resistance together contributed to the increase in cardiac output.

Noradrenaline infusion did not change the cardiac index in rats with chronic arteriovenous fistulae. Administration of noradrenaline 1) increased total peripheral resistance and resistance to venous return; 2) increased heart rate and cardiac contractility based on dP/dt values recorded; and 3) did not change total blood volume but increased mean circulatory filling pressure. The positive effects of noradrenaline on heart rate,

cardiac contractility, and total body venous tone tended to increase cardiac index. However, the increase of cardiac index was prevented due to the increase in total peripheral resistance and resistance to venous return.

Administration of arginine vasopressin did not change the cardiac index in rats with chronic arteriovenous fistulae. Upon arginine vasopressin infusion, 1) total peripheral resistance and resistance to venous return increased; 2) heart rate did not change while cardiac contractility increased based on dP/dt value recorded; and 3) total blood volume and mean circulatory filling pressure increased. Unlike its actions in the intact animal, administration of arginine vasopressin in rats with chronic arteriovenous fistulae reversed the preload-afterload mismatch by increasing mean circulatory filling pressure and total blood volume with little effect on resistance to venous return. Therefore, cardiac index did not fall despite of elevation of the afterload.

Administration of sodium nitroprusside had limited effects on cardiac index in rats with chronic arteriovenous fistula. The circulatory parameters altered in the following manner: 1) total peripheral resistance and resistance to venous return remained at the baseline level; 2) heart rate did not change while cardiac contractility decreased at the highest dose based on dP/dt value recorded; and 3) total blood volume and mean circulatory filling pressure was kept unchanged. Due to the special condition in our experimental model such as low vascular resistance and maximal vasodilation, administration of sodium nitroprusside had limited haemodynamic effects.

In conclusion, in rats with well compensated chronic arteriovenous fistulae vasoactive agents have little effects on cardiac output. Therefore, in diseases resembling the pathophysiology of a chronic arteriovenous fistula such as septic shock administration of these drugs may have limited effect and little benefit.

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LIST OF ABBREVIATIONS

Arginine Vasopressin	AVP
Arteriovenous fistula	AVF
Cardiac index	CI
Mean Circulatory Filling Pressure	P_{mcf}
Pressure Gradient for Venous Return	PGVR
Sodium Nitroprusside	SNP
Total peripheral resistance	TPR

1. Introduction

1.1. Regulation of Cardiac Output

Cardiac output is the quantity of blood pumped into the aortic arch each minute by the heart (Guyton & Hall, 2000a). This also is the amount of blood that flows through the whole circulation (Guyton & Hall, 2000a). The stream of the blood flow transports nutrients, wastes and hormones from one part of the body to another therefore maintaining a constant internal environment (Guyton *et al.*, 1973a). A substantial decrease in cardiac output can lead to detrimental effects on cellular functions because of failure to deliver adequate nutrients or timely remove wastes (Guyton *et al.*, 1973a). Due to its important role, cardiac output is delicately regulated to provide enough blood to individual tissues to maintain their functions (Guyton, 1981). According to Guyton (1981), cardiac output is regulated by four basic circulatory factors: total peripheral resistance (TPR), heart pumping capacity (heart rate and cardiac contractility), blood volume and vascular compliance.

Venous return is the quantity of blood flowing from the veins into the right atrium each minute (Guyton & Hall, 2000a). It should equal cardiac output in the steady state with exceptions when blood is temporarily being stored in or removed from the heart and lungs during a few heart beats (Guyton & Hall, 2000a). Venous return is determined by the balance between the pressure gradient that drives the venous return and the resistance

to venous return.

1.1.1 Resistance

Resistance is the impediment to blood flow in a vessel (Guyton & Hall, 2000b). It can not be measured by direct means but calculated from the equation below (Guyton & Hall, 2000b):

$$\text{Resistance} = \text{Pressure gradient} \div \text{Blood flow}$$

The resistance of the entire systemic circulation is called total peripheral resistance (TPR) (Guyton & Hall, 2000b). Similarly, resistance in arteries and veins is called arterial resistance and venous resistance, respectively. Moreover, resistance to venous return is the combined resistance of all the vessels up to the right atrium from venous locations where the intravascular pressure is equal to the pressure when circulation is stopped and equilibrated (Bower & Law, 1993). It has been suggested that resistance to venous return accounts for approximately 5% of the TPR (Bower & Law, 1993).

At each level of atrial pressure, the ventricular output is nearly reciprocally related to the square root of the resistive load (Guyton *et al.*, 1973b). It was postulated that resistance may alter cardiac output either by changing the load on the ventricle or the ease with which blood flows through the vessels back to the heart (Guyton *et al.*, 1973b). To support their suggestion, Guyton *et al.* (1959) conducted a series of experiments in which

the total vascular resistance was increased by elevating arterial resistance and venous resistance. Comparatively, a substantial increase in the TPR caused by arterial embolization resulted in only a moderate reduction in cardiac output (i.e. a 400% increase in TPR only decreased cardiac output by 30%) (Guyton et al., 1959). In contrast, an increase of TPR by 25% above the control level by the inflation of a cuff on vena cava reduced cardiac output by approximately 30% (Guyton *et al.*, 1959). Based on these observations, it is evident that an increase in resistance in veins has a much greater impact on the cardiac output. This is because in spite of elevation of the impediment to blood flow, the pressure gradient for venous return (PGVR) was decreased simultaneously in the presence of venous resistance augmentation (Guyton *et al.*, 1959).

In contrast, when TPR is decreased greatly by opening a large arteriovenous fistula (AVF), the cardiac output immediately increases and remains at this higher level as long as the fistula is maintained (Guyton & Sagawa, 1961; Huang *et al.*, 1992). The reduced TPR decreases the impedance to the blood flowing back to the heart. In combination with volume expansion, more blood is able to flow back into the heart. In turn, the heart responds by pumping out extra blood in accordance with Frank-Starling's law (Guyton & Sagawa, 1961; Huang *et al.*, 1992).

1.1.2. Heart pumping capacity

Cardiac output is also finely regulated by the sympathetic and parasympathetic nervous system which innervates the heart at different levels (Guyton & Hall, 2000c). The neurotransmitters released by nerves that innervate the heart alter the pumping effectiveness. This is chiefly achieved in two ways: 1) changing the heart rate and/or 2) changing the strength of the contractile force of the myocardium (Guyton & Hall, 2000c).

There is substantial evidence to suggest that an increase in heart rate has a biphasic impact on the cardiac output. In experimental situation in dogs, simple elevation of heart rate from 55 to 108 beats/min only results in 7.7% increase in cardiac output in experimental dogs (Nakano, 1964; Cowley & Guyton, 1971). Cardiac output then is maintained at the highest level when heart rate is between 108 and 148 before it declines (Nakano, 1964; Cowley & Guyton, 1971). Therefore, the shape of the heart-rate-cardiac-output curve resembles a "bell". The 7.7% increase of cardiac output (the left side of the "bell" curve) is brought about by an elevation of the heart rate without substantial reduction in stroke volume. The highest cardiac output achieved during the optimal range for heart rate (the plateau of the "bell" curve) is the consequence of simultaneous reduction in stroke volume and elevation of heart rate (Cowley & Guyton, 1971). The decrease of cardiac output when the heart rate increases

beyond a critical value (the right side of the "bell" curve) is caused, at least, partially by shortened diastolic filling period of ventricles as well as incomplete ventricular relaxation (Nakano, 1964; Sugimoto *et al.*, 1966; Cowley & Guyton, 1971).

There is evidence to support the view that cardiac contractility is positively correlated to cardiac output if other circulatory factors remain constant. In an isolated canine heart which was paced at a constant rate (211 beats/min), fed with fixed venous reservoir and connected to unchanged peripheral resistance (controlled by the valve in the artificial circuit), reduction of the contractility due to efferent vagal stimulation consistently diminished stroke volume resulting in a reduction in the ventricular output (Degeest *et al.*, 1965). In a similar preparation of isolated, perfused, working guinea-pig heart, when preload and afterload were kept constant, 2×10^{-7} M of digoxin (a positive inotropic agent) increased cardiac output without altering heart rate (Zannad *et al.*, 1982). The enhanced performance of the heart was suggested to be the result of increased contractility induced by digoxin (Zannad *et al.*, 1982). The basis for the relationship between contractility and cardiac output is that decreased strength of heart beat limits the amount of the blood volume being expelled from the heart to the systemic circulation thus leading to decreased cardiac output (Guyton *et al.*, 1973c). On the other hand, enhanced heart beat displaces extra quantity of blood from the heart to the remainder of the circulation, which is then responsible for the increase in cardiac output (Guyton *et al.*,

1973c).

It has been suggested that the effect of heart rate on the cardiac output is dependent on the level of venous return. The heart was reported to pump more quickly and deliver more blood at the higher venous return level which can be caused by opening a large AVF, noradrenaline infusion, or sympathetic stimulation (Sugimoto *et al.*, 1966; Cowley & Guyton, 1971). In the latter condition, the left side of the "bell" curve will be moved further upward and rightward (Cowley & Guyton, 1971). Meanwhile, the effect of the heart rate on the cardiac output is more obvious when venous return is increased (Sugimoto *et al.*, 1966).

Similarly, in one investigation in which Guyton *et al.* (1957) replaced the right ventricle with a mechanical pump, the effect of unlimited increase in both heart rate and cardiac contractility was examined. In the latter experiments, as heart pumping capacity increased, venous return curve reached the maximum value and remained at a plateau level when the right atrium pressures were more negative than -2 to -4 mmHg (Guyton *et al.*, 1957). This implies that no matter how hard the heart pumps the cardiac output would not exceed the plateau level of the venous return curve at the steady state. The simple explanation is that the amount of blood the heart pump is much more than the venous system can feed the heart.

The rate of change of the ventricular pressure with respect to time (i.e. dP/dt) can be an indicator of the level of contractility (Guyton & Hall, 2000c). In experimental studies, the peak dP/dt represents the maximal rate of rise in ventricular pressure and is often used for comparing the contractility of hearts in different functional states (Mason, 1969; Guyton & Hall, 2000c). However, the dP/dt value can be affected by factors other than the strength of the heart beat such as the loading conditions of the heart (Mason, 1969; Guyton & Hall, 2000c). It has been shown that a substantial increase in ventricular preload or a huge rise in ventricular afterload is capable of increasing dP/dt without a real elevation of cardiac contractility (Mason, 1969). The increment of preload can be reflected by a climb of the ventricular end-diastolic pressure or an increase in blood volume whereas the augmentation of the afterload can be manifested as an increment of the arterial diastolic pressure (Mason, 1969). Therefore, dP/dt may not increase upon the elevation of the cardiac contractility if the afterload falls extensively (Mason, 1969). On the other hand, dP/dt can increase while cardiac contractility is decreasing (Mason, 1969). This might happen when there is a large increase in preload (Mason, 1969). Furthermore, a direct linear correlation has been shown between peak dP/dt and heart rate in an areflexic canine preparation (Mason, 1969). Therefore, caution must be exercised when evaluating changes in cardiac contractility using dP/dt as the parameter in vivo.

1.1.3. Blood Volume

Acute volume expansion can immediately increase cardiac output as well as the mean arterial pressure (Prather *et al.*, 1969; Cevese & Guyton, 1976). The hyperdynamic effect is more significant in headless dogs than in anaesthetized ones (Prather *et al.*, 1969; Cevese & Guyton, 1976). The acute volume expansion first increases the filling of the systemic circulation. Subsequently, the elevation of the fullness of systemic circulation increases the preload of the heart. This consequently increases the cardiac output due to Frank-Starling's law (Guyton *et al.*, 1973d). However, it has also been established that after 90-120 minutes, cardiac output is able to fall back to the control level despite the persistent elevation of blood volume (Prather *et al.*, 1969; Cevese & Guyton, 1976). Nervous and/or stress relaxation of the capacitance blood vessels and trans-capillary fluid exchange are thought to contribute to the reduction of cardiac output after a prolonged period of volume expansion (Prather *et al.*, 1969; Guyton *et al.*, 1973d). It is also recognized that if the pumping capacity remains normal, acute hemorrhage decreases cardiac output while quick transfusion reverses the deficiency in cardiac output (Guyton *et al.*, 1958).

1.1.4. Vascular compliance

Vascular compliance is the total quantity of blood that can be stored in a given portion of the circulation for each millimeter of mercury rise in pressure (Guyton & Hall, 2000d).

However, it is difficult to quantify vascular compliance in vivo. According to Tabrizchi and Pang (1992), Grodins formulated four equations to quantify the vascular factors influencing the systemic circulation:

$$Q = (P_a - P_v)/R \quad (1)$$

$$P_a = BV_a/C_a \quad (2)$$

$$P_v = BV_v/C_v \quad (3)$$

$$BV = BV_a + BV_v \quad (4)$$

Where Q is cardiac output during the steady state; R is systemic resistance; P_a and P_v are arterial and venous pressures; C_a and C_v are arterial and venous compliances; BV_a and BV_v are arterial and venous blood volumes, respectively. By rearranging the four equations (1-4), equation (5) and (6) are obtained (Tabrizchi & Pang, 1992):

$$P_a = BV/(C_a + C_v) + C_vRQ/(C_a + C_v) \quad (5)$$

$$P_v = BV/(C_a + C_v) - C_aRQ/(C_a + C_v) \quad (6)$$

When Q = 0, the corollary is that (Tabrizchi & Pang, 1992):

$$P_a = P_v = BV/(C_a + C_v) \quad (7)$$

This equilibrium of the pressure after blood flow cessation is synonymous with Guyton's term "mean circulatory filling pressure" (P_{mcf}) (Guyton *et al.*, 1954). It is apparent from equation (7) that the P_{mcf} is directly proportional to the total blood volume and inversely proportional to the overall vascular compliance. Since venous compliance is many times greater than arterial compliance, P_{mcf} is mainly a reflection of the body's total

venous tone provided blood volume remains unchanged (Tabrizchi & Pang, 1992).

Mathematically, P_{mcf} reflects the ratio of the total blood volume to the ability of the circulatory system to hold the volume (Prather *et al.*, 1969). Total spinal anaesthesia lowers P_{mcf} while increasing vasomotor tone or acute volume expansion increases P_{mcf} (Guyton *et al.*, 1954). Experimental evidence seems to suggest that the relationship between blood volume and P_{mcf} is almost linear (Holman, 1940; Guyton *et al.*, 1954; Guyton *et al.*, 1973e). Furthermore, without reflexes, only slight alterations of the blood volume can markedly affect the P_{mcf} (Holman, 1940). It has been shown that venous return increases approximately in proportion to the pressure difference between P_{mcf} and right atrial pressure if resistance to venous return remains constant (Guyton *et al.*, 1955). The pressure gradient is called PGVR. Therefore, in the latter condition, cardiac output will be also increased by the increment of PGVR at a steady state. This is so because P_{mcf} increment increases cardiac output by intensifying the force that pushes the blood to the right atrium. In addition, a rise in P_{mcf} will distend the veins leading to the right atrium. The venodilation consequently decreases the impedance to venous return which also contribute to the elevation of cardiac output (Guyton *et al.*, 1955).

In summary, 1) a rise in vascular resistance tends to decrease cardiac output and a fall in

vascular resistance tends to increase cardiac output; 2) heart rate has a biphasic impact on cardiac output in a way that the heart-rate-cardiac-output curve resembles a "bell"; 3) cardiac contractility is positively related to cardiac output when preload, afterload, and heart rate are kept constant; 4) acute change of blood volume alters cardiac output in the same direction; 5) P_{mcf} is an indicator of total body venous tone provided blood volume is unchanged; 6) an increase in P_{mcf} is a contributory factor to increase cardiac output.

1.2. Haemodynamic effects of noradrenaline

Noradrenaline (norepinephrine, levarterenol) is a major chemical transmitter liberated by mammalian postganglionic sympathetic nerves (Von Euler, 1952; Von Euler, 1955; Westfall & Westfall, 2006). Noradrenaline elicits vasoconstriction and raises the blood pressure by stimulating α-adrenergic receptor (Bellomo & Giantomasso, 2001) and increases coronary blood flow possibly through direct β_2-adrenaceptor activation (Westfall & Westfall, 2006). As a pressor hormone and physiological vasoconstrictor, noradrenaline plays an important role in blood pressure homeostasis (Von Euler, 1952; Von Euler, 1955). Also, it is the standard therapy in the situation when there is a need to increase blood pressure or stimulate the heart (Von Euler, 1952; Von Euler, 1955; Westfall & Westfall, 2006). As well, in clinical situations in which the endogenous noradrenaline is depleted supplemental noradrenaline is recommended (Von Euler, 1952; Von Euler, 1955). However since excessive amounts of noradrenaline can cause severe

hypertension, careful blood pressure monitoring is generally recommended during systemic administration of this agent (Westfall & Westfall, 2006). Reduction of blood flow to organs such as kidney and intestines is another potential disadvantage of noradrenaline administration (Bellomo & Giantomasso, 2001; Westfall & Westfall, 2006).

Noradrenaline seems to have dose dependent biphasic action on cardiac output (Takacs, 1965; Tabrizchi, 2001). Reportedly, cardiac output is not changed significantly during intravenous infusion of noradrenaline at moderate doses (0.3, 0.4, and 1.0 μg/kg/min or intraperitoneally 20 and 100 μg/kg) (Takacs, 1965; Tabrizchi, 2001). However, cardiac output was markedly increased when noradrenaline was infused intraperitoneally at a dose of 500 μg/kg (Takacs, 1965). Similarly, Imms *et al.* (1974) reported a significant increase in cardiac index (CI), which is cardiac output divided by body weight, at the infusion rate of 1.7 μg/min. Moreover, noradrenaline has been shown to increase ventricular output in the isolated perfused rat heart with constant preload and afterload by enhancing stroke volume and heart rate (Geier *et al.*, 2002). Noradrenaline infusion (0.4–3.0 μg/kg/min) also results in a significant increase in cardiac output in cats with suppressed autonomic reflexes and respiratory movements (Bower & Law, 1993).

Noradrenaline dose dependently increases the mean arterial pressure, systolic pressure, diastolic pressure and pulse pressure (Takacs, 1965; Young *et al.*, 1992; Bower & Law,

1993; Westfall & Westfall, 2006). Arterial resistance and resistance to venous return were shown to increase significantly at doses of 0.3 and 1.0 μg/kg/min (Imms *et al.*, 1974; Pang & Tabrizchi, 1986; Tabrizchi, 2001). In contrast, TPR did not change at the higher dose due to simultaneous increase in mean arterial pressure and cardiac output (Imms *et al.*, 1974; Pang & Tabrizchi, 1986; Tabrizchi, 2001).

Noradrenaline significantly increases the heart rate in intact anaesthetized rats while it lowers the heart rate in rats treated with a β-blocker (Imms *et al.*, 1974; Pang & Tabrizchi, 1986; Tabrizchi, 2001). Compensatory vagal reflex activity also slows the heart rate, overcoming the direct cardioaccelerating (increase of heart rate) action of noradrenaline (Young *et al.*, 1992; Westfall & Westfall, 2006). Administration of noradrenaline increased dP/dt in intact anaesthetized rat (Tabrizchi, 2001). Moreover, noradrenaline was found to increase both dP/dt_{max} and dP/dt_{min} in isolated perfused heart with constant preload and afterload indicating a rise in cardiac contractility (Geier *et al.*, 2002).

Haematocrit was found to increase during noradrenaline administration implying a decrease in plasma volume (Floyer & Morris, 1976; Aziz & Sommer, 1982). The explanation for the latter phenomenon is suggested to be that potent post-capillary vasoconstriction elicited by noradrenaline increases the filtration pressure and mobilizes the fluid out of the vessel (Floyer & Morris, 1976; Aziz & Sommer, 1982). In addition,

noradrenaline significantly increases the P_{mcf} but has limited effect on right atrial pressure (Pang & Tabrizchi, 1986; Bower & Law, 1993; Tabrizchi, 2001). Therefore, the PGVR is elevated (Bower & Law, 1993). Simply put, total body venous tone was substantially elevated by the administration of noradrenaline.

In summary, noradrenaline may either maintain or increase the cardiac output. The haemodynamic factors can change in the following manner: 1) TPR and resistance to venous return increases; 2) the whole heart pumping ability which is indicated by heart rate and cardiac contractility is elevated; 3) total body venous tone rises. The underlying mechanism for the cardiac output alteration is that the increase in both arterial resistance and resistance to venous return during noradrenaline administration tend to lower cardiac output, while the positive inotropic effect of noradrenaline and the increase of total body venous tone help to increase the cardiac output. At a moderate dose of noradrenaline, the level of driving force to increase cardiac output is balanced with that of lowering cardiac output; therefore the output of the heart is unchanged (Tabrizchi, 2001). However, at the higher dose or in animals with suppressed autonomic regulation, the circulatory changes that favor cardiac output increase overwhelms the cardiac output lowering effect of other circulatory factors; as a result cardiac output is increased.

1.3. Haemodynamic effects of arginine vasopressin

Arginine vasopressin (AVP), also known as antidiuretic hormone, is essential for both osmotic and cardiovascular homeostasis (Share, 1988). Two major physiological roles of the circulating AVP are 1) maintaining the arterial blood pressure when blood volume is decreased; and 2) protection of the plasma osmolality when plasma solute concentration is increased (Share, 1988). The different physiological effects of AVP are mediated via stimulation of the Vasopressin 1 (V_1) receptor (or V_{1a}, vascular), the Vasopressin 2 (V_2) receptor (renal), the Vasopressin 3 (V_3) receptor (or V_{1b}, pituitary), the oxytocin receptor, and purinergic 2 receptor (P_2R) (Holmes *et al.*, 2003). The antidiuretic effect of this peptide has been exploited clinically to treat diabetes insipidus for over half a century (Holmes *et al.*, 2003). Recently, it was found that plasma AVP was inappropriately low in advanced vasodilatory septic shock and exogenous AVP infusion increased and maintained blood pressure and reduced the use of catecholamine in such population of patients (Landry *et al.*, 1997). Therefore, this rationale has been the basis for the use of AVP in the treatment of septic shock. However, limited controlled clinical trials are available on the effectiveness, efficacy, and safety of the use of AVP for treatment of patients with shock (Holmes & Russell, 2004). For this reason, it is not recommended that AVP be used as a first-line or routine therapy in patient with septic shock (Holmes & Russell, 2004).

Reduction of cardiac output has long been observed in subjects when AVP is administered (Share, 1988). In ganglion-blocked conscious rats as well as anaesthetized rats, cardiac output was decreased during the infusion of AVP (Share, 1988; Hernandez *et al.*, 1991; Tabrizchi & Ford, 2004). When AVP is administered and its plasma concentration is maintained within the physiological level (2.0 fmol/ml compared with 1.9 fmol/ml in control dogs), TPR increases and cardiac output decreases (Montani *et al.*, 1980; Tipayamontri *et al.*, 1987). The increase in vascular resistance is most prominent in stomach, small and large intestines, muscles, and skin vascular beds (Charocopos *et al.*, 1982). However, the mean arterial pressure does not change due to the baroreflex (Ohta *et al.*, 1991). It is suggested that the reduction of cardiac output is mediated centrally while the elevation of TPR is due to the vasoconstrictor effects of AVP through the V_1 receptor (Montani *et al.*, 1980; Ohta *et al.*, 1991). At pharmacological dose levels, AVP has been shown to increase the mean arterial pressure, the arterial resistance and the resistance to venous return in a dose dependent manner (Walker, 1986; Lee *et al.*, 1988; Martin & McNeill, 1990; Hernandez *et al.*, 1991; Hernandez *et al.*, 1994; Tabrizchi & Ford, 2004).

In conscious rats, heart rate was dose dependently decreased following infusion of AVP (Hernandez *et al.*, 1991; Hernandez *et al.*, 1994). In contrast, the peptide exerts no effect on the heart rate in ganglion-blocked conscious rats and anaesthetized rats

(Hernandez *et al.*, 1991; Tabrizchi & Ford, 2004). The reduction of the heart rate is believed to be mediated both by the neurogenic mechanism and the decrease in coronary blood flow (Share, 1988; Hernandez *et al.*, 1994). When coronary perfusion is kept constant, the contractility of isolated rat heart displays biphasic reaction to AVP infusion: while dP/dt_{max} increased at the lower concentrations (50 and 100 pg/ml) it decreased at higher concentration of 400 and 500 pg/ml infusion (Walker *et al.*, 1988). It is suggested that the direct cardiac effects of AVP were mediated by the V_1 receptor. In vivo, AVP infusion significantly increased dP/dt compared to corresponding saline values (Tabrizchi & Ford, 2004). It has been known that when both preload and afterload are kept constant, an increase in dP/dt means an increment in cardiac contractility. However, during AVP administration, afterload increases with limited effects on preload. Therefore, the elevation of dP/dt may be due to an increase of afterload or positive inotropic effects or both (Lee *et al.*, 1988; Tabrizchi & Ford, 2004).

AVP has limited effects on P_{mcf} and central venous pressure when infused acutely, therefore the PGVR was found to be unaffected (Trippodo, 1981; Pang & Tabrizchi, 1986; Tipayamontri *et al.*, 1987; Share, 1988; Lee *et al.*, 1988; Hernandez *et al.*, 1991; Ohta *et al.*, 1991; Hernandez *et al.*, 1994; Tabrizchi & Ford, 2004). This is because AVP has less constrictor effects on veins compared to arteries (Trippodo, 1981). However, Tipayamontri *et al.* (1987) reported that chronic AVP infusion (2.6 ng/kg/min, 50 ml each

day for 4 days) reduced P_{mcf}. It was stipulated that suppression of renin-angiotensin system and/or sympathetic nervous system was responsible for the effects of AVP in reducing P_{mcf} (Tipayamontri *et al.*, 1987). Acute infusion of AVP (1, 3, 10 ng/kg/min for 20 min) has no significant impact on the total blood volume and unstressed blood volume in intact anaesthetized animals (Lee *et al.*, 1988; Tabrizchi & Ford, 2004). As well, the haematocrit did not change significantly in rats in the latter experiments (Hernandez *et al.*, 1994; Tabrizchi & Ford, 2004). Therefore, the total body venous tone did not change during short term AVP infusion (Lee *et al.*, 1988; Martin & McNeill, 1990). Nevertheless, Floyer and Morris (1976) reported a decrease in haematocrit during AVP infusion (60 μIU/min in 0.15 M-NaCl) indicating an increase in plasma volume in female Wistar rats (Floyer & Morris, 1976). The kidneys were suggested to play an important role in the reduction in haematocrit because the latter phenomon disappeared in nephrectomized rats (Floyer & Morris, 1976). The source of plasma expansion is suggested to derive from the interstitial space (Floyer & Morris, 1976). The discrepancies on haematocrit alteration may be due to the way that AVP and animals were prepared, different doses as well as species differences. Simply, the total body venous tone may not change or increase during infusion of AVP.

In summary, AVP administration decreases cardiac output, and the haemodynamic parameters change in the following ways: 1) TPR increases; 2) heart rate decreases and

cardiac contractility may either increase or decrease; and 3) total body venous tone remains unchanged or increases. The reduction in cardiac output is most likely due to an inadequate venous return and a failure of the left ventricle to overcome the increase in afterload (Hernandez *et al.*, 1991). Inadequate venous return is the result of no change in PGVR and increased resistance to venous return. The elevated afterload signifies the increase in TPR. This situation is also referred to as cardiac preload-afterload mismatch (Hernandez *et al.*, 1994). In addition, supraphysiological dose of AVP may exert negative chronotropic and inotropic effects directly on the heart (Walker *et al.*, 1988). This also can be responsible for the reduction of cardiac output. Finally, it is also suggested that AVP may decrease cardiac output by baroreflex regulation (Montani *et al.*, 1980).

1.4. Haemodynamic effects of sodium nitroprusside

Sodium nitroprusside (SNP) is a nitrovasodilator that acts by releasing nitric oxide (Hoffman, 2006). Nitric oxide activates the guanylyl cyclase-cyclic GMP-cyclic GMP-dependent protein kinase pathway leading to vasodilation (Hoffman, 2006). SNP dilates both arterioles and venules, and the consequent haemodynamic effects result mainly from a combination of venous pooling and reduced arterial resistance (Hoffman, 2006). It has been used for 1) rapid reduction of arterial pressure in the presence of hypertensive crisis regardless of etiology; 2) diminution of myocardial oxygen demand

by decreasing afterload in the situation of low cardiac output when blood volume is normal or increased; and 3) reducing blood loss during operation (Friederich & Butterworth, 1995; Hoffman, 2006). Compared with other vasodilators, SNP offers the advantage of exquisitely precise dose titration for critically ill patients (Mills, 2003). However, the drug administration needs invasive monitoring and skilled nursing (Mills, 2003). Moreover, cyanide toxicity also limits the clinical use of SNP for prolonged periods (Friederich & Butterworth, 1995; Hoffman, 2006).

Administration of SNP has long been observed to decrease the cardiac output in subjects with normal left ventricle function (Gmeiner *et al.*, 1975). In cats with suppressed autonomic reflexes and spontaneous respiratory movement, cardiac output was changed little by the infusion of SNP (Bower & Law, 1993). Similar results were also found in hexamethonium treated dogs (Ogilvie & Zborowska-Sluis, 1991). Increasing the dose of SNP results in progressive fall in mean arterial pressure, TPR and resistance to venous return (D'Oyley *et al.*, 1989; Bower & Law, 1993). The reduction in resistance is linearly related to the logarithm of the SNP dose (correlation coefficients $r > 0.73$, $P < 0.01$; SNP, 0.4 – 40 nmol/kg/min) (Bower & Law, 1993). In contrast, TPR and resistance to venous return was found unchanged during the treatment of SNP in pentobarbital anaesthetized, ganglion blocked, ventilated, and splenectomized dogs (SNP dose 1 μg/kg/min) (Ogilvie & Zborowska-Sluis, 1991).

Sodium nitroprusside dose-dependently increases the heart rate due to sympathetic reflex (D'Oyley *et al.*, 1989). In cats with suppressed autonomic reflexes, heart rate did not alter significantly (Ogilvie & Zborowska-Sluis, 1991; Bower & Law, 1993). No direct positive chronotropic action of the drug has been observed in isolated rat heart (Gmeiner *et al.*, 1975). As well, a wide range of SNP concentration (1-1000 ng/ml) did not alter dP/dt_{max} (Gmeiner *et al.*, 1975). The effect of SNP on P_{mcf} is limited in conscious intact rats (D'Oyley *et al.*, 1989). P_{mcf} was dose dependently decreased in ganglion blocked rats and cats (D'Oyley *et al.*, 1989; Ogilvie & Zborowska-Sluis, 1991; Bower & Law, 1993). The lack of change in P_{mcf} in intact rats might be due to the compensatory neural reflex (D'Oyley *et al.*, 1989).

Simply put, SNP lowers the cardiac output in subjects with normal ventricular function. SNP 1) dose dependently decreases the TPR; 2) has no direct effects on the heart rate and cardiac contractility but induces tachycardia due to reflex sympathetic nerve activation; and 3) reduces total body venous tone in ganglion blocked animals but does not affect venous tone in intact animals. The reduction of cardiac output is caused by a fall in left ventricular filling pressure (Gmeiner *et al.*, 1975). Also, SNP is able to pool blood in the capacitance vessels by relaxing systemic veins and thereby reducing venous return (Gmeiner *et al.*, 1975). In ganglion-blocked animals, the substantial fall in P_{mcf} further decreases cardiac output by lowering the PGVR (Bower & Law, 1993).

1.5. Haemodynamics in rat with arteriovenous fistula

An arteriovenous fistula (AVF) is an abnormal direct passage between an artery and a vein (Holman, 1965). It can be congenital or acquired (Holman, 1965). AVF generates a high output low TPR state that resembles the hyper-haemodynamic condition of several diseases such as vascular injury or congenital malformation, chronic severe anemia, severe hepatic or renal disorders, and septic shock (Anand & Florea, 2001). The effect of AVF on the haemodynamics of the circulation is mainly determined by the amount of blood diverting through it (Holman, 1940).

The cardiac output increases immediately within the first two to three heart beats after the opening of a fistula in experimental dogs (Guyton & Sagawa, 1961). The increment in cardiac output is similar to the amount of fistula flow as long as the fistula flow remains less than 19% of the cardiac output prior to fistula creation (Frank *et al.*, 1955; Guyton & Sagawa, 1961). Thereafter, the cardiac output experiences an additional rise in the next 15-30 seconds in dogs with intact reflexes but not in the areflexic dogs (Guyton & Sagawa, 1961). Since there is limited change in the heart rate in short term AVF, it is suggested that the augmentation in the cardiac output is mainly due to the increase in stroke volume. Once the shunt flow exceeds 27% of the control cardiac output, the increase in cardiac output will no longer compensate the fistula flow (Guyton & Sagawa, 1961). In such condition, the perfusion to tissues is compromised as there is

diminished blood flow in systemic circulation due to the diversion of blood flow through the fistula. The blood through the fistula repeatedly flows in the short circuit with high speed. Consequently, the systemic circulation has to increase the resistance to maintain the arterial pressure (Frank *et al.*, 1955). When the perfusion to tissues is compromised due to large AVF, infusion of isotonic saline will increase cardiac output dramatically (Frank *et al.*, 1955). Approximately, 88% of the elevated output is shown to be diverted to the systemic circulation with a decrease in the systemic resistance (Frank *et al.*, 1955). The infusion of the isotonic saline returns the systemic flow to the level prior to fistula introduction in both areflexic and reflexic dogs (Guyton & Sagawa, 1961). Such evidence would suggest that the availability of blood volume for the venous return instead of the cardiac reserve is one limiting factor of the increase in cardiac output in acute uncompensated AVF (Frank *et al.*, 1955). In a rat model of chronic AVF in which the shunt flow varied from 52% to 77% of the cardiac output prior to fistula introduction, systemic flow decreased significantly compared with the cardiac output in rats without AVF 1 hour and 1 week after fistula creation (Huang *et al.*, 1992). However, the systemic flow returned to the same level as in the rat without AVF at the fifth week (Huang *et al.*, 1992). The recovery of the systemic flow at the fifth week was explained by compensatory increase in blood volume and heart hypertrophy (Huang *et al.*, 1992).

Perhaps not surprisingly, the TPR is decreased immediately after the opening of the

fistula and it is maintained at the lower level as long as the fistula exists (Guyton & Sagawa, 1961). Although the mean arterial blood pressure decreases shortly after the opening of the fistula, it returns to the level just below the control level during the ensuing 30 seconds (Guyton & Sagawa, 1961). This return of the pressure is thought to be mediated by a neural reflex (Guyton & Sagawa, 1961). Huang *et al.* (1992) have shown that in rats with fistula the mean arterial pressure was reduced significantly in the first week but returned to the control level at the fifth week post-shunt. The underlying mechanism responsible for the restoration of the mean arterial pressure may involve blood volume expansion (Huang *et al.*, 1992).

Opening and closing AVF has limited effects on the heart rate in experimental dogs and rats (Nickerson *et al.*, 1951a; Huang *et al.*, 1992; Wang *et al.*, 2003). Guyton & Sagawa (1961) reported an average increase of 13% in heart rate upon the opening of the fistula with variation of 0 to 87%. Similar results have been reported in rats (Liu *et al.*, 1991). Flaim *et al.* (1979) however recorded significant reduction of the heart rate 2 months after creation of the AVF. A number of reasons may account for the contradictory findings which include 1) different species, 2) size of the fistula, and/or 3) different length of recovery following introduction of AVF (Flaim *et al.*, 1979).

In rats with chronic AVF, left ventricular function is depressed as evidenced by a

reduction in left ventricular dP/dt and left ventricular systolic pressure, and an increase in the left ventricular end diastolic pressure (Liu *et al.*, 1991; Wang *et al.*, 2003). According to Wang *et al.* (2003), no changes in cardiac contractility were detected at the first, second, and fourth weeks following the introduction of AVF, but thereafter, a progressive decline in cardiac contractility was observed based on dP/dt values recorded. The right ventricular dP/dt_{max}, right ventricular systolic pressure and right ventricular end diastolic pressure are all found to be significantly increased at both 1 week and 1 month after creation of fistula (Liu *et al.*, 1991). In isolated rat heart, the deviation of these haemodynamic values from the norm occurs earlier (4 vs 8 week) and is more dramatic compared with those from intact rats (Wang *et al.*, 2003). This can perhaps be best explained by the existence of compensatory mechanism in vivo (Wang *et al.*, 2003).

In experimental dogs, total blood volume is increased several weeks after creation of AVF (Epstein & Ferguson, 1955). This is accompanied by an increase in plasma volume but not the total red cell volume (Epstein & Ferguson, 1955; Gratama *et al.*, 1992). There is a positive correlation between the increase in plasma volume and the magnitude of the fistula flow ($r = 0.81$, $P < 0.01$) (Gratama *et al.*, 1992). Renal retention of salt and water stimulated by reduced renal flow is believed to be the contributory factor for the volume expansion (Hilton *et al.*, 1955; Gratama *et al.*, 1992). In addition, the amount of intravascular protein is found to be significantly higher in

lambs with AVF (5.0 +/- 0.3 vs. 3.5 +/- 0.2 g/kg body mass, $P < 0.001$) (Gratama *et al.*, 1992). The increased amount of intravascular protein provide oncotic pressure to retain fluid in the vascular compartment (Gratama *et al.*, 1992). The latter fact may be another mechanism responsible for the plasma volume expansion (Gratama *et al.*, 1992).

Once the fistula is introduced, an additional volume of blood leaks into the venous side. This is believed to be the contributory factor to increase PGVR. When the size of the fistula is relatively small, that is, the volume of the blood diverting through it is less than 19% of control cardiac output, the increase in venous return to the right heart equals that of the fistula flow (Warren *et al.*, 1951). According to the Frank-Starling mechanism, the heart pumps out the extra venous return to prevent the reduction of the systemic flow. If the size of the fistula further enlarges, there will not be sufficient blood supply for the pumping heart (Warren *et al.*, 1951). The flow to the systemic organs is then reduced and shunted to the venous side through fistula. According to Guyton *et al.* (1973e), the venous return is almost directly proportional to the PGVR as long as the resistance of the circulation remains constant. In dogs, P_{mcf} is significantly increased upon opening of the fistula due to the sympathetic nerve activation (Guyton & Sagawa, 1961). Opening of the fistula also has a slight net increase in right atrial pressure (Guyton & Sagawa, 1961). However, other researchers did not find any change in the atrial pressure (Nickerson *et al.*, 1951b). The variation of the fistula size might explain the different

observations regarding changes in the atrial pressure. In conclusion, AVF increases venous return due to the rise of PGVR.

In summary, a high cardiac output state is generated by the existence of AVF. In such hyper-haemodynamic condition, 1) TPR decreases; 2) heart rate and cardiac contractility do not change significantly in acute AVF but cardiac function exhausts in the chronic state; 3) a simultaneous increase occurs in P_{mcf} and total blood volume. The augmentation of cardiac output is caused by a decrease in TPR and an increase in PGVR. Blood volume expansion also contributes to the elevation of cardiac output in chronic AVF.

1.6. Nature of the Problem

Although the pathophysiology of AVF on the circulatory system has been well described, very few pharmacological studies have been conducted to document the effect of vasoactive agents in this paradigm.

It has been reported that intravenous administration of 1 μg/kg of noradrenaline increased cardiac output in dogs with AVF (Nakano, 1968). This dose of noradrenaline decreased heart rate by 20 beats/min following an initial increase of 12 beats/min. (Nakano, 1968). As well it increased mean arterial pressure, shunt flow, and systemic flow (Nakano,

1968). Sympathomimetics such as adrenaline was found to increase cardiac output, systemic flow, fistula flow, and heart rate in volume overload dogs (Nakano, 1968). Also, adrenaline increases the arterial pressure due to increases in cardiac output and TPR in the same animal model (Nakano, 1968).

In conscious dogs with AVF, intravenous infusion of SNP at 1.2 μg/kg/min decreased left ventricular end diastolic pressure without any significant change in left ventricular end diastolic dimension or left ventricular dP/dt_{max} (O'Rourke *et al.*, 1982). A dose of 1.8 μg/kg/min SNP seemed to further decrease left ventricular end diastolic pressure, left ventricular systolic pressure and aortic pressure (O'Rourke *et al.*, 1982). A fall in left ventricular end diastolic dimension and an increase in left ventricular dP/dt_{max} also occurs during administration of SNP (O'Rourke *et al.*, 1982). The reduction in the left ventricular filling pressure was suggested to be the result of the reduced venous return due to venodilation (O'Rourke *et al.*, 1982). The rise in left ventricular dP/dt_{max} at the dose of 1.8 μg/kg/min may be caused in part by baroreceptor reflex activation because the elevation of dP/dt_{max} disappeared following the treatment with a beta blocker (O'Rourke *et al.*, 1982).

Nitroglycerin (30 μg/kg) and bradykinin increased cardiac output, systemic flow while reducing fistula flow, heart rate and arterial pressure in dog with large AVF (Nakano,

1968). Consistently, in the rat model of chronic AVF (6.4 months), cardiac output was not altered by nitroglycerin infusion at 32 μg/kg but decreased significantly at 2 and 8 μg/kg (Flaim, 1982). In the latter study, there was a significant increase in systemic vascular resistance while the mean arterial pressure and central venous pressure did not change (Flaim, 1982). The vasodilating effect of nitroglycerin may be masked by the increased sympathetic tone to maintain the blood pressure (Flaim, 1982). In contrast, nitroglycerin (8 μg/kg) did not significantly increase cardiac output due to reduced systemic vascular resistance in acute AVF rats (20 days). The contradictory results of the systemic vascular resistance response in acute vs. chronic AVF rats to nitroglycerin might be that both fistula and nitroglycerin is tending to reduce vascular resistance but the sympathetic activation was not sufficient enough to overcome the resistance lowering effect from both of them (Flaim *et al.*, 1981). In chronic AVF, blood volume expansion and venous return elevation compensated the low resistance caused by AVF and return blood pressure and sympathetic tone to normal. Therefore, vascular resistance was increased by reflex activation of sympathetic nervous system during nitroglycerin infusion in animals with chronic AVF.

Isoproterenol which is a hypotensive agent in normal dogs increased arterial pressure in fistula dogs by enhancing cardiac output and increasing systemic flow, fistula flow, heart rate, myocardial contractility, and venous return while reducing TPR (Nakano, 1968). It

is suggested that flow through the fistula is always positively related to systemic arterial pressure but is not influenced by changes in either systemic blood flow or cardiac contractility (Nakano, 1968). In addition, it is reported that transdermal glyceryl trinitrite increased the blood flow through the surgically generated fistula in patients receiving haemodialysis therapy (Akin *et al.*, 2002).

1.7. Objectives

A working hypothesis for the study based on Guyton's theory of cardiac output regulation (Guyton, 1981) would predict infusion of noradrenaline will increase cardiac output by enhancing heart pumping performance and increasing venous return which overcomes the elevation of TPR; AVP would be expected to decrease cardiac output due to increment in TPR without any change in venous return; and SNP to a) increase cardiac output by decreasing TPR; b) decrease cardiac output by reducing venous return; or c) maintain the cardiac output by simultaneous reduction of TPR and elevation of venous return. In the latter cases, cardiac output decreases whenever resistance to venous return increases. However, Guyton's logic was based on experimental data from normal animals. In such a condition as chronic AVF, lack of venous return is not a limiting factor to control cardiac output anymore. On the contrary, the blood flow from the venous side is at a relative high level and drives the heart to pump super-normally according to the Frank-Starling mechanism. To pump all the venous return becomes the

dominant factor of cardiac output regulation before the heart fails. The other point that does not occur in Guyton's experimental animal is that the TPR is set at a very low level due to the existence of fistula. According to Ohm's law no matter how strong an effect of vasoactive agents on the systemic vessels, the resistive load against the pumping heart will not change as much as in animals without fistula. Since the situation we were dealing with is totally different from which Guyton's theory was based on, we may expect different results. Therefore, the aim of the present study was to investigate how cardiac output and other circulatory factors alter during the administration of vasoactive agents.

2. Materials and Methods

2.1. Preparation of animals with arteriovenous fistula

We have adopted the method described by Garcia and Diebold (1990) for the introduction of a shunt between abdominal aorta and inferior vena cava by inserting a 20-gauge needle from the abdominal aorta into inferior vena cava in order to create the arteriovenous shunt. 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/ml/kg) by injecting heparin subcutaneously. A laparotomy was performed and both vena cava and abdominal aorta were exposed. At positions caudal to the renal arteries and cephalic to the aortic bifurcation, two vascular clamps were placed on both vena cava and abdominal aorta. Subsequently, the aorta was punctured with a 20 gauge disposable needle (Becton–Dickinson, Franklin Lakes, NJ) at the place two thirds caudal to the renal artery and one third cephalic to the aortic bifurcation (Garcia & Diebold, 1990; Huang *et al.*, 1994). 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 one 6-0 vascular suture (Prolene, Johnson & Johnson, Somerville, NJ). The vascular clamps were removed 30 s after completion of the anastomosis. The whole process of circulation

cessation was less than 5 minutes. The patency of the fistula was verified visually by 1) swelling of the vena cava; 2) the mixing of the bright arterial blood with dark venous blood. Subsequently, the internal abdominal wall was closed with absorbable suture (Surgigut, Norwalk, CT). The skin cut was closed by single stitches with silk (3-0). Each animal was allowed to recover for five weeks. For 8 sham-operated rats, the needle was inserted into the abdominal aorta but not pushed into the inferior vena cava. Following this surgical procedure, each animal was treated with an analgesic, buprenorphine (0.5 mg/kg s.c.; q12 hr for 48 hr).

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 of mean arterial pressure. Subsequently, sodium pentobarbitone (65 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 (Schenk *et al.*, 1992) and injection of radioactive-labeled microspheres. A saline-filled balloon-tipped catheter was advanced into the right atrium through the right external jugular vein. Inflation of the balloon transiently stops the circulation and the cessation of circulation is necessary for the P_{mcf} measurement (Pang & Tabrizchi, 1986). Afterward, a laparotomy was performed and the abdominal aorta and inferior vena cava were exposed. A transit sonic flow probe was placed on the aorta cephalic to the fistula. The blood flow in the aorta above fistula is regarded as an index of flow through the fistula. Finally, the abdominal cavity was closed. All catheters were filled with heparinised (25 IU/ml) normal saline (0.9% NaCl). The animals were tracheotomized and allowed to stabilize for 1 hr when arterial pressure, venous pressure, ventricular pressure, fistula flow index 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 and central venous pressure were recorded with a pressure transducer (Gould Statham, USA; Model PD23B). The pressure transducer was connected to an amplifier (DA 100A) that in turn was linked to a universal interface module (UIM 100). Meanwhile, the interface module was interfaced with an acquisition unit (MP 100) (Tabrizchi, 2001). 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 (Tabrizchi, 2001).

2.3. Measurement of cardiac output

This technique has been described in detail elsewhere (Pang, 1983; Tabrizchi & Pugsley, 2000). Briefly, suspensions of micro-spheres (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 mean circulatory filling pressure

The technique of P_{mcf} measurement developed by Yamamoto *et al.* (1980) was employed in the present study. In detail (Yamamoto *et al.*, 1980; Pang & Tabrizchi, 1986), by injecting a small volume of fluid into the balloon, which was pre-positioned in the right atrium, for a period of 5-7 s, the blood flow through the right atrium was stopped. As a consequence, the circulation was arrested completely. Right after the balloon inflation, mean arterial pressure decreased and central venous pressure increased simultaneously.

Both pressures reached plateau levels within 4-5 s which are referred to as final arterial pressure (FAP) and venous plateau pressure (VPP) respectively.

2.5. 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 heparinised 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 - $(\text{Hct} \times \text{tp} \times F_{cells})$.

2.6. Measurement of the organ weight

After completion of each experiment and under anaesthesia, the rat was euthanized by

opening the chest. Heart, lung, and both kidneys 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 hrs and the dry weight was also recorded.

2.7. Experimental designs and protocols

After the surgery of AVF introduction, the body weight was recorded every day. The weights were compared between shunted and sham-operated groups. The existence of AVF was proved by a higher fistula flow index which is the flow in the abdominal aorta cephalic to the fistula. Five weeks after the introduction of AVF, shunted rats were randomly assigned to four groups (n = 5-7). There are 8 sham-operated rats working as control group. After surgical preparation, animals were stabilized for 1 hr when arterial pressure, venous pressure, ventricular pressure, fistula flow index and heart rate were monitored continuously. Subsequently, they were infused with one of the following solutions: saline (0.9% NaCl; 0.002, 0.006 and 0.02 ml/kg/min), noradrenaline (1, 3, 10 μg/kg/min), AVP (10, 30, 100 ng/kg/min), or SNP (100, 300, 1000 ng/kg/min) respectively at three increasing doses. The duration of each infusion at each dose was 18-20 min. Two sets of control measurement which constitutes of cardiac output and P_{mcf} measurements and 100-μl-blood sample were taken prior to administration of saline/drug and another three sets of measurements were repeated during the saline/drug

administration. An additional group (n=8) that was composed of sham-operated rats was infused with saline at the rate of 0.002, 0.006 and 0.02 ml/kg/min.

2.8. Chemicals

All drugs were made up fresh daily and dissolved in normal saline (0.9% NaCl). Noradrenaline was purchased from Sigma Chemical Company (St Louis, MO, U.S.A.), AVP from Sigma Chemical Company (Ontario, Canada), and SNP from Research Biochemicals International (Natick, MA, U.S.A.).

2.9. 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; systemic flow (ml/min) was calculated as cardiac output divided by fistula flow index. CI is cardiac output per 100 g body weight, systemic flow index is systemic flow per 100 g body weight, and fistula flow index is fistula flow per 100 g body weight. P_{mcf} (mmHg) was calculated as the one sixtieth of difference between final arterial pressure and venous plateau pressure plus venous plateau pressure (Samar & Coleman, 1978; Yamamoto *et al.*, 1980). Resistance to venous return (mmHg $\times$ min/ml) was calculated as the difference of P_{mcf} and central venous pressure divided by cardiac output (Bower & Law, 1993), and arterial resistance (mmHg $\times$ min/ml) was obtained by dividing blood pressure by cardiac output

(Tabrizchi & Ford, 2004); systemic resistance (mmHg × min/ml) was calculated by blood pressure divided by the difference between cardiac output and fistula flow; resistance of fistula (mmHg × min/ml) was calculated as the difference of blood pressure and central venous pressure divided by cardiac output.

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 (El Sayed *et al.*, 1995). In two groups of experiments using sham operated rats (n=21) and fistula rats (n=32), 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 hr (at 2, 60, 90, 120 min). A plot of Log [Evans blue dye] in plasma against time indicated first order elimination kinetics ($C = C_0e^{-kt}$), and the information from the plot were 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 ± SEM; n = 21) for α and β respectively in sham-operated rats and 0.2912 ± 0.0209 and 0.1791 ± 0.0132 (mean ± SEM; n = 32) 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. In all cases, a probability of error less than 0.05 was selected as the criterion for statistical significance.

3. Results

3.1. Haemodynamics in rats with chronic AVF

There was no difference between the daily body weights of rats with AVF and sham-operated rats (Figure 1). The control value of CI of rats with chronic AVF was 36.65 ± 2.28 ml/min per 100 g (mean ± SEM; n = 24) in comparison to 20.04 ± 0.86 ml/min per 100 g in sham-operated rats (mean ± SEM; n = 8) (Table 1). The value was 82.9% higher than the mean CI of sham-operated rats. In rats with chronic AVF, blood pressure, pulse pressure and venous pressure were not significantly changed from sham-operated rats (Table 2). Both TPR and resistance to venous return decreased significantly in rats with chronic AVF compared to sham-operated rats (Table 3). Heart rate was not significantly altered in rats with chronic AVF and dP/dt was found to be at the same level when compared to the respective value in sham-operated group (Table 1). The fistula flow index (i.e. blood flow in abdominal aorta just below the renal artery) and P_{mcf} were significantly higher in AVF group than the respective values in sham-operated group (Table 1, 2).

3.2 Haemodynamic effects of noradrenaline in rats with chronic AVF

The baseline values are given in Table 4. All three doses of noradrenaline

Table 1. Cardiac index (CI; ml/min per 100 g), fistula flow index (FFI; ml/min per 100 g), heart rate (HR; beat/min) and dP/dt (mmHg/sec) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (n = 6; 5 weeks post shunt) and sham-operated rats (n = 8) infused with saline (C, control measurement; M1, measurement 1 during the fist dose of infusion; M2, measurement 2 during the second dose of infusion; M3, measurement 3 during the third dose of infusion). The duration of each infusion was 18-20 min. Each value represents mean ± SEM.

		CI	FFI	HR	dP/dt
Fistula	C	36.65 ± 2.28*	24.97 ± 3.44*	339 ± 11	6277 ± 362
	M1	36.96 ± 3.33*	25.28 ± 3.38*	342 ± 16	6169 ± 483
	M2	36.11 ± 2.46*	23.85 ± 3.24*	345 ± 9	6117 ± 414
	M3	36.81 ± 3.58*	22.89 ± 3.55*	346 ± 12	6099 ± 348
Sham	C	20.04 ± 0.86	2.03 ± 0.40	333 ± 11	6373 ± 351
	M1	21.32 ± 1.05	1.87 ± 0.37	318 ± 21	6445 ± 510
	M2	20.09 ± 0.75	1.85 ± 0.33	318 ± 16	6556 ± 507
	M3	20.21 ± 0.95	1.87 ± 0.32	309 ± 14	6282 ± 430

***Significant different from respective value in sham-operated group; $P < 0.05$**

Table 2. Blood pressure (BP; mmHg), pulse pressure (Pulse P; mmHg), venous pressure (VP; mmHg), and mean circulatory filling pressure ($P_{mcf;}$ mmHg) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (n = 6; 5 weeks post shunt) and sham-operated rats (n = 8) infused with saline (C, control measurement; M1, measurement 1 during the first dose of infusion; M2, measurement 2 during the second dose of infusion; M3, measurement 3 during the third dose of infusion). The duration of each infusion was 18-20 min. Each value represents mean ± SEM.

		BP	Pulse P	VP	P_{mcf}
Fistula	C	97.17 ± 5.44	93.00 ± 9.93	2.15 ± 0.37	7.04 ± 0.50*
	M1	96.00 ± 6.17	93.17± 11.26	2.14 ± 0.39	6.88 ± 0.49*
	M2	94.17 ± 6.01	94.33 ± 11.48	2.10 ± 0.38	6.66 ± 0.36*
	M3	94.83 ± 5.71	99.67 ± 12.59	2.02 ± 0.48	7.09 ± 0.38*
Sham	C	104.9 ± 5.00	77.88 ± 7.94	1.27 ± 0.18	5.05 ± 0.14
	M1	105.9 ± 7.87	77.13 ± 7.47	1.29 ± 0.17	5.09 ± 0.21
	M2	110.0 ± 8.60	82.13 ± 6.66	1.19 ± 0.16	4.96 ± 0.18
	M3	109.25 ± 8.69	81.13 ± 8.18	1.13 ± 0.16	4.92 ± 0.18

***Significant different from the respective value in sham-operated group; $P < 0.05$**

Table 3. Total peripheral resistance (TPR; mmHg × min per ml) and resistance to venous return (RVR; mmHg × min per ml) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (n = 6; 5 weeks post shunt) and sham-operated rats (n = 8) infused with saline (C, control measurement; M1, measurement 1 during the first dose of infusion; M2, measurement 2 during the second dose of infusion; M3, measurement 3 during the third dose of infusion). The duration of each infusion was 18-20 min. Each value represents mean ± SEM.

		TPR	RVR
Fistula	C	0.5847 ± 0.038*	0.0274 ± 2.83 $\times 10^{-3}$*
	M1	0.5710 ± 0.081*	0.0273 ± 3.94 $\times 10^{-3}$*
	M2	0.5535 ± 0.051*	0.0262 ± 1.56 $\times 10^{-3}$*
	M3	0.5697 ± 0.077*	0.0287 ± 2.27 $\times 10^{-3}$*
Sham	C	1.1587 ± 0.063	0.0420 ± 3.23 $\times 10^{-3}$
	M1	1.1219 ± 0.125	0.0401 ± 3.58 $\times 10^{-3}$
	M2	1.1639 ± 0.084	0.0412 ± 2.27 $\times 10^{-3}$
	M3	1.2140 ± 0.126	0.0416 ± 2.54 $\times 10^{-3}$

Table 4. Baseline values of cardiac index (CI; ml/min per 100 g), fistula flow index (FFI; ml/min per 100 g), systemic flow index (SFI; ml/min per 100 g), blood pressure (BP; mmHg), pulse pressure (Pulse P; mmHg), venous pressure (VP; mmHg), mean circulatory filling pressure (P_{mcf}; mmHg), heart rate (HR; beat/min), dP/dt (mmHg/sec), total peripheral resistance (TPR; mmHg × min per ml), resistance to venous return (RVR; mmHg × min per ml), systemic resistance (Syst R; mmHg × min per ml), and resistance of fistula ($R_{fistula}$; mmHg × min per ml) before administration of noradrenaline (NA; n = 7); arginine vasopressin (AVP; n = 5); sodium nitroprusside (SNP; n = 6); saline (n = 6) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt). Each value represents mean ± SEM.

	NA	AVP	SNP	Saline
CI	38.04 ± 2.37	41.62 ± 3.32	39.36 ± 1.90	40.12 ± 4.09
FFI	21.74 ± 2.13	21.48 ± 3.05	23.13 ± 2.78	27.41 ± 3.88
SFI	16.28 ± 1.49	15.84 ± 2.30	20.43 ± 1.19	15.72 ± 1.96
BP	101.3 ± 5.93	96.8 ± 2.60	102.5 ±3.94	102.2 ± 5.12
Pulse P	92 ± 5.5	104 ± 8.2	105 ± 11.5	99.0 ± 10.85
VP	1.76 ± 0.33	1.72 ± 0.53	2.17 ± 0.45	1.66 ± 0.28
P_{mcf}	6.28 ± 0.36	6.64 ± 0.45	6.72 ± 0.35	7.22 ± 0.46
HR	340 ± 11.5	292 ± 4.9	332 ± 12.4	354 ± 11.4
dP/dt	6407 ± 315	6443 ± 252	6651 ± 302	6700 ± 230
TPR	0.5791 ± 0.0755	0.6640 ± 0.0769	0.4977 ± 0.0346	0.5772 ± 0.0412
RVR	$0.0252 \pm 2\times10^{-3}$	$0.0307 \pm 4\times10^{-3}$	$0.0230 \pm 3\times10^{-3}$	$0.0299 \pm 2\times10^{-3}$
Syst R	1.1955 ± 0.0853	1.4808 ± 0.2330	1.0659 ± 0.0735	1.4283 ± 0.114
$R_{fistula}$	1.0640 ± 0.1903	1.0815 ± 0.1959	0.9628 ± 0.1011	0.8384 ± 0.1122

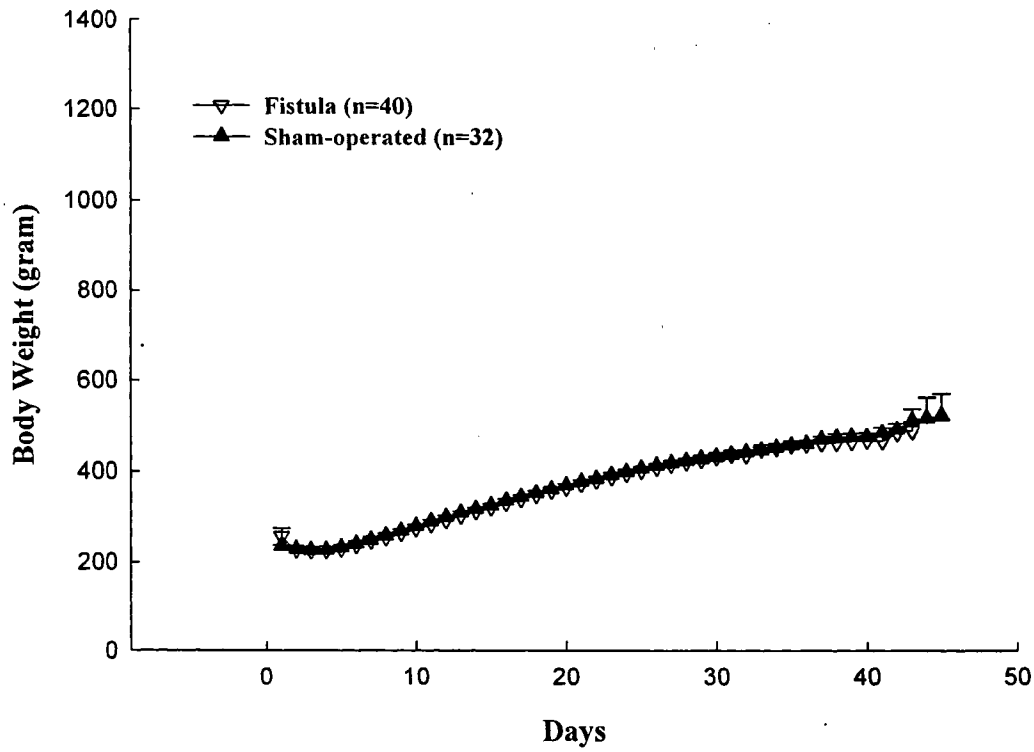

Figure 1 Daily body weight of rats with chronic arteriovenous fistula and sham-operated rats. Data represents mean value ± SEM.

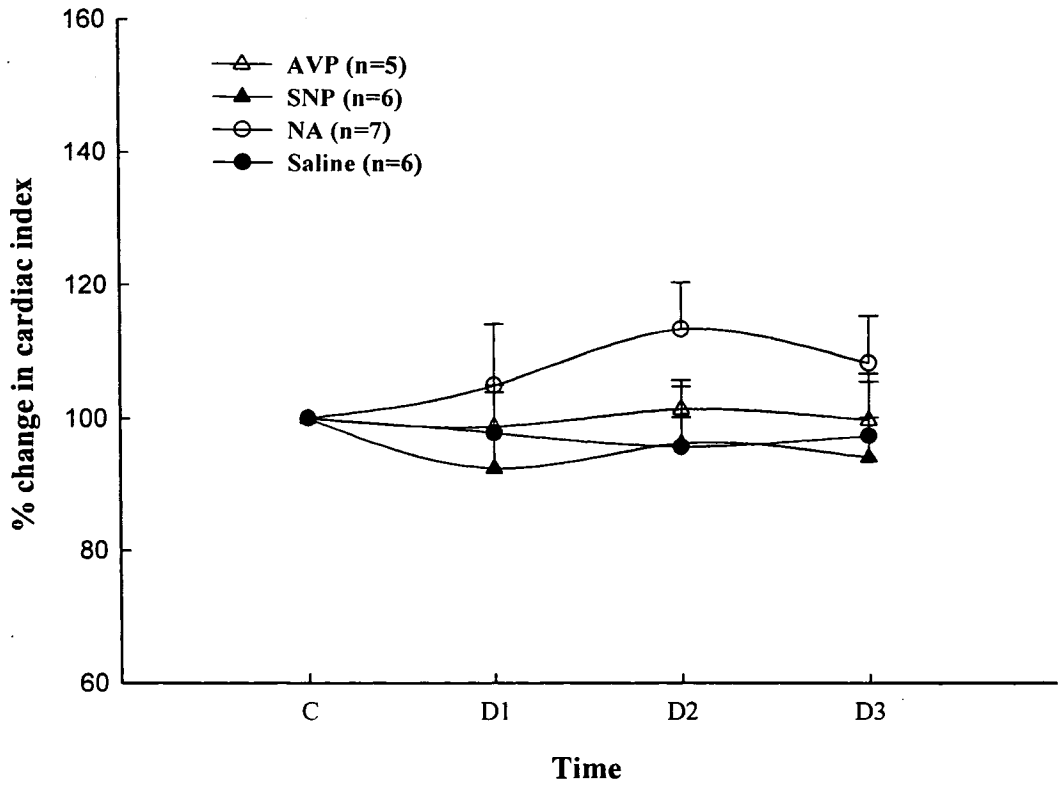

Figure 2 Percent change in cardiac index during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 μg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaethetized rats with chronic arteriovenous fistula (5 weeks post shunt). C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

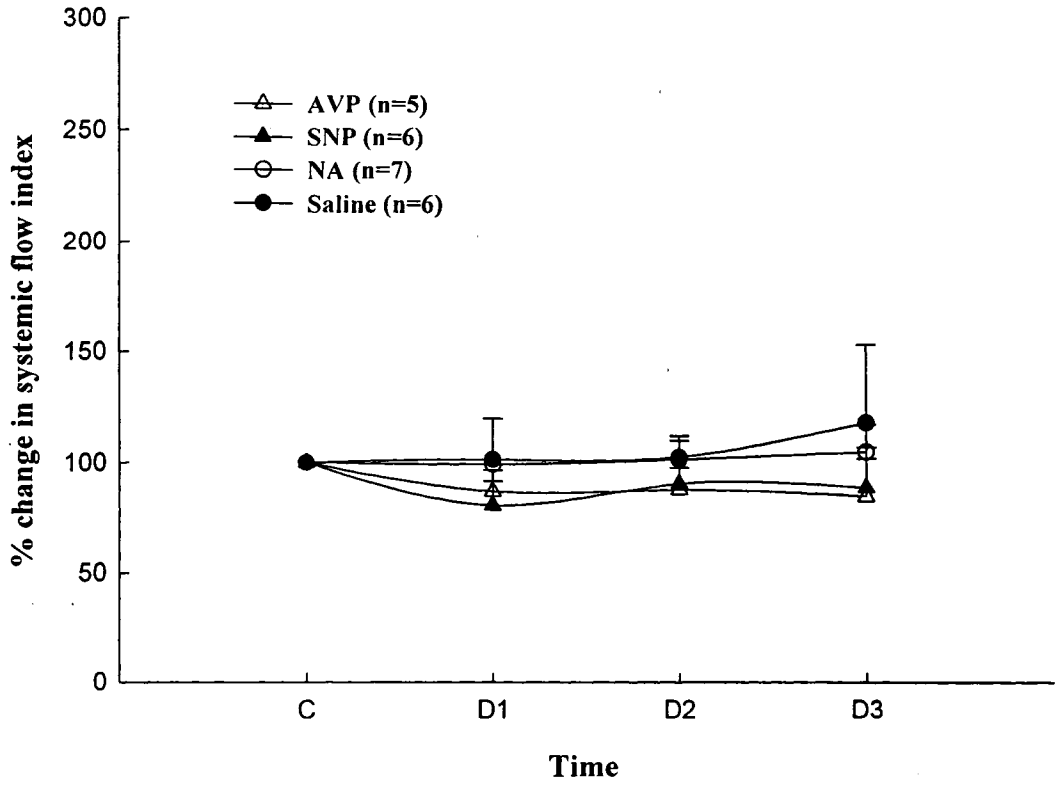

Figure 3 Percent change in systemic flow index during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 μg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaethetized rats with chronic arteriovenous fistula (5 weeks post shunt). C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

(1, 3, 10 μg/kg/min) did not significantly change CI and systemic flow index in rats with chronic AVF when compared to both baseline level and respective values in saline treated rats (Figure 2, 3). Fistula flow index was significantly increased compared to both baseline level and respective values in saline treated rats by all three doses of noradrenaline administration (Figure 4). As well, blood pressure was significantly increased by all three doses compared to both baseline level and respective values in saline treated rats (Figure 5). Similarly, pulse pressure was significantly increased compared to baseline level at all three doses and only increased significantly higher than respective values in saline treated group at the lowest and middle doses (Figure 6). Infusion of noradrenaline at the highest dose also increased venous pressure significantly when compared to both baseline level and the respective value in saline treated group (Figure 7). Administration of noradrenaline also significantly increased P_{mcf} compared to baseline level at all three doses (Figure 8). Moreover, administration of noradrenaline at the last two doses significantly increased P_{mcf} when compared to respective values in saline treated group (Figure 8). Administration of noradrenaline at the lowest and highest doses resulted in significantly increase in dP/dt compared to both baseline level and respective values in saline treated group (Figure 9). In contrast, dP/dt remained at the baseline level at the middle dose and was not significantly different from the respective value in saline treated rats (Figure 9). The middle and highest dose of noradrenaline significantly increased heart rate compared to both baseline level and

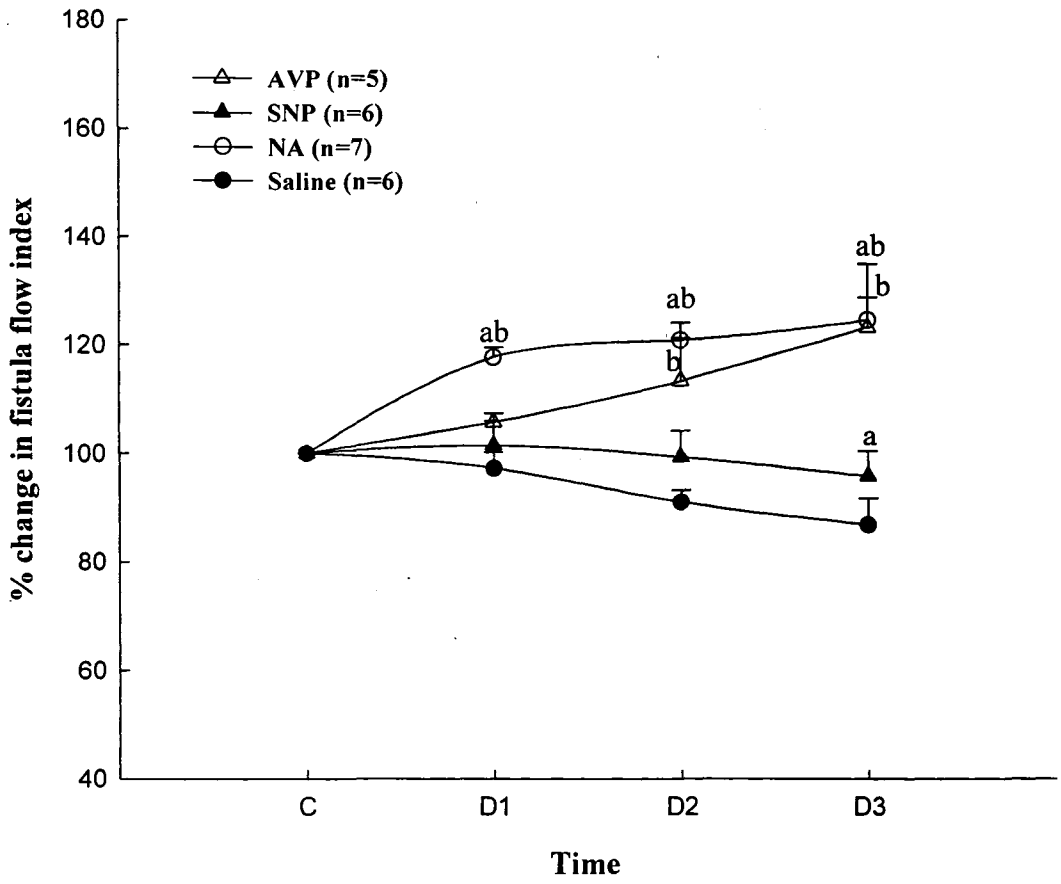

Figure 4 Percent change in fistula flow index during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 μg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; $P < 0.05$

[b]Significantly different from the respective value in saline treated group; $P < 0.05$

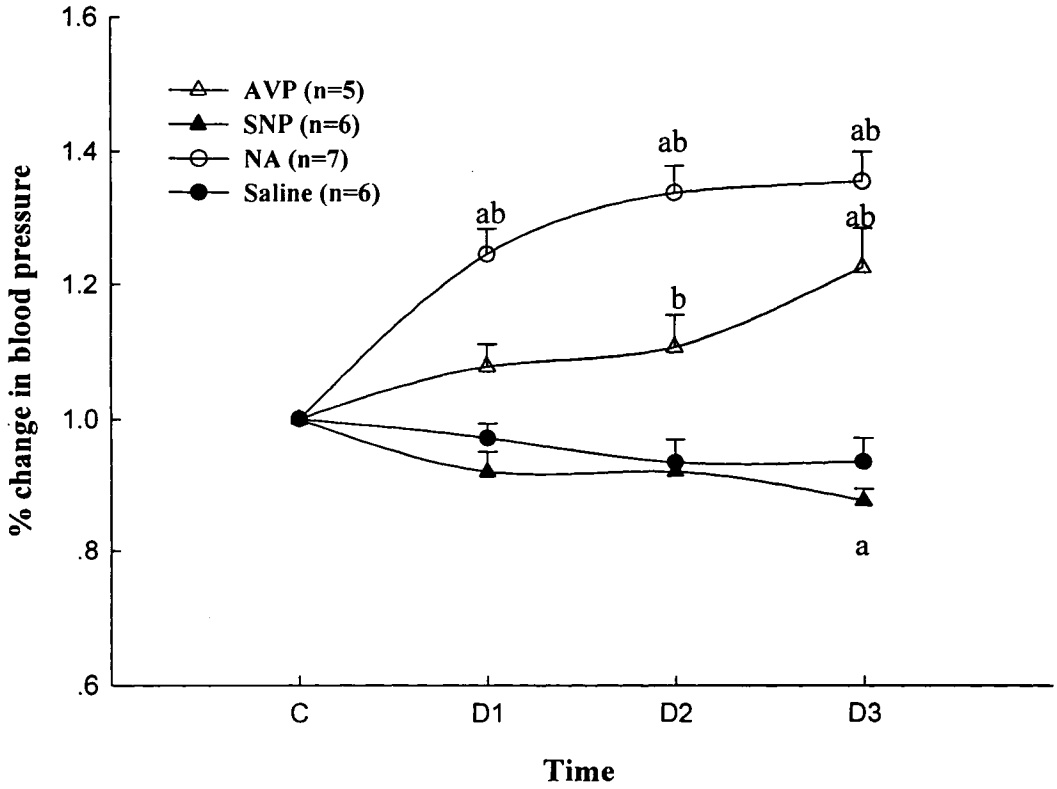

Figure 5 Percent change in blood pressure during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; P < 0.05

[b]Significantly different from the respective value in saline treated group; P < 0.05

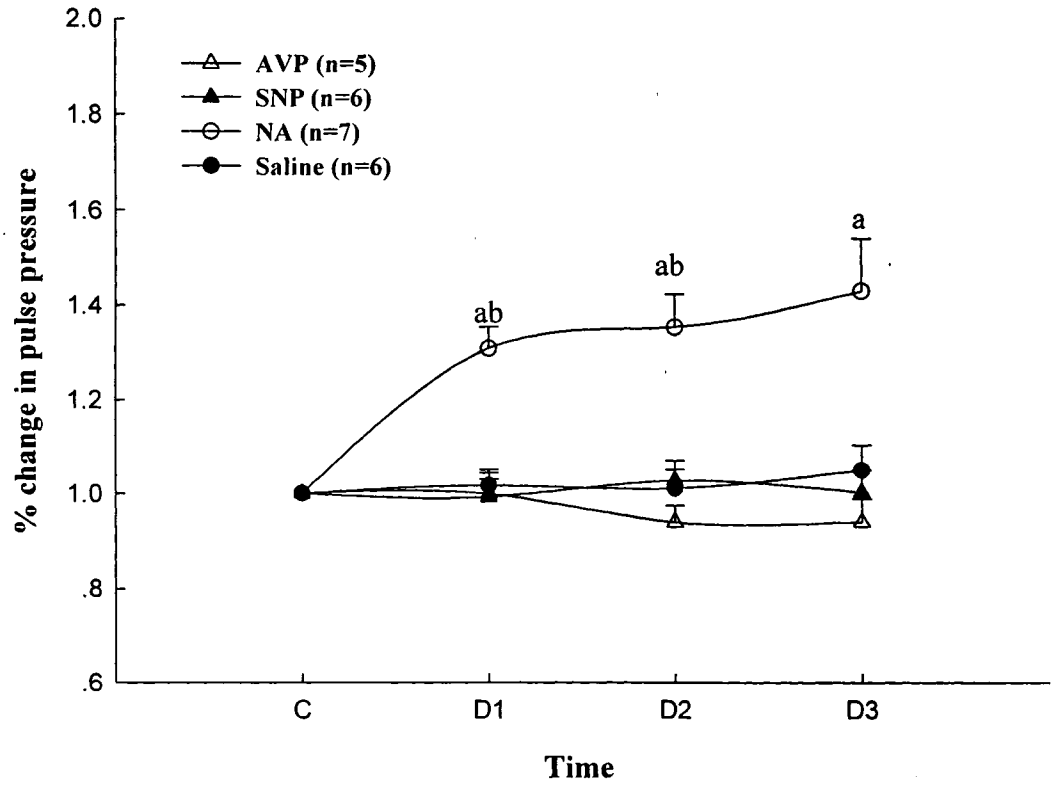

Figure 6 Percent change in pulse pressure during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 μg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; $P < 0.05$

[b]Significantly different from the respective value in saline treated group; $P < 0.05$

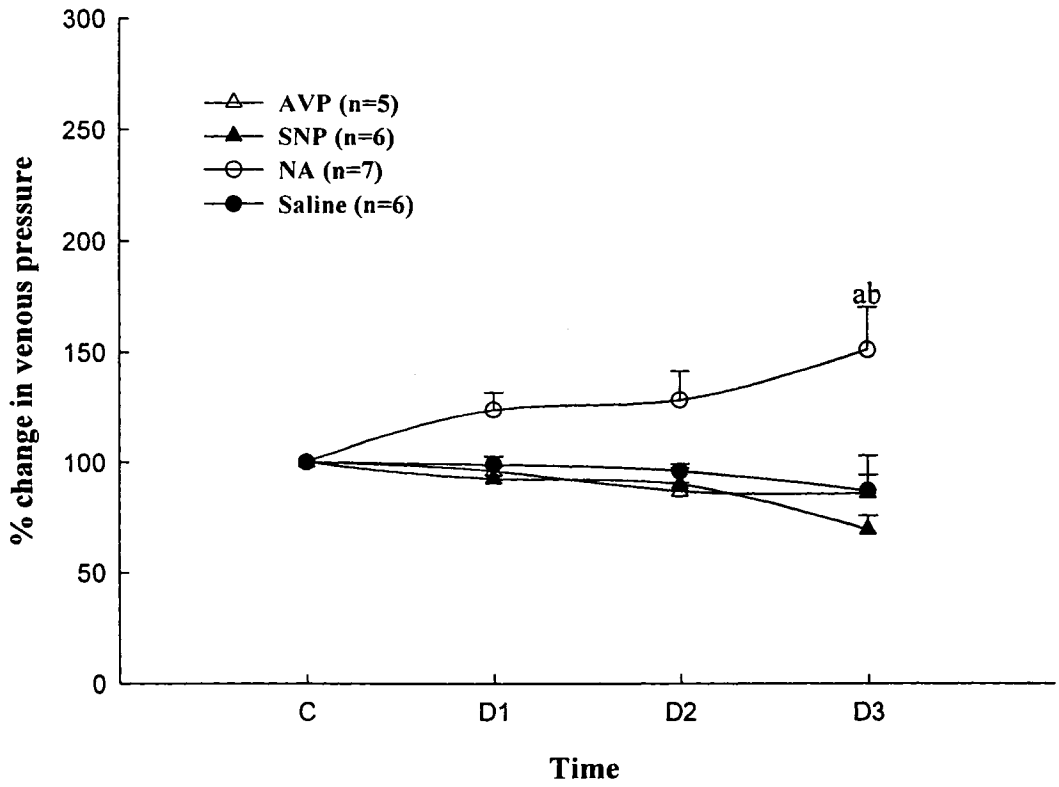

Figure 7 Percent change in venous pressure during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; P < 0.05

[b]Significantly different from the respective value in saline treated group; P < 0.05

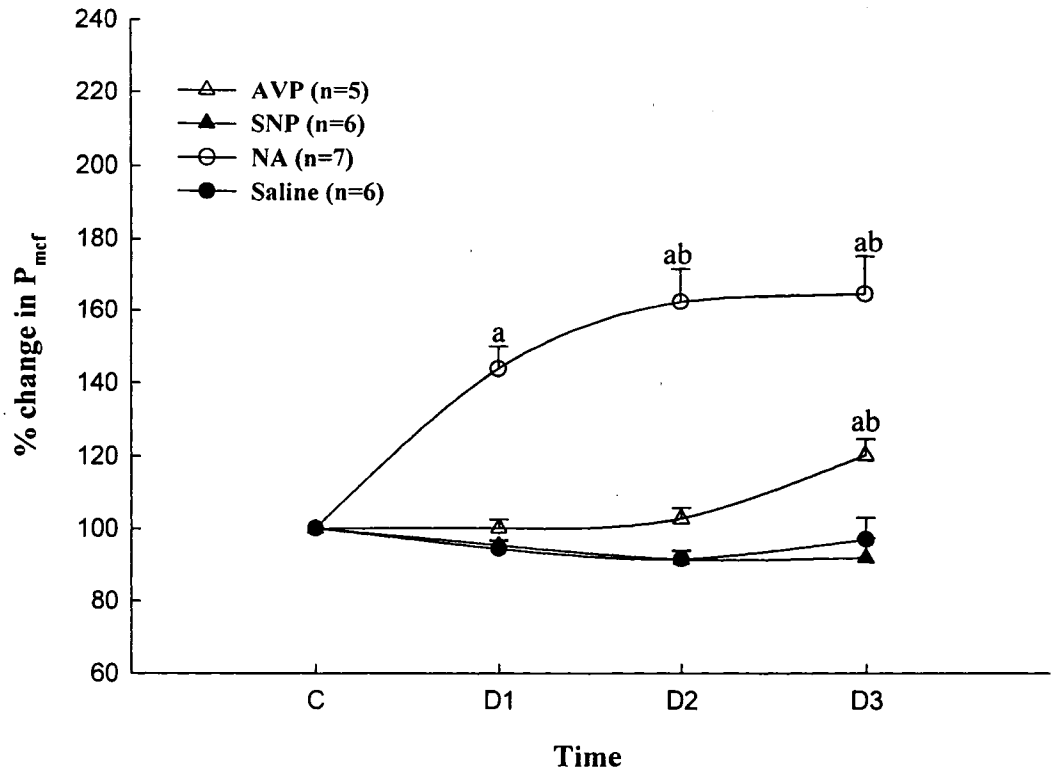

Figure 8 Percent change in P_{mcf} during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; $P < 0.05$

[b]Significantly different from the respective value in saline treated group; $P < 0.05$

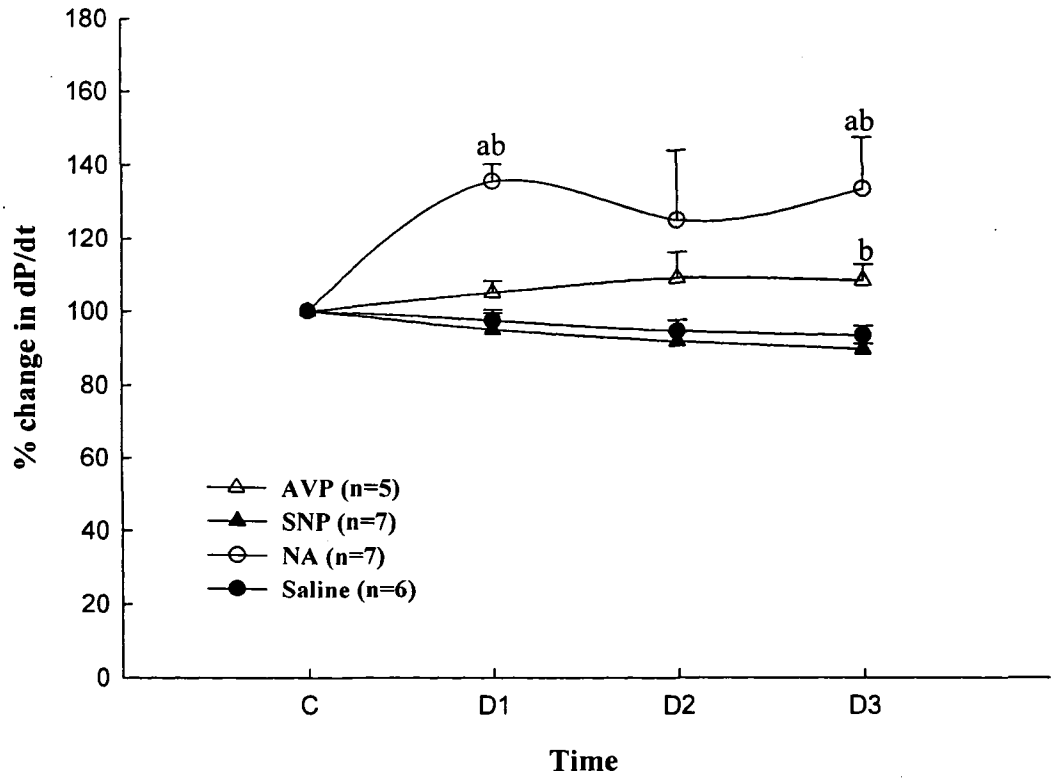

Figure 9 Percent change in dP/dt during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; P < 0.05

[b]Significantly different from the respective value in saline treated group; P < 0.05

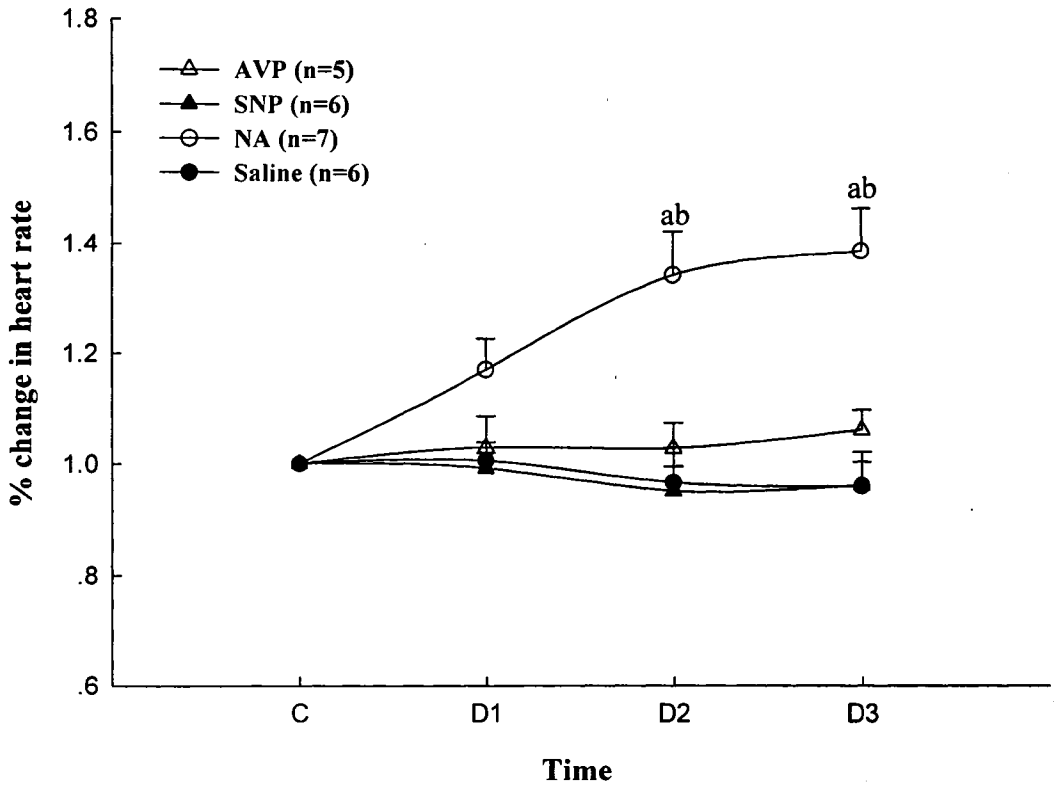

Figure 10 Percent change in heart rate during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; P < 0.05

[b]Significantly different from the respective value in saline treated group; P < 0.05

respective values in saline treated group (Figure 10). Noradrenaline infusion significantly increased TPR compared to baseline at the lowest and highest doses as well as elevated resistance to venous return at all three doses (Figure 11, 12). Moreover, infusion of noradrenaline also significantly increased resistance to venous return when compared to respective values in saline infused group at all doses (Figure 12). Infusion of noradrenaline did not change resistance of fistula when compared to either baseline level or respective values in saline treated rats (Figure 13). Systemic resistance was significantly higher compared to baseline level at the lowest and highest dose while it was only significantly higher than the respective value in saline treated group at the highest dose (Figure 14).

3.3. Haemodynamic effects of arginine vasopressin in rats with chronic AVF

The baseline values are available from Table 4. Impact of AVP on cardiac output was very limited (Figure 2). At the middle and highest doses of AVP infusion, fistula flow index increased significantly compared to respective values in saline treated group (Figure 4). However, there was not any alteration in systemic flow index compared to both baseline level and respective values in saline treated rats (Figure 3). Infusion of AVP at the last two doses significantly increased blood pressure when compared to respective values in saline treated group (Figure 5). Moreover, AVP infusion at the highest dose significantly increased blood pressure when compared to the baseline value

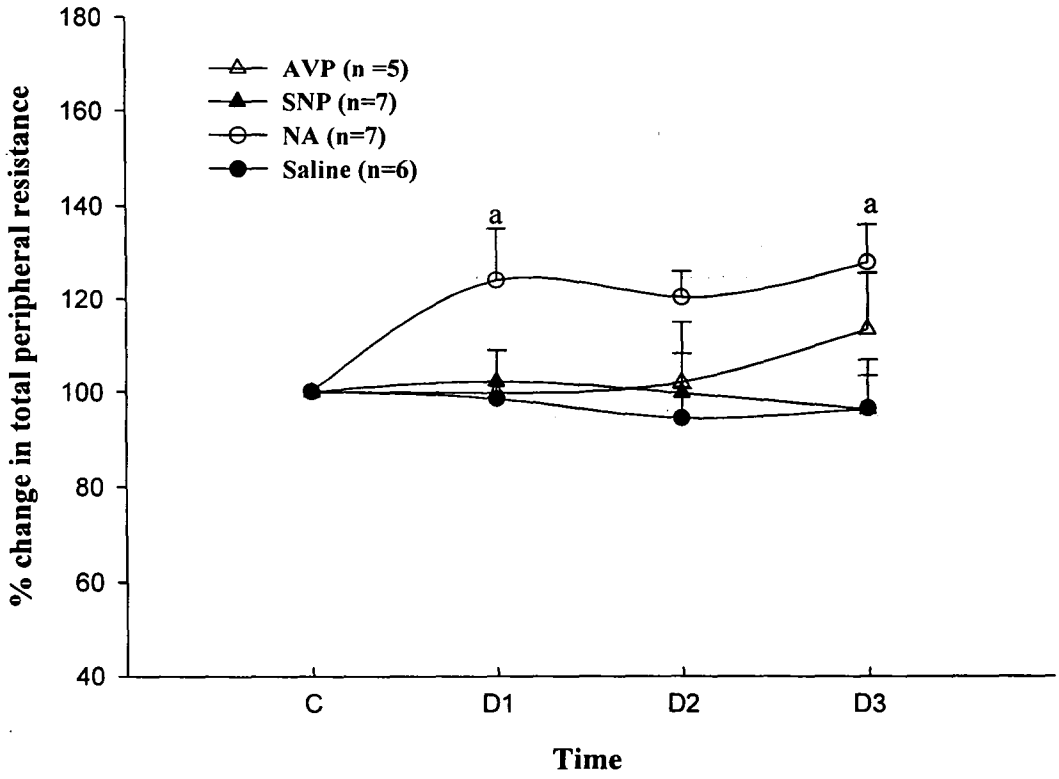

Figure 11 Percent change in total peripheral resistance during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; $P < 0.05$

[b]Significantly different from the respective value in saline treated group; $P < 0.05$

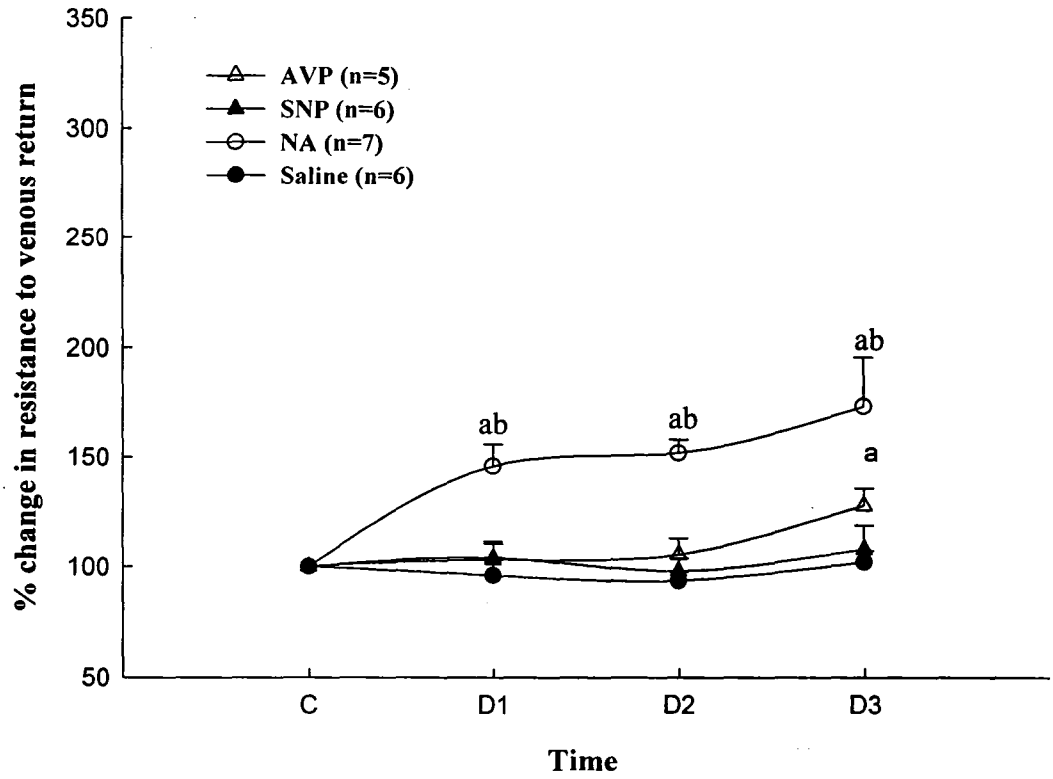

Figure 12 Percent change in resistance to venous return during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; P < 0.05

[b]Significantly different from the respective value in saline treated group; P < 0.05

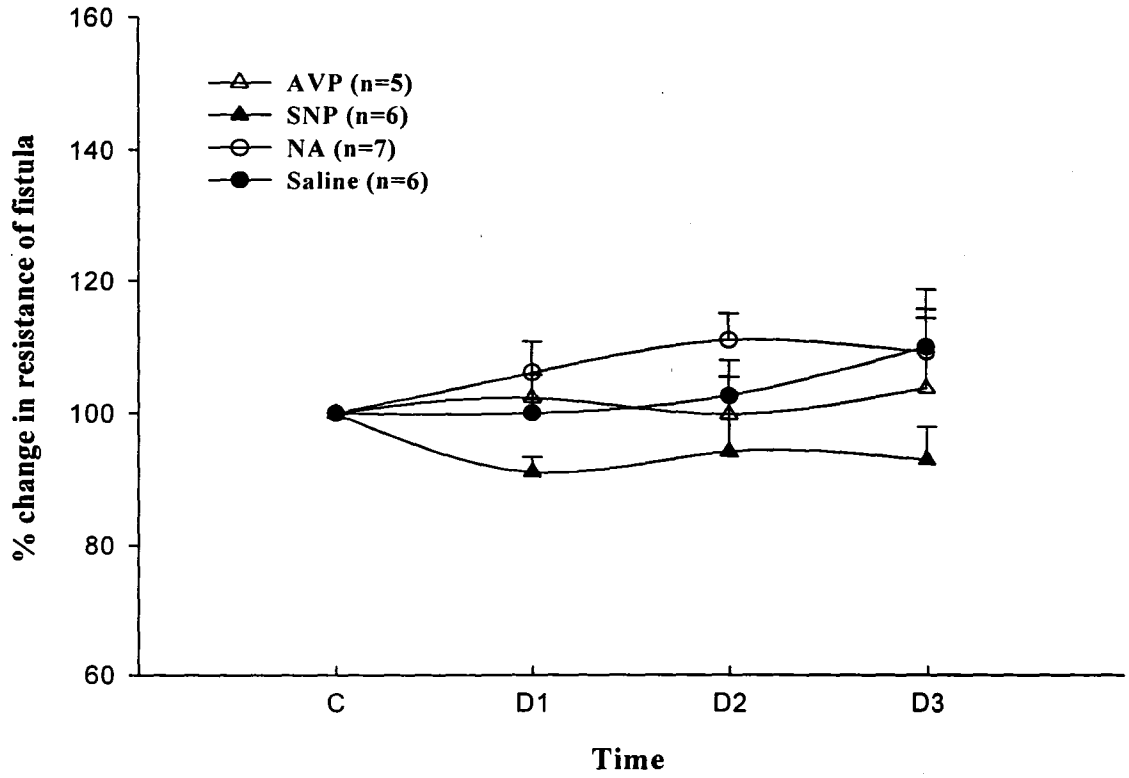

Figure 13 Percent change in resistance of fistula during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; $P < 0.05$

[b]Significantly different from the respective value in saline treated group; $P < 0.05$

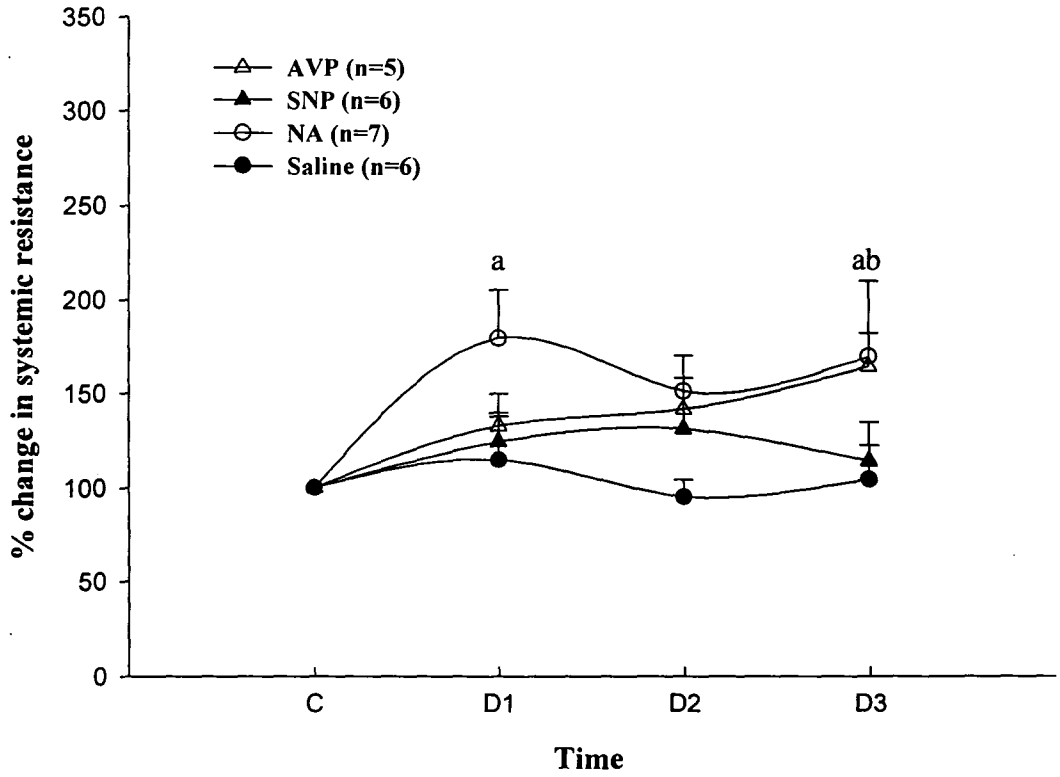

Figure 14 Percent change in systemic resistance during administration of arginine vasopressin (AVP; 10, 30, 100 ng/kg/min), sodium nitroprusside (SNP; 100, 300, 1000 ng/kg/min), noradrenaline (NA; 1, 3, 10 µg/kg/min), and saline (0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) in three increasing doses over the course of time. C, control; D1, dose 1; D2, dose 2; D3, dose 3. The duration of each infusion was 18-20 min. Data represents mean value ± SEM.

[a]Significantly different from the baseline level; $P < 0.05$

[b]Significantly different from the respective value in saline treated group; $P < 0.05$

(Figure 5). In contrast, administration of AVP did not significantly alter pulse pressure and venous pressure compared to both baseline level and respective values in saline treated rats (Figure 6, 7). The highest dose of AVP significantly increased P_{mcf} compared to both baseline value and the respective value in saline treated group (Figure 8). Infusion of AVP at the highest dose also significantly increased dP/dt when compared to the respective value in saline treated group (Figure 9). However, there was no alteration in heart rate during the infusion of AVP (Figure 10). At the highest administrated dose of AVP, resistance to venous return increased significantly when compared to the baseline level (Figure 12). Administration of AVP at the highest dose did not significantly change TPR and systemic resistance compared to both baseline levels and respective values in saline treated rats (Figure 11, 14). As well, resistance of fistula did not alter significantly compared to both baseline levels and respective values in saline treated rats (Figure 13).

3.4. Haemodynamic effects of sodium nitroprusside in rats with chronic AVF

The baseline values are available from Table 4. Administration of SNP did not change cardiac output significantly (Figure 2). While there was no change in systemic flow index, the highest dose of SNP administration significantly decreased fistula flow index when compared to the baseline level (Figure 3, 4). The highest dose of SNP significantly decreased blood pressure compared to the baseline level (Figure 5). In

contrast, administration of SNP did not alter pulse pressure (Figure 6). As well, infusion of SNP at the highest dose did not significantly change the venous pressure when compared to the respective value in the saline treated group (Figure 7). Similarly, SNP infusion had very limited effect on P_{mcf} (Figure 8). The highest administrated dose of SNP significantly decreased dP/dt when compared to the baseline level (Figure 9). In contrast, infusion of SNP did not alter heart rate (Figure 10). Similarly, infusion of SNP did not change TPR, resistance to venous return, systemic resistance and resistance of fistula when compared either to baseline level or respective values in saline treated group (Figure 11, 12, 13, and 14).

3.5. Haematocrit, plasma volume and total blood volume

In both fistula and sham-operated group, haematocrit decreased significantly compared to baseline level at the highest dose of saline infusion (Table 5). However, there is no significant difference between the two groups. Plasma volume and total blood volume were significantly lower in sham-operated group compared to respective values in fistula-saline treated group. AVP dose dependently decreased haematocrit compared to baseline level and respective values in saline treated group. Similarly, administration of AVP increased plasma volume significantly compared to baseline level. Moreover, the middle dose of AVP infusion increased total blood volume significantly when compared

Table 5 Haematocrit (Hct), plasma volume and total blood volume values during administration of noradrenaline (NA; n = 7; 1, 3, 10 μg/kg/min), arginine vasopressin (AVP; n = 5; 10, 30, 100 n4g/kg/min), sodium nitroprusside (SNP; n = 6; 100, 300, 1000 ng/kg/min), and saline (n = 6; 0.002, 0.006, 0.02 ml/min) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) and sham-operated rats infused with saline (n = 8; 0.002, 0.006, 0.02 ml/min) (C, control measurement; M1, measurement 1; M2, measurement 2; M3, measurement 3) (n =8; 0.002, 0.006, 0.02 ml/min). The duration of each infusion was 18-20 min. Each value represents mean ± SEM.

	Hct	Plasma volume	Total blood volume
NA			
C	0.53 ± 0.007	23.2 ± 0.99	37.1 ± 1.54
M1	0.53 ± 0.005	24.0 ± 1.59	38.6 ± 2.43
M2	0.54 ± 0.008	23.5 ± 1.63	37.9 ± 2.52
M3	0.54 ±0.009	23.2 ± 1.78	37.8 ± 2.79
AVP			
C	0.53 ± 0.012	20.6 ± 1.21	32.9 ± 2.00
M1	0.48 ± 0.009[a]	24.3 ± 1.48[a]	37.0 ± 2.17
M2	0.47 ± 0.007[ab]	24.8 ± 1.77[a]	37.3 ± 2.63[a]
M3	0.46 ± 0.007[ab]	24.8 ± 1.71[a]	36.8 ± 2.68
SNP			
C	0.54 ± 0.011	23.2 ± 1.22	37.7 ± 1.63
M1	0.52 ± 0.014	23.8 ± 1.46	37.8 ± 1.95
M2	0.52 ± 0.013[a]	25.5 ± 1.25[a]	40.4 ± 1.55
M3	0.51 ± 0.012[a]	26.5 ± 2.37[a]	41.5 ± 3.23
Saline			
C	0.53 ±0.011	22.2 ± 1.54	35.7 ± 2.25
M1	0.53 ± 0.011	20.8 ± 1.00	33.1 ± 1.46
M2	0.51 ± 0.008	21.5 ± 0.90	33.6 ± 1.33
M3	0.51 ± 0.007[a]	22.0 ± 1.12	34.3 ± 1.53
Sham			
C	0.54 ± 0.012	19.5 ± 1.00[b]	31.6 ± 1.87[b]
M1	0.51± 0.009	20.0 ± 1.26[b]	31.6 ± 1.88[b]
M2	0.51± 0.006	19.7 ± 1.25[b]	30.8 ± 1.92[b]
M3	0.50 ± 0.007[a]	19.2 ± 1.16[b]	29.8 ± 1.81[b]

[a]Significantly different from the baseline level; P < 0.05

[b]Significantly different from the respective value in saline treated group; P < 0.05

to the baseline level. As well, infusion of SNP decreased haematocrit and therefore increased plasma volume significantly compared to baseline level. In contrast, SNP infusion at all three dose levels did not significantly change total blood volume. Administration of noradrenaline had very limited effects on haematocrit, plasma volume, and total blood volume (Table 5).

3.6. Organ weight and body weight

The weight of the right ventricle in rats with chronic AVF (n =6) was 48.5% higher than the respective value in sham-operated group (n = 8). As well, left ventricle plus septum in rats with chronic AVF was 21.2% higher than respective value in sham-operated group (Table 6). Not surprisingly, the weight ratio of right ventricle to left ventricle plus septum increased significantly in rats with chronic AVF infused with saline when compared to the respective value in sham-operated group. In the group administrated with noradrenaline, the weights of left and right kidneys were heavier than respective values in saline treated group by 14.2% and 14.9% respectively (Table 6).

Table 6. Weights of wet lung (WL; g), dry lung (DL; g), weight ratio of dry lung to wet lung (DL/WL), weights of right kidney (RK; g), left kidney (LK; g), right ventricle (RV; g), left ventricle plus septum (LVS; g), and weight ratio of right ventricle to left ventricle plus septum (RV/LVS) after administration of noradranline (NA; n = 7), arginine vasopressin (AVP; n = 5), sodium nitroprusside (SNP; n = 6), and saline (n = 6) in thiobutabarbital anaesthetized rats with chronic arteriovenous fistula (5 weeks post shunt) and sham-operated rats infused with saline (n = 8; 0.002, 0.006, 0.02 ml/min) and mean body weight in each group (BW; g). Each value represents mean ± SEM.

	AVP	SNP	NA	Saline	Sham
WL	4.62 ± 0.44	3.54 ± 0.12	3.94 ± 0.44	3.50 ± 0. 30	3.07 ± 0.31
DL	1.02 ± 0.20	0.84 ± 0.05	1.20 ± 0.32	1.06 ± 0.22	0.82 ± 0.04
DL/WL	0.26 ± 0.04	0.23 ± 0.03	0.24 ± 0.01	0.28 ± 0.07	0.28 ± 0.03
RK	3.61 ± 0.19	3.35 ± 0.12	4.16 ± 0.09*	3.62 ± 0.19	3.48 ± 0.45
LK	3.72 ± 0.26	3.41 ± 0.12	4.03 ± 0.07*	3.53 ± 0.24	3.81 ± 0.13
RV	1.05 ± 0.06	1.05 ± 0.06	0.87 ± 0.11	0.98 ± 0.06	0.66 ± 0.04*
LVS	2.82 ± 0.09	2.59 ± 0.13	2.88 ± 0.07	2.63 ± 0.14	2.17 ± 0.06*
RV/LVS	0.37 ± 0.03	0.33 ± 0.02	0.32 ± 0.02	0.37 ± 0.02	0.30 ± 0.01*
BW	457 ± 18	480 ± 8	481 ± 14	480 ± 21	457 ± 16

***Significantly different from respective values in saline treated group; $P < 0.05$**

4. Discussion

In the present study, the haemodynamic changes in rats with chronic AVF were investigated. Specifically, the effect of vasoactive agents, NA, AVP, and SNP were studied. The most striking observation was the lack of significant change in cardiac output following treatment with vasoactive agents despite significant changes in preload and afterload in the presence of chronic AVF.

In accordance with Huang *et al.*, (1992) the daily increase in body weight of rats with chronic AVF was similar with the increment in body weight in sham-operated rats (Huang *et al.*, 1992). Perhaps, the two most reasonable explanations for such an observation are: 1) the rats with chronic AVF did not develop heart failure, therefore no fluid retention occurred; and 2) the rats with chronic AVF gained weight by fluid retention due to high output heart failure but tend to lose adipose tissue due to the stress of having a fistula. However, it is unlikely that the existence of heart failure was responsible for an edematous state since there was no change in the weight ratio of dry lung to wet lung between rats with chronic fistula compared to sham-operated rats.

The CI of rats with chronic AVF in the present study was 36.65 ml/min per 100 g which translated into an 82.9 % increase compared to the respective value in sham-operated group at baseline. The level of CI was higher than reported by Flaim *et al.* (1979) but

lower than that reported by Huang *et al.* (1992) and Liu *et al.* (1991). Similar to previous reports, blood pressure, pulse pressure and venous pressure were not significantly changed in rats with chronic AVF when compared to sham-operated rats (Guyton & Sagawa, 1961; Flaim *et al.*, 1979; Huang *et al.*, 1992). As expected, TPR decreased significantly in rats with chronic fistula. As well, resistance to venous return also decreased significantly in rats with chronic fistula compared to the respective value in sham-operated rats, while there is no report of this parameter being measured in rats with chronic AVF in the current literature. Elevation of PGVR and expansion of blood volume may be the contributory factors for the reduction in resistance to venous return. Also, chronic AVF had limited impact on the heart rate (Nickerson *et al.*, 1951a; Huang *et al.*, 1992; Wang *et al.*, 2003) and observations in the present study support the view. Although there is no apparent change in dP/dt in rats with chronic AVF compared to sham-operated rats, the alteration of cardiac contractility in rats with chronic AVF may have been masked by the simultaneous increase in preload and decrease in afterload. An increase in contractility could likely be the result of cardiac hypertrophy in rats with chronic AVF. This view is supported by the fact that the ratio of right ventricle weight to left ventricle plus septum weight increased in rats with chronic AVF (0.37 ± 0.03; mean ± SEM, $n = 6$) compared to the respective value in sham-operated rats (0.30 ± 0.01; mean ± SEM, $n = 8$). As reported previously, total blood volume and plasma volume increased significantly in rats with chronic fistula when compared to respective values in

sham-operated rats (Epstein & Ferguson, 1955; Gratama *et al.*, 1992). However, haematocrit in the present study did not change significantly which indicated a simultaneous elevation of blood cell volume. In contrast, Epstein & Ferguson (1955) reported a significant decrease in arterial haematocrit after creation of fistula in experimental dogs compared with haematocrit prior to fistula. Similarly, Gratama *et al.* (1992) recorded lower haematocrit in lambs with aorto-pulmonary left-to-right shunt than control. A reasonable explanation for the discrepancy could be due to difference in species, size and duration of the AVF. In accordance with the previous study done by Guyton & Sagawa (1961), we found an increase in P_{mcf} by 38.2% in rats with chronic AVF compared to P_{mcf} in sham-operated rats. The increase in P_{mcf} is most likely due to the increase in blood volume.

In the present investigation, the existence of chronic fistula increased cardiac output. The following haemodynamic factors changed compared to respective values in sham-operated rats: 1) TPR and resistance to venous return decreased; 2) heart rate did not change and there was no apparent change in cardiac contractility based on dP/dt values recorded; and 3) blood volume and P_{mcf} increased. The augmentation of blood volume, P_{mcf} and reduction in vascular resistance together contributed to the increase in cardiac output.

Here, administration of noradrenaline did not significantly increase CI, moreover, noradrenaline infusion also did not change systemic flow index while increasing fistula flow index. The latter observation contrast those reported by Nakano (1968) in which administration of noradrenaline increased cardiac output, shunt flow and systemic flow in dogs. This difference could be due to different species and experimental design. It is evident that Nakano (1968) quickly administrated noradrenaline (1 μg/kg) as a single bolus injection. In contrast, in the present study, noradrenaline was infused continuously for 18-20 min to reach a steady state. Similar to his study, blood pressure was significantly increased by all the three doses (Nakano, 1968). Contrary to our study, heart rate was decreased in Nakano's study. Since noradrenaline was administrated in a single dose injection, the positive chronotropic action of the drug might be masked by acute compensatory vagal reflex activity (Young *et al.*, 1992; Westfall & Westfall, 2006).

The positive inotropic effect of noradrenaline is mediated by activation of β-adrenoceptors in the human ventricular myocardium (Bristow *et al.*, 1990). However, in some cardiovascular diseases such as myocardial infarction, cardiomyopathy, the β-adrenoceptors are altered in a manner that the degree of cardiac contraction upon β-adrenergic activation is reduced (Bristow *et al.*, 1990). The chronic exposure to increased amounts of neurotransmitter released upon myocardial sympathetic activation may be responsible for the insensitivity of β-adrenoceptors (Bristow *et al.*, 1990). In

contrast, Wang *et al.* (2003) reported a hypersensitivity to β-adrenoceptor stimulation in rats with chronic AVF. Similarly, in the present study we find that dP/dt is increased in rats with chronic AVF during noradrenaline infusion. The increase in the degree of cardiac contraction is suggested to be caused by the up-regulation of β_1-adrenoceptor density in the heart (Wang *et al.*, 2005). Such unique change in β_1-adrenoceptor density in the rat with chronic AVF compared to other cardiovascular stresses may be related to the lack of direct injury to the heart or associated with decreased catecholamine level (Wang *et al.*, 2005).

Administration of noradrenaline in rats with chronic AVF also elicits different haemodynamic effects when compared with normal rats. In rats with chronic AVF, TPR and systemic resistance were significantly elevated at the lowest and highest dose while these were maintained at the control level at the middle dose. The slightly increase in cardiac output at the middle dose of noradrenaline infusion may lower the vascular resistance. During noradrenaline administration, resistance of fistula did not change due to the simultaneous increase in fistula flow index and blood pressure difference between vein and artery. The latter phenomenon indicates the absence of α-adrenoceptor in big vessels or the non-distensible property of the fistula. Administration of noradrenaline significantly increased venous pressure in rats with chronic AVF. The reason for the latter might be that the high arterial pressure was

transmitted into the venous system directly through the AVF.

Infusion of noradrenaline increased blood pressure, pulse pressure (Takacs, 1965; Bower & Law, 1993), P_{mcf}, resistance to venous return which is similar to its action in normal rats (Imms *et al.*, 1974; Pang & Tabrizchi, 1986; Bower & Law, 1993; Tabrizchi, 2001). Haematocrit did not change during noradrenaline administration (Floyer & Morris, 1976; Aziz & Sommer, 1982). Since there is a fall in haematocrit value in saline treated sham-operated rats and rats with chronic AVF in the present study, administration of noradrenaline should have a hemo-concentrating effect.

In the present investigation, the weight of right and left kidneys were significantly increased in animals that received noradrenaline compared to respective values in saline treated group. As early as 1922, there was description of swelling of kidney during catecholamine infusion (Richards & Plant, 1922). The possible explanation could be that administration of noradrenaline: 1) increased capillary pressure filtration fraction by constricting efferent glomerular arterioles which resulted in fluid retention in kidneys; 2) activated the renin-angiotensin-aldosterone system through β-adrenoceptor stimulation; or 3) directly elicited tubular electrolyte and water reabsorption (Osswald & Greven, 1981). Moreover, it has long been recognized that reduction of blood flow to the kidney may lead to ischemia injury (Bellomo & Giantomasso, 2001; Westfall & Westfall, 2006).

Also, noradrenaline has been used to establish a reversible model of acute renal failure (Cronin *et al.*, 1978). In the latter study, acute renal failure was induced by the infusion of noradrenaline (0.75 μg/kg/min) directly into renal artery for 40 minutes. Although different from the present experimental design, in which noradrenaline was administrated at higher doses (1, 3, 10 μg/kg/min) and for a longer period (60 minutes for three doses), suspicion of renal ischemia could be raised. The ischemia injury led to a decrease in endothelial cell motility and surface area (Kaufman *et al.*, 1987). The damage of endothelium resulted in an increase in vascular permeability and leak of fluid into the interstitial tissue which is indicated by renal weight gain (Kaufman *et al.*, 1987).

In summary, administration of noradrenaline did not change CI in rats with chronic AVF. The positive effects of noradrenaline on heart rate, cardiac contractility, and total body venous tone tended to increase CI. However, the increase of CI at the highest dose may have been prevented due to the increase in TPR and resistance to venous return.

Unlike its actions in animals without chronic AVF, administration of AVP did not change CI in rats with chronic AVF (Share, 1988). As previously reported, blood pressure increased during administration of AVP (Tabrizchi & Ford, 2004). In the present animal model, blood pressure increased while pulse pressure, venous pressure, and heart rate were unaffected during AVP infusion.

Consistent with a previous report, infusion of AVP increased dP/dt (Tabrizchi & Ford, 2004). It has been demonstrated that AVP, via V_1-receptor activation, evokes concentration-dependent increases in $[Ca^{2+}]_i$ in cultured neonatal rat myocardiocytes (Xu & Gopalakrishnan, 1991; Liu *et al.*, 1999). Therefore, administration of AVP is able to increase cardiac contractility by stimulating V_1-receptor. In contrast, left ventricular dP/dt_{max} dose dependently decreased during administration of AVP (0.12-4 mU/kg/min) in pentobarbitone-anaesthetised dogs (Yatsu *et al.*, 2002). Myocardial ischemia and cardiosuppression secondary to the parasympathetic stimulation may contribute to the reduction in dP/dt (Yatsu *et al.*, 2002). When coronary perfusion is kept constant (15 ml/min), the contractility of isolated rat heart displays biphasic effect upon AVP infusion, while dP/dt_{max} increased at the lower concentrations (50 and 100 pg/ml) it decreased at higher concentrations of 400 and 500 pg/ml infusion (Walker *et al.*, 1988). In the present study, the increment in P_{mcf} and total blood volume increase the fullness of the circulation. This in turn would elevate the coronary filling pressure. As a consequence, coronary vasoconstriction and myocardial ischemia will be reduced. Cardiac contractility increased due to direct cardiac stimulating action of AVP via V_1 receptor.

In accordance with Floyer and Morris (1976), we reported a decrease in haemactocrit

together with an increase in plasma volume and total blood volume. According to Tabrizchi & Ford (2004), there is no change in haematocrit, plasma volume, and total blood volume. The existence of chronic AVF and higher dose of AVP administration may be the reason for the difference from the previous work in our lab (1, 3, 10 ng/kg/min vs. 10, 30, 100 ng/kg/min) (Tabrizchi & Ford, 2004). The increase in total blood volume was supposed to be mediated via V_2 receptor (Jackson, 2006). Exposure to AVP for a period of 40 min leads to translocation of water channel-containing vesicles to plasma membrane (Nielsen *et al.*, 1995; Jackson, 2006). Because water channel-containing vesicles contain functional water channels (aquaporin 2), the water permeability of renal collecting duct cells increases greatly (Jackson, 2006). Moreover, V_2-receptor activation also increases Na^+ transport in the thick ascending limb and collecting duct which also helps to increase water reabsorption (Jackson, 2006). As well, we recorded a significant increase in P_{mcf} at the highest dose of AVP infusion in rats with chronic AVF. There might be two explanations: 1) the highest dose of AVP administration contracted veins directly; and 2) an increase in total blood volume elevated the fullness of the vasculature.

In contrast to noradrenaline administration, the weights of kidneys did not increase significantly after AVP treatment when compared to saline treated group. This is because AVP increase vascular resistance most prominently in stomach, small and large

intestines, muscles, and skin vascular beds (Charocopos *et al.*, 1982). Therefore, no change in the wet weights of kidneys was observed in AVP administrated rats.

In summary, in rats with chronic AVF, administration of AVP did not change the CI. Unlike its actions in normal rats, administration of AVP in rats with chronic AVF reverse the preload-afterload mismatch by increasing P_{mcf} and total blood volume with little effect on resistance to venous return. Therefore, CI did not fall despite elevation of the afterload.

Administration of SNP had limited effects on CI in our present study. As reported by O'Rourke *et al.* (1982), infusion of SNP did not change blood pressure at 1.2 μg/kg/min but decreased blood pressure at the dose of 1.8 μg/kg/min in conscious dogs with chronic AVF. In present study, blood pressure also decreased at the highest dose while no alterations in pulse pressure, venous pressure, and P_{mcf} were observed. The lack of decrease in P_{mcf} may be caused by an increase in plasma volume. Since SNP has a vasodilator effect, it might reduce the capillary pressure and remove the fluid from interstitial to the intravascular space according to Starling equation (Gabel & Drake, 1979). The latter process may be mediated by the kidney because no change in haematocrit and plasma volume was observed in nephrectomized rats (Almeida *et al.*, 1986). Fistula flow index decreased significantly at the highest dose due to the

reduction in the pressure difference between the artery and vein. As well, similar to its actions in pentobarbital anaesthetized dogs without AVF, SNP administration did not alter TPR and resistance venous return due to baroreflex activation (Ogilvie & Zborowska-Sluis, 1991). Contrary to the study of O'Rourke *et al.* (1982) in which left ventricle dP/dt_{max} was increased, we found no change in dP/dt at the lowest and middle dose of SNP administration but a significant decrease in dP/dt at the highest dose. It has been demonstrated that there are no direct inotropic and chronotropic actions of SNP on isolated heart. Also, in the present study there is limited change in preload and afterload during the administration of SNP. Therefore, the fall in dP/dt may due to the increase of compliance in the wall of heart caused by nitric oxide pathway.

In summary, administration of SNP has limited effects on CI in rats with chronic AVF. In rats with chronic AVF, the transmission of arterial pressure to distensible large veins increased venous capacitance (Epstein & Ferguson, 1955). As well, TPR is set at very low level due to the existence of AVF, further infusion of SNP had little impact on TPR based on Ohm's law. Since SNP reduces venous return by increasing venous capacitance and reduce TPR, the effects of the drug were nullified in our working animal model. As well, it has been demonstrated that many blood vessels dilate in response to an increase in blood flow (Corretti *et al.*, 2002). A principal mediator of flow mediated vasodilation is supposed to be endothelium-derived nitric oxide (Corretti *et al.*, 2002).

Moreover, endothelial nitric oxide synthase gene transcription is activated when exposed to high flow for a longer period of time (hours) and this can result in continued increase in nitric oxide generation (Corretti *et al.*, 2002). The up-regulation of nitric oxide synthesis might be another reason for the lack of response to SNP administration in rats with chronic AVF. In conclusion, due to the special condition in our experimental model, administration of SNP had limited haemodynamic effects on rats with chronic AVF.

In the present study we managed to increase the vascular resistance by administration of noradrenaline or AVP, elevate heart pumping capacity and total body venous tone by infusion of noradrenaline. Nevertheless, despite these circulatory alterations, CI did not change. One explanation could be that heart was pumping at the maximal level due to the large increase in venous return in rats with chronic AVF according to Frank-Starling mechanism. In another words, the heart working reserve was used up to compensate the AVF. Higher cardiac performance was not achievable. In the latter condition, further increase in cardiac output was prevented by the limited heart pumping capacity. Therefore, further increment in heart rate, cardiac contractility, and total body venous tone were incapable of elevating CI. Since there was an increase in resistance to venous return during the infusion of noradrenaline, CI did not fall. This may be the result of simultaneous augmentation of venous return caused by PGVR. Moreover, elevation of

systemic resistance (resistance of the circulation except the fistula) by 79% and 69% only increased TPR by 24% and 28%, respectively, during infusion of noradrenaline at the lowest and highest doses. Therefore, although strong vasoconstrictors such as noradrenaline and AVP increased systemic resistance greatly, the increment in resistive load against the pumping heart was relatively minor. As a consequence, the CI was not changed by noradrenaline or AVP infusion. Unfortunately, we failed to decrease the TPR and/or total body venous tone during administration of SNP. This is possibly caused by an activation of sympathetic tone (Flaim, 1982). We would be very interested to see whether the CI will increase by further decreasing TPR or decrease upon the reduction in total body venous tone. We postulate that at a lower dose of vasodilator such as SNP or nitroglycerin, cardiac output will decrease due to the decrease in ventricular filling. However, when the drug concentration reaches a level high enough to reduce blood pressure, systemic vasoconstriction occurs due to sympathetic activation to maintain the blood pressure in baroreceptor level and counteracts the cardiac output lowering effect of the vasodilators. The latter postulation was supported by the work of Flaim (1982), in which administration of nitroglycerin at 2, 8 μg/kg/min reduced cardiac output without affecting mean arterial pressure while failing to reduce the cardiac output and mean arterial pressure at 32 μg/kg/min infusion. Furthermore, we predict that if we increase the dose of vasodilator to the level to reduce blood pressure, sympathetic activation will not keep the blood pressure at the baroreceptor level and cardiac output

will fall.

In conclusion, in rats with well compensated chronic AVF, vasoactive agents have little effects on cardiac output. Therefore, in diseases resembling the pathophysiology of chronic AVF such as septic shock, administration of these drugs has little benefit. The present work not only sheds light on the future medical intervention in clinical conditions resembling the hyper-haemodynamics of chronic AVF but also expands our knowledge on the cardiovascular system.

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