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Attenuated arterial and venous constriction in conscious rats with streptozotocin-induced diabetes

Xing Cheng, Susan W.S. Leung, Su L. Lim, Catharine C.Y. Pang*

Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, 2176 Health Sciences Mall, Vancouver, B.C. Canada, V6T 1Z3

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Abstract

We examined if arterial or venous constriction is impaired in early diabetes. Dose-pressor and mean circulatory filling pressure (index of venous tone) response curves to noradrenaline and angiotensin II were constructed in four groups of conscious, instrumented, Wistar rats pretreated with streptozotocin (60 mg/kg i.v.) or vehicle at 2 weeks prior to the study. Rats with diabetes, relative to controls, had increased ED_{50} (reduced potency) for the pressor (2.5-fold of control) and mean circulatory filling pressure (4.3-fold of control) response to noradrenaline, as well as reduced maximum pressor response (efficacy) to noradrenaline (diabetic, 74 ± 8 mm Hg; control, 96 ± 5 mm Hg). Diabetic rats also had reduced potency (ED_{50}, 5-fold of control) of the pressor response to angiotensin II; however, maximum pressor response and dose-mean circulatory filling pressure curve to angiotensin II were similar in both groups. Therefore, arterial and venous constrictions are impaired at an early phase of type I diabetes.

Keywords: Angiotensin; Capacitance; Diabetes; Circulatory filling pressure, mean; Noradrenaline; Vasoconstriction

1. Introduction

Neuropathy is a common morbidity in patients with insulin-dependent (type I) as well as insulin-independent (type II) diabetes, and it may affect both the peripheral sensorimotor and autonomic nervous systems (Sima and Sugimoto, 1999; Vinik et al., 2000). Autonomic neuropathy, on the other hand, may involve the degeneration of pre- as well as post-ganglionic fibres (Schmidt and Scharp, 1982). Serious vascular consequences of autonomic neuropathy may include the impairment of capillary circulation and orthostatic hypotension (Vinik et al., 2000; Stanberry et al., 1997; Watkins and Thomas, 1998); the latter is primarily due to diminished constriction of capacitance vessels following a change in posture from the supine to upright position (Pang, 2001). Indeed, plasma concentration of noradrenaline is generally lower in diabetic patients than controls (Watkins and Thomas, 1998; Hilsted, 1995), and this may cause circulatory abnormality in diabetes.

Although autonomic neuropathy and reduced transmitter release undoubtedly impair constriction of both resistance and capacitance vessels at the late phase of diabetes, attenuated constriction to vasoactive agents may also be a contributing factor to circulatory abnormality. Indeed, pressor response to noradrenaline is decreased as early as 2 to 6 weeks after injection of streptozotozin in rats (Foy and Lucas, 1976; Jackson and Carrier, 1983; Lucas, 1985; Yu and McNeill, 1992). This period is likely prior to the onset of nerve damage, since autonomic neuropathy is detected at 6 but not 3.5 months after injection of streptozotozin (Schmidt and Scharp, 1982). It is unclear if constriction of capacitance vessels to noradrenaline is also impaired at the early phase of diabetes.

The purpose of this study was to examine if pressor and mean circulatory filling pressure (index of body venous tone) responses to noradrenaline and angiotensin II are altered at the early phase of streptozotozin-induced diabetes. Mean circulatory filling pressure is the driving force of venous return, and is experimentally the equilibrium pressure that exists in the circulation immediately after an abrupt cessation of blood flow (Guyton, 1963). Mathematically, mean circulatory filling pressure is inversely proportional to venous compliance (Grodins, 1959), and can be used as an index of body venous tone or the driving force of venous return (Pang, 2000). Central venous pressure, being a

* Corresponding author. Tel: +1-604-822-2039; fax: +1-604-822-6012.
E-mail address: ccypang@interchange.ubc.ca (C.C.Y. Pang).

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downstream venous pressure, is not a reliable measure of upstream venous pressure or body venous tone due to the existence of venous resistance. As it is technically difficult to measure upstream venous pressure, venous plateau pressure during mechanically induced circulatory arrest is conventionally used to estimate upstream venous pressure (Pang, 2000).

2. Materials and methods

2.1. Experimental animals and induction of diabetes

Male Sprague–Dawley rats (300–350 g) were obtained from Charles River Canada. The rats were maintained under a 12:12-h light–dark cycle (lights on from 7 a.m. to 7 p.m.) and supplied with a standard laboratory chow diet (PMI Feeds) and water ad libitum.

The rats were randomly divided into four groups and injected with streptozotozin (60 and 1 ml/kg i.v.) or an equal volume of vehicle (0.9% NaCl) via the tail vein under light halothane anaesthesia. The rats were considered to be diabetic and used for the study if they had hyperglycemia (>15 mmol/l) at 48 h after injection of streptozotozin as detected by AccuSoft test strips (Hoffmann-La Roche) (Sambandam et al., 2000; McNeill, 1999). Plasma glucose was measured by the glucose oxidase method (Sigma, Trinder 100 kit) via the use of a Spectrarainbow (ART F039039, Austria). The rats were studied at 2 weeks after injection of streptozotozin.

The experiment has been carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institute of Health.

2.2. Surgical preparation

Streptozotozin-induced diabetic and control rats were surgically prepared under halothane anaesthesia. A saline-filled, balloon-tipped catheter was inserted into the right atrium through the right external jugular vein. Cannulae were also inserted into an iliac artery for the measurement of mean arterial pressure, into the right iliac vein for the withdrawal of blood (0.1 ml) for the measurement of plasma glucose and infusion of drugs, and into the inferior vena cava via the left iliac vein for the measurement of central venous pressure. All cannulae were tunnelled subcutaneously to the back of the neck and exteriorised. The rats were recovered from surgery and anaesthesia for at least 6 h prior to the study.

2.3. Experimental protocol

Each rat was placed in a small cage and allowed to wander freely during the study. The arterial and venous catheters were connected to pressure transducers (P23DB, Gould Statham, Oxnard, CA). The rats were equilibrated for 1 h prior to the study. Two groups of rats (diabetics and controls, $n=6$ each) were first pretreated with propranolol (8×10^{-7} mol/kg i.v. bolus, followed by 3.4×10^{-7} mol/kg/min continuous infusion) to prevent the stimulation of β-adrenoceptors by noradrenaline. At 10 min later, dose–response curves of noradrenaline ($2.2–300\times10^{-9}$ mol/kg/min) were constructed in both groups. Another two groups of diabetic and control rats ($n=7$ each) were infused with angiotensin II ($1.3–550\times10^{-11}$ mol/kg/min). Mean arterial pressure and central venous pressure were measured at the baseline condition, at 10 min after the start of infusion of propranolol (if applicable), and at the plateau phase of response (2 to 10 min after the start of infusion) to various doses of noradrenaline and angiotensin II. Each dose of drug was followed by a recovery period of 10–15 min.

2.4. Mean circulatory filling pressure measurements

Central venous pressure was measured after transient stopping of the circulation through injection of a small volume of fluid into the right atrial balloon. Within 5 s following inflation of the balloon, mean arterial pressure decreased to a plateau value (referred to as final arterial pressure), while central venous pressure increased to a plateau value (referred to as venous plateau pressure). Mean circulatory filling pressure was calculated as follows: Mean circulatory filling pressure = venous plateau pressure + 1/60 (final arterial pressure − venous plateau pressure), using 1:60 as the ratio of arterial to venous compliance (Rothe, 1993; Yamamoto et al., 1980).

2.5. Statistical analysis

Data were log-transformed prior to statistical analysis to obtain ED_{50} values from pairs of control and diabetic rats using the GraphPad Prism program. $ED_{2.5\ mm\ Hg}$ values (doses that increased mean circulatory filling pressure by 2.5 mm Hg) were obtained in instances where maximum responses could not be attained. ED_{50}, $ED_{2.5\ mm\ Hg}$ and E_{max} values between control and diabetic rats were analysed by two-tailed, non-paired *t*-tests with $P<0.05$ selected as the criterion for statistical significance.

3. Results

3.1. Baseline values

Rats injected with streptozotozin had higher plasma concentration of glucose at 24 h later compared to control rats (21.1 ± 2.6 versus 5.6 ± 0.8 mM). At 2 weeks after induction, the diabetic rats had lower body weight and higher plasma glucose, but similar mean arterial pressure, heart rate and mean circulatory filling pressure relative to controls (Table 1). There are no significant differences in

Table 1
Baseline values of body weight, plasma glucose, mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious, diabetic and control rats

	Control ($n=13$)	Diabetes ($n=13$)
Body weight (g)	416 ± 15	361 ± 21[a]
Plasma glucose (mM)	5.7 ± 1.4	23.5 ± 4.3[a]
MAP (mm Hg)	107 ± 3	102 ± 4
HR (beats/min)	375 ± 11	339 ± 15
MCFP (mm Hg)	6.5 ± 0.2	6.2 ± 0.3

The measurements were made at 2 weeks following injection of vehicle (0.9% NaCl, i.v., control) or streptozotocin (60 mg/kg, i.v., diabetes), respectively.
All values are means ± S.E.M.
[a] Denotes significant difference from controls ($P<0.05$).

any of the above baseline values between the subgroups of diabetic rats (or control rats) to be infused with noradrenaline or angiotensin II.

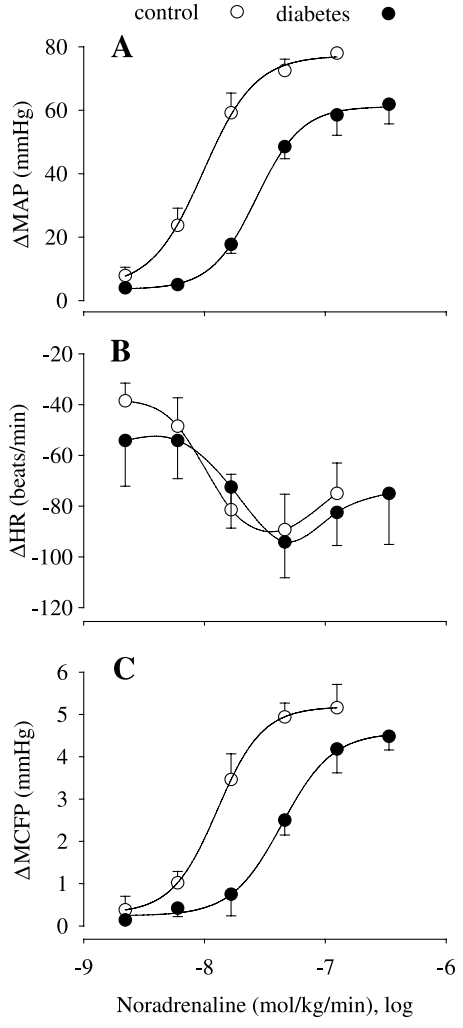

Fig. 1. Dose–response curves for the effects (mean ± S.E.M, $n=6$ per group) of i.v. infused noradrenaline on (A) mean arterial pressure (MAP), (B) heart rate (HR) and (C) mean circulatory filling pressure (MCFP) in conscious rats injected with streptozotocin (60 mg/kg, closed circle, diabetic group) or vehicle (0.9% NaCl, open circle, control group) at 2 weeks prior to the study.

3.2. Cardiovascular response to noradrenaline

Propranolol did not significantly alter mean arterial pressure and mean circulatory filling pressure (results not shown), but caused an insignificantly smaller decrease in heart rate in control (− 27 ± 4 beats/min) relative to diabetic (− 46 ± 14 beats/min) rats at 10 min after the start of infusion.

Noradrenaline caused dose-dependent increases in mean arterial pressure and mean circulatory filling pressure in both control and diabetic rats (Fig. 1A and B). The rats with diabetes, relative to controls, had significantly higher ED_{50} for the mean arterial pressure (2.5-fold) as well as mean circulatory filling pressure (4.3-fold) responses to noradrenaline, and significantly lower E_{max} for the mean arterial pressure (77% of maximum), but similar E_{max} for the mean circulatory filling pressure response to noradrenaline (Table 2). Heart rate was similarly decreased by noradrenaline in both groups (Fig. 1C).

3.3. Cardiovascular response to angiotensin II

Angiotensin II also increased mean arterial pressure and mean circulatory filling pressure in a dose-dependent manner in control and diabetic rats (Fig. 2A and B). Relative to the control rats, the diabetic rats had greater ED_{50} but similar E_{max} for the mean arterial pressure response to angiotensin II (Table 2). The control and diabetic rats had apparently similar dose-mean circulatory filling pressure response curves to angiotensin II (Fig. 2B). Maximum mean circulatory filling pressure responses could not be determined for angiotensin II, however, since three of seven controls and five of seven diabetic rats died during inflation of the atrial balloon at the next higher dose of angiotensin II (than the highest dose shown on the graphs). Consequently, the $ED_{2.5\ mm\ Hg}$ values (doses that increased mean circulatory filling pressure by 2.5 mm Hg) were obtained as the index of potency (Laurence and Carpenter, 1998). The diabetic rats had slightly, but insignificant, greater $ED_{2.5\ mm\ Hg}$ for the

Table 2
Potency (ED_{50}) and efficacy (E_{max}) for the mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) response curves of noradrenaline and angiotensin II in conscious rats

	ED_{50} (nmol/kg)		E_{max} (mm Hg)	
	Control	Diabetes	Control	Diabetes
Noradrenaline				
MAP	12 + 4	30 + 10[a]	96 ± 5	74 ± 8[a]
MCFP	12 + 3	51 + 16[a]	6.5 ± 0.5	5.7 ± 0.6
Angiotensin II				
MAP	0.04 + 0.01	0.20 + 0.06[a]	69 ± 5	65 ± 5

The rats were injected with streptozotocin (60 mg/kg, diabetes) or vehicle (0.9% NaCl, control) at two weeks prior to the study ($n=6$ or 7 per group). All values are means ± S.E.M. All data of ED_{50} were log transformed prior to statistical analysis.
[a] Denotes significant difference from control ($P<0.05$).

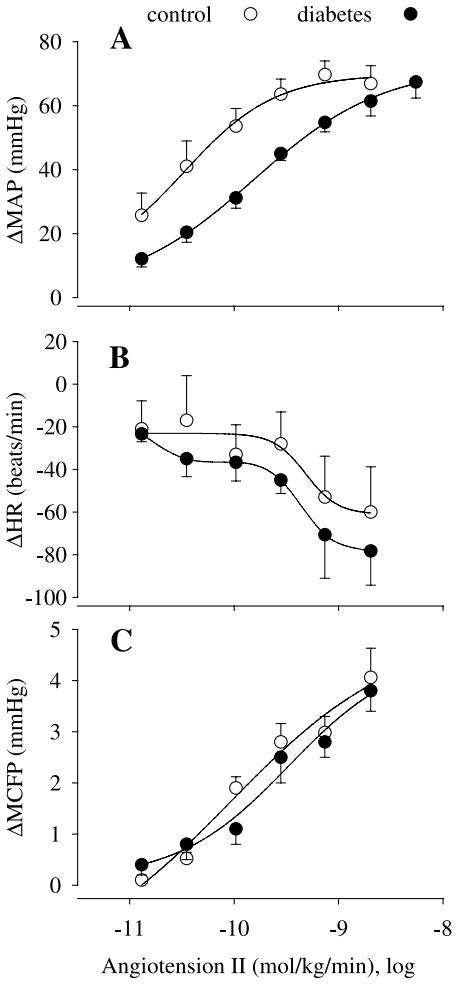

Fig. 2. Dose–response curves for the effects (mean ± S.E.M, $n = 7$ per group) of i.v. infused angiotensin II on (A) mean arterial pressure (MAP), (B) heart rate (HR) and (C) mean circulatory filling pressure (MCFP) in conscious rats injected with streptozotocin (60 mg/kg, closed circle, diabetic group) or vehicle (0.9% NaCl, open circle, control group) at 2 weeks prior to the study.

mean circulatory filling pressure response to angiotensin II relative to the readings in the control rats (0.40 ± 0.17 and 0.30 ± 0.11 nmol/kg/min, respectively).

4. Discussion

Our results show that rats at an early phase (2 weeks) of streptozotozin (60 mg/kg)-induced type I diabetes and the age-matched control rats had similar mean arterial pressure, heart rate and mean circulatory filling pressure. The systolic and diastolic blood pressures of the diabetic and control rats were also similar (results not shown). It is of interest that rats at the early phase of streptozotozin (60 mg/kg)-induced diabetes have been shown to have similar, slightly reduced, or slightly increased blood pressure relative to the age-matched controls. For example, the mean arterial pressure of conscious rats with streptozotozin-induced diabetes for 4–6 weeks had mean arterial pressure similar to (Yu and McNeill, 1992) or lower (Jackson and Carrier, 1983) than the pressure of controls. The systolic blood pressure and heart rate of conscious rats with streptozotozin-induced diabetes for 3–6 weeks were however, slightly lower than those of age-matched controls (Hebden et al,. 1987; Yu and McNeill, 1992). In contrast, the conscious rats with streptozotozin-induced diabetes for 4 weeks had higher mean arterial pressure relative to that of the controls (Hayashi et al., 1983). Litwin et al. (1991) have shown that thiobutabarbitone-anaesthetised rats (220–250 g) with streptozotozin-induced diabetes for 4 weeks had similar mean arterial pressure, heart rate and mean circulatory filling pressure relative to those of age-matched controls, however, the mean arterial pressure of the diabetic rats was lower than that of the controls following thoracotomy. The small differences in baseline mean arterial pressure between controls and diabetic rats among the various studies are likely due to the use of different strain or age of rats and/or varying experimental conditions.

The diabetic rats in the present study had reduced potency (higher ED_{50}) of mean arterial pressure and mean circulatory filling pressure responses to infused noradrenaline, as well as reduced efficacy (E_{max}) of mean arterial pressure response to noradrenaline. These results reflect reduced constriction to α-adrenoceptor activation, since the rats were pretreated with propranolol to block the chronotropic and inotropic effects of β-adrenoceptor activation by noradrenaline. Indeed, baseline heart rate in both the diabetic and control rats were decreased, to a similar extent, by pretreatment with propranolol. Furthermore, both groups had similar dose-dependent bradycardic responses to noradrenaline, which were likely due to pressor response-induced parasympathetic activation (and not sympathetic withdrawal, since the rats were pretreated with propranolol). Reduced pressor response to noradrenaline has been reported in pithed rats at 2 weeks following induction with streptozotozin (Foy and Lucas, 1976; Lucas, 1985), in conscious rats at 4–5 weeks (Jackson and Carrier, 1983), and 6 weeks (Yu and McNeill, 1992) following induction with streptozotozin; however, unlike the present study, these rats were not pretreated with a β-adrenoceptor antagonist. Our results further show that along with reduced potency as well as efficacy of the pressor effect of noradrenaline, there was reduced potency of the mean circulatory filling pressure response to noradrenaline. The latter suggests reduced sensitivity of venoconstriction to noradrenaline, since mean circulatory filling pressure reflects total body venous tone (Pang, 2000). Furthermore, mean circulatory filling pressure has been shown to be proportional to venous return (Pang, 2001), and is an important determinant of cardiac output (Guyton et al., 1954). Our results therefore suggest that not only are constrictions of arterial resistance vessels reduced, but constriction of capacitance vessels to α-adrenoceptor activation are also impaired as early as 2 weeks after the onset of diabetes. The mechanism responsible for reduced

α-adrenoceptor response is unclear since no measurement of neuronal noradrenaline uptake or release was made. It is of interest that autonomic neuropathy was not yet detected in rats at 3.5 months, but existed at 6 months, after injection of streptozotozin (Schmidt and Scharp, 1982).

It is unclear what mechanisms were responsible for the attenuated response to infused noradrenaline. It has been reported that the potency of response to noradrenaline, but not phenylephrine, was increased in the dorsal hand vein of diabetic patients with symptoms of orthostatic hypotension relative to those of controls, and this was attributed to defective neuronal uptake of noradrenaline (Eichler et al., 1992). Extrapolation of these human results to help interpret our data is inappropriate in this respect, since the disease conditions are very different between the studies. Furthermore, responses of the hand vein may not reflect those of other veins, since cutaneous veins are primarily involved in thermoregulation. Indeed, changes in sympathetic outflow to the cutaneous veins are often opposite to those of resistance vessels and capacitance vessels (Tkachenko and Chernjavskaja, 1971; Shepherd and Vanhoutte, 1975). Reduced vasoconstriction may be due to postjunctional changes resulting in attenuated vascular contraction or increased neuronal uptake of infused noradrenaline. Our results also show that the potency, but not efficacy, of pressor response to angiotensin II was also reduced in the diabetic rats, relative to the response in the control rats. Our mean arterial pressure results are in accordance to those of Jackson and Carrier (1983), which show that pressor response to i.v. bolus injections of noradrenaline as well as angiotensin II are attenuated in rats with streptozotozin-induced diabetes. It should be noted that the rats used in the Jackson and Carrier (1983) study were younger than ours (body weight 150–180 g) at the time of induction, and the duration of diabetes was longer (4–5 weeks), relative to the rats in the present study. Potency of mean circulatory filling pressure response to angiotensin II was insignificantly reduced in the diabetic than control rats, as reflected by the small (insignificantly) higher $ED_{2.5\ mm\ Hg}$ readings in the diabetic animals. These results suggest that venoconstriction to angiotensin II is not yet altered at 2 weeks after the induction of diabetes. More studies are needed to elucidate the mechanisms responsible for altered responses to noradrenaline and angiotensin II at an early stage of diabetes, and to find out if diabetes of a longer duration causes greater impairment of venoconstriction to these agents.

To summarize, propranolol-treated rats with streptozotozin-induced diabetes had attenuated potency and efficacy of pressor responses to noradrenaline, as well as reduced potency of mean circulatory filling pressure response to noradrenaline. Furthermore, the diabetic rats had reduced potency of mean arterial pressure response to angiotensin II. The results suggest that functional impairment of arterial and venous constrictions occur at the early phase of type I diabetes.

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