Adrenomedullin increases fluid extravasation from the splenic circulation of the rat

S. Kaufman, P. Andrew, R. Sultanian and Y. Deng

Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

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1. We studied the effect of adrenomedullin (ADM) on fluid efflux from the splenic vasculature into extravascular spaces.

2. Splenic arterial infusion of ADM (1, 3 and 9 ng min^{-1}; $n = 9$, 11 and 10, respectively) caused a dose-dependent increase in intrasplenic fluid efflux ($+0.6 \pm 0.3$ (saline) *vs.* $+2.0 \pm 0.3$ ml min^{-1} (9 ng min^{-1} ADM), $P < 0.05$), and in splenic (venous minus arterial) haematocrit ($+0.8 \pm 0.1$ (saline, $n = 6$) *vs.* $+3.1 \pm 0.3$% (9 ng min^{-1} ADM, $n = 7$), $P < 0.05$). There was no change in splenic weight (0.99 ± 0.02 (saline, $n = 6$) *vs.* 0.99 ± 0.02 g (9 ng min^{-1} ADM, $n = 7$), $P > 0.05$).

3. There was no change in MAP before (97.5 ± 2.2 mmHg), during (98.4 ± 3.4 mmHg), or after (100.2 ± 2.2 mmHg) intrasplenic infusion of ADM (9 ng min^{-1}) ($n = 11$, $P < 0.05$).

4. ADM (9 ng min^{-1}) caused an increase in intrasplenic microvascular pressure (11.3 ± 0.3 (saline, $n = 5$) *vs.* 13.0 ± 0.3 mmHg (9 ng min^{-1} ADM, $n = 6$), $P < 0.05$).

5. ADM (1×10^{-11} to 1×10^{-6} M) induced greater vasorelaxation of isolated preconstricted splenic resistance arteries than veins (maximal relaxation: 60 ± 0.9 (artery, $n = 9$) *vs.* 43 ± 1.7% (vein, $n = 8$), $P < 0.05$). L-NMMA (10^{-4} M) partially inhibited the ADM-induced relaxation in splenic arteries (maximal relaxation: 38 ± 3 (ADM + L-NMMA, $n = 5$) *vs.* 60 ± 3% (ADM + D-NMMA, $n = 5$), $P < 0.05$).

6. It is concluded that ADM increases fluid efflux from the splenic vasculature by differentially reducing pre- *vs.* post-capillary resistance, thus increasing intrasplenic microvascular pressure.

Adrenomedullin (ADM) is a vasorelaxant peptide (Kitamura *et al.* 1993) that is produced and secreted by many tissues including endothelial and vascular smooth muscle cells (Sakata *et al.* 1994; Sugo *et al.* 1994*a*,*b*). Secretion is stimulated by such factors as tumour necrosis factor-α, interleukin-1, angiotensin II, endothelin-1 and atrial natriuretic factor (Tasaka & Kitazumi, 1994; Sugo *et al.* 1994*b*, 1995; Minamino *et al.* 1995). There are two signal transduction pathways mediating ADM-induced vasorelaxation, namely stimulation of NO biosynthesis and increased cAMP production (Shemakake *et al.* 1995; Minamino *et al.* 1998).

ADM is known to contribute to the physiological regulation of fluid and electrolyte homeostasis (Samson, 1999) by inducing natriuresis and diuresis (Ebara *et al.* 1994), and by inhibiting water intake (Murphy & Samson, 1995) and salt appetite (Samson & Murphy, 1997). All these actions, in concert, reduce extracellular fluid volume and blood volume. In addition, elevated levels of ADM in the tissues and blood have been reported in several pathophysiological conditions including hypertension, septic shock and congestive heart failure (Hirata *et al.* 1996; Kobayashi *et al.* 1996; Kohno *et al.* 1996).

We have shown that the spleen plays an important role in controlling blood volume by regulating the translocation of protein-rich fluid from the intravascular space into the systemic lymphatic system (Chen & Kaufman, 1996). An increase in intrasplenic microvascular pressure (P_C) has been proposed as the driving force for this fluid efflux (Sultanian *et al.* 2001). We have suggested that the increase in intrasplenic P_C is caused by differential vasoreactivity of pre- and post-capillary splenic resistance vessels to vasoactive factors. Recently, we have reported that NO increases intrasplenic fluid efflux in the rat (Andrew *et al.* 2001). Given that ADM-induced vasorelaxation may be mediated through increased NO biosynthesis (Shemakake *et al.* 1995; Minamino *et al.* 1998), we wished to investigate the effect of ADM on intrasplenic haemodynamics and fluid extravasation.

We hypothesized that ADM would increase intrasplenic fluid efflux, and that this would be due to an increase in intrasplenic P_C, resulting from relatively greater dilatation of the splenic resistance arterioles (hilar arteries) than the venules (hilar veins). Splenic arterial and venous blood flows were measured during infusion of ADM into the splenic artery of anaesthetized male rats. It

was reasoned that a significant increase in the arteriovenous (A − V) flow differential would confirm an ADM-induced increase in fluid efflux from the splenic vasculature. Changes in intrasplenic P_C, in response to close arterial infusion of ADM, were measured in an isolated, blood-perfused spleen using the double occlusion technique (Townsley *et al.* 1986; Sultanian *et al.* 2001). Isometric tension, measured using a wire myograph system, was used to determine the vascular reactivity of isolated splenic hilar arteries and veins to ADM. We hypothesized that differential vasoreactivity between isolated hilar arteries and veins to ADM would be in a manner consistent with an ADM-induced increase in intrasplenic P_C and subsequent intrasplenic fluid efflux, i.e. that the maximal relaxation of hilar veins would be less than that of hilar arteries, and/or that the hilar arteries would be more sensitive to ADM-induced vasorelaxation.

METHODS

The experiments described in this paper were examined by the local animal welfare committee and found to be in compliance with the guidelines issued by the Canada Council on Animal Care. At the completion of the studies in experiments A and B, all animals were killed with an anaesthetic overdose (0.3 ml I.V. Euthanyl, MTC Pharmaceuticals, Cambridge, Ontario, Canada). In experiment C, the animals were killed by decapitation using a small animal guillotine.

Animals

Male Long-Evans rats (450–600 g) were obtained from Eastern Canada (Charles River, St Foy, Quebec, Canada). They were held in the University Animal Facility for at least 1 week prior to any surgical or experimental procedure. The animal room was temperature and humidity controlled, and kept on a 12 h light–12 h dark cycle. The rats were given a 0.28% sodium diet (Purina) and water *ad libitum*.

Experiment A: effect of ADM on splenic blood flow

Surgery. Anaesthesia was induced with sodium pentobarbitone (60 mg (kg body weight (BW))$^{-1}$ I.P.) and maintained at a surgical plane (no paw-pinch response) with Inactin (ethyl-(1-methyl-propyl)-malonyl-thio-urea, 80 mg (kg BW)$^{-1}$ I.P.). Body temperature was maintained by placing the rat on a heating pad (Deltaphase Isothermal pad, Braintree Scientific, Inc., Braintree, MA, USA). Access to the abdominal organs was through a mid-line laparotomy. In order to ensure that the splenic artery and vein supplied and drained only the spleen, all branches running from the splenic vessels to the pancreas, stomach and other surrounding tissue were ligated and divided.

The spleen was carefully cleared from its attachments to the stomach. The stomach was then delivered through the abdominal incision, and laid on the thorax of the rat. The gastric artery was carefully cleared, great care being taken to handle the vessels as little as possible since they are capable of extreme vasoconstriction. The gastric artery was cannulated with drawn-out Tygon tubing (nominal dimensions: 0.25 mm i.d., 0.5 mm o.d.).

Polyethylene (0.58 mm i.d., 0.97 mm o.d.) and Silastic (Dow Corning, USA, 0.51 mm i.d., 0.94 mm o.d.) cannulae were placed in the femoral artery and vein, respectively. The arterial cannula was used for measuring blood pressure. The venous cannula was used for infusing saline.

Blood flow probes. Flow probes (Transonic transit time ultrasonic probes, 1RB series; Transonic Systems, Ithaca, NY, USA) were attached to micromanipulators and aligned to receive the splenic artery and vein. The probes were then positioned under the splenic vascular arcade, and the splenic artery and vein were slipped into the probe windows. The stomach was replaced over the splenic vessels and probes, and the wound covered with moist sponges and plastic film. The use and calibration of these probes has previously been described (Chen & Kaufman, 1996).

Data acquisition and analysis. The femoral artery cannula was connected to a Statham pressure transducer. Blood pressure and flow were recorded on-line using a data acquisition board (DI-400, DATAQ Instruments, Akron, OH, USA). The data were collected and analysed using DATAQ's own software (WINDAQ).

Protocol. After cannulation of the femoral vein, saline infusion was started (3 ml h^{-1}). The femoral artery was then cannulated, and blood pressure recorded. The flow probes were positioned around the splenic artery and vein, after which the preparation was allowed to stabilize for 45 min. Vehicle (isotonic saline) was infused for 30 min, during which time baseline blood flows and pressure were recorded. ADM (Phoenix Pharmaceuticals Inc., Mountain View, CA, USA) was then infused for 5 min at doses of 0 (saline), 1, 3 or 9 ng min^{-1}, with a 30 min recovery period between each dose. A maximum of two doses, given in randomized order, was administered to any given animal; this ensured that the experiment did not run for more than 2 h. The mean flow rates were computed over the last 5 min of the baseline and recovery periods. At the end of the experiment, dye was infused into the splenic artery to confirm vascular isolation of the spleen. In separate groups of animals, the highest dose of ADM (9 ng min^{-1}) or saline was infused into the splenic artery, and the arterial and venous haematocrits were measured. The splenic vessels were then ligated, and the spleen removed and weighed.

Statistical analysis. The changes in splenic arterial and venous blood flows were compared using one-way ANOVA. The changes in splenic (A − V) flow differential were compared using ANOVA on ranks since the data were not normally distributed. This was followed by Dunn's procedure for pairwise multiple comparisons. The differences between arterial and venous haematocrits were assessed using Student's t test for paired data. The differences between the experimental (ADM) and control (saline) groups with respect to arteriovenous differential of haematocrit and splenic weight were analysed using Student's t test for unpaired data. The level of significance was defined at $P < 0.05$.

Experiment B: effect of ADM on splenic microvascular pressure

Surgery. Anaesthesia was induced with isoflurane (2.5%; IsoFlo, Abbott Laboratories, USA) and continued until the femoral vein was cannulated, at which time Somnotol (sodium pentobarbitone, 65 mg ml^{-1} I.V., MTC Pharmaceuticals, Cambridge, Ontario, Canada) was injected (50 mg kg^{-1}). Inactin (80 mg (kg BW)$^{-1}$ S.C., BYK, Germany), given at the end of surgery, maintained the rat under a surgical plane of anaesthesia (no paw-pinch response) for the duration of the experiment.

Vascular isolation of the spleen was implemented and verified as previously described for Experiment A. Silastic (Dow Corning, USA; 0.51 mm i.d., 0.94 mm o.d.) and PE-50 (Intramedic, USA; 0.58 mm i.d., 0.965 mm o.d.) cannulae were placed in the femoral vein and artery, respectively. MAP was monitored at the femoral artery, and Somnotol was administered through the femoral vein. The venous line was also used to infuse saline (3 ml h^{-1}) to maintain adequate hydration of the animal throughout the duration of the experiment. The right common carotid artery was occlusively cannulated using

PE-90 (0.86 mm i.d., 1.27 mm o.d.) to provide the source of oxygenated blood for splenic perfusion.

The gastric artery was cannulated with drawn-out PE-50 tubing (0.58 mm i.d., 0.965 mm o.d.), while the gastric vein was cannulated with micro-renethane (Braintree Scientific, Inc.; 0.30 mm i.d., 0.64 mm o.d.). The gastric artery cannula was connected, via a three-way adapter, to a pressure transducer (which monitored splenic arterial perfusion pressure), and to a peristaltic pump. The venous cannula was advanced to the junction of the gastric and splenic veins, and was connected to a pressure transducer (which monitored venous pressure of the blood-perfused spleen). When the surgery was completed, splenic perfusion was started. At the start of splenic perfusion (1.0 ml min^{-1}), heparin (0.15 ml; 10 000 i.u. ml^{-1} I.V.) was injected. The splenic perfusion consisted of oxygenated blood taken from the carotid artery, and perfused into the splenic artery via the peristaltic perfusion pump (1.0 ml min^{-1}). Systemic pressure and splenic arterial and venous perfusion pressures were monitored on-line using a data acquisition system (D1-400, DATAQ Instruments) and recorded using WINDAQ.

Microvascular pressure. In the blood-perfused spleen, the microvascular pressure (P_C) was determined using the double vascular occlusion technique (Townsley *et al.* 1986). After stabilization, both inflow and outflow cannulae were simultaneously occluded. Arterial pressure (P_A) and venous pressure (P_V) equilibrated rapidly to a value reflective of P_C. If P_A and P_V did not exactly equilibrate to the same pressure upon double occlusion, then the mean pressure was determined and defined as P_C (Barman, 1997). Results of previous studies have shown that microvascular pressures measured by double vascular occlusion are equivalent to those measured by other classic methods, such as the micropuncture technique (Hakim & Kelly, 1989).

Protocol. Animals were allowed to stabilize for 30 min before any haemodynamic variables were measured. ADM (9 ng min^{-1}) was then infused into the splenic artery. Five minutes later, double vascular occlusions were applied by simultaneously tightening a snare placed around the splenic vein while the perfusion pump was stopped and the tubing clamped; arterial inflow was blocked for a period of ~5 s (Hakim & Kelly, 1989). Control animals, implanted with the same cannulae and treated in the same manner as the experimental animals, were infused with saline and subjected to the same protocol.

The circulation of blood through the spleen may be represented by a simple linear model where P_A is separated from P_C by a pre-capillary resistance (R_A), and P_C is separated from P_V by a post-capillary resistance (R_V) (Barman, 1997). The pre- and post-capillary resistances may be calculated using the following equations:

$$R_A = (P_A - P_C)/Q \quad \text{and} \quad R_V = (P_C - P_V)/Q,$$

where Q is blood flow (ml min^{-1}).

Statistical analysis. The significance of the ADM-induced alterations in pre- and post-capillary resistances, and intrasplenic microvascular pressure were assessed using one-way ANOVA. The level of significance was defined at $P < 0.05$.

Experiment C: vasoactivity of isolated splenic resistance vessels

Vessel preparation. The rats were decapitated and the vascular arcade serving the spleen was rapidly removed and placed in ice-cold Hepes-buffered phosphate saline (Hepes-PSS). Hilar arteries (125–200 μm) and veins (350–450 μm) were dissected free, cut into 2 mm lengths and mounted in an isometric myograph system (Kent Scientific, Litchfield, CA, USA). Venous and arterial segments were studied simultaneously in two organ baths. The change in developed isometric force was recorded on-line.

Resting length–tension curve. After mounting, vessels were allowed to stabilize for 30 min in Hepes-PSS buffer under no tension, during which time the buffer solution was changed at 10 min intervals. This was followed by a preconditioned stretch of approximately 0.6 mN, after which the vessels were rested at 0.1–0.2 mN mm^{-1} for a further 10 min. From Laplace's law, the L_{100} could be calculated from the exponential fit of tension generated *vs.* internal vessel circumference, where L_{100} is defined as the circumference that the vessel would have at a transmural pressure of 100 mmHg (arteries) or 5 mmHg (veins). Preliminary studies on splenic arteries and veins established that maximum active tension with least passive tension was developed in the arteries and veins at $0.65L_{100}$ and $0.8L_{100}$, respectively (Andrew *et al.* 2001). Vessels were allowed to stabilize at these settings for 30 min.

Protocol. After characterization of the active–passive tension curves for each vessel, a cumulative dose–response curve to phenylephrine (1×10^{-8} to 1×10^{-2} M) was generated, from which the dose required to achieve 80% maximal constriction (EC_{80}) was determined. It is acknowledged that the absolute tension would be greater in hilar arteries than veins. However, an 80% preconstriction value (EC_{80}) was chosen in order to assess the vasorelaxant properties of ADM on vessels that had the same *degree* of preconstrictor tone relative to maximal constriction for that vessel. After a 30 min stabilization period, during which time the organ bath was changed with fresh buffer every 10 min, phenylephrine (the EC_{80}) was used to preconstrict the vessel for determination of the vasodilatory response to ADM (1×10^{-11} to 1×10^{-6} M). The effect of NO synthase inhibition was investigated by incubating hilar arteries with L-NMMA (10^{-4} M) or the inactive enantiomer D-NMMA for 15 min before preconstriction and construction of the ADM dose–response curve. At the end of each experiment, the vessels were exposed to methacholine (10^{-4} M) to confirm that they were still responsive.

Solutions and drugs. The Hepes-PSS (Hepes-buffered phosphate saline solution) was maintained at pH 7.4. It contained (mM): 142 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 1.56 $CaCl_2$, 1.18 K_2PO_4, 10 Hepes and 5.5 glucose. Stock solutions of ADM (Phoenix Pharmaceuticals Inc.) and L-phenylephrine hydrochloride (Sigma Chemical Co., St Louis, MO, USA), were prepared in distilled water at concentrations of 6.98×10^{-4} M and 10 mM, respectively. Subsequent dilutions were made using Hepes-PSS. L-NMMA (N^G-monomethyl-L-arginine, di-*p*-hydroxyazobenzene-*p*-sulfonate salt) and D-NMMA (N^G-monomethyl-D-arginine, monoacetate salt) (Calbiochem, La Jolla, CA, USA) were both prepared in distilled water at a concentration of 10^{-4} M. Acetyl-β-methacholine chloride (Sigma) was prepared in distilled water (10^{-2} M) and diluted to 10^{-4} M immediately before use.

Statistical analysis. The significance of changes in tension at each of the doses of ADM were analysed by one-way repeated measures ANOVA, followed by Student-Newman-Keuls test to identify the individual points of significance. The significance of differences between the vasodilatory response of arteries and veins was analysed by two-way repeated measures ANOVA. The level of significance was defined at $P < 0.05$.

RESULTS

Experiment A: effect of ADM on splenic blood flow

In the saline-infused control group ($n = 5$), splenic arterial blood flow was significantly higher than venous blood flow (2.3 ± 0.4 *vs.* 1.7 ± 0.2 ml min^{-1}, respectively, $P < 0.05$). Intrasplenic infusion of ADM caused a dose-dependent increase in the difference between splenic arterial inflow and venous outflow, i.e. ADM increased fluid extravasation from the splenic vasculature (Fig. 1*C*).

This resulted from the increase in splenic arterial flow (Fig. 1*A*) in the face of unchanged venous outflow (Fig. 1*B*). There was no change in MAP before (97.5 ± 2.2 mmHg), during (98.4 ± 3.4 mmHg), or after (100.2 ± 2.2 mmHg) infusion of the highest dose of ADM ($P > 0.05$). As blood passed through the spleen, the loss of erythrocyte-free fluid caused an increase in haematocrit from 41.2 ± 0.2 to 42.0 ± 0.3% ($n = 6$, $P < 0.05$) in the saline-infused control animals, and from 40.7 ± 0.2 to 43.8 ± 0.3% ($n = 7$, $P < 0.05$) in the ADM-infused (9 ng min^{-1}) animals. This arteriovenous difference in haematocrit was significantly greater in the ADM-infused group than in the control group (+0.8 ± 0.1 % (saline, $n = 6$) *vs.* +3.1 ± 0.3 % (9 ng min^{-1} ADM, $n = 7$), $P < 0.05$). There was no significant difference in wet weight between the ADM- and saline-infused spleens: 0.99 ± 0.02 g (saline, $n = 6$) *vs.* 0.99 ± 0.02 g (9 ng min^{-1} ADM, $n = 7$), $P > 0.05$).

Experiment B: effect of ADM on splenic microvascular pressure

Splenic arterial infusion of ADM (9 ng min^{-1}, $n = 6$) caused a significant decrease in pre-capillary resistance ($P < 0.05$) (Fig. 2*A*), no change in post-capillary resistance ($P > 0.05$) (Fig. 2*B*), and a significant increase in intrasplenic P_C ($P < 0.05$) (Fig. 2*C*) compared with the saline-infused control group ($n = 5$).

Experiment C: effect of ADM on vasoactivity of isolated splenic resistance vessels

ADM caused a dose-dependent relaxation of preconstricted splenic resistance arteries and veins. However, the maximal relaxation was greater in the arteries (60 ± 0.9 %, $n = 9$) than in the veins (43 ± 1.7 %, $n = 8$) ($P < 0.05$; Fig. 3), i.e. the vasorelaxant efficacy of ADM was greater in the arteries than in the veins. L-NMMA, but not D-NMMA, partially inhibited the ADM-induced relaxation of arteries (maximal relaxation: 38 ± 3 % (ADM + L-NMMA, $n = 5$) *vs.* 60 ± 3 % (ADM + D-NMMA, $n = 5$), $P < 0.05$; Fig. 4). Neither L- nor D-NMMA caused any change in wall tension of the EC_{80} phenylephrine concentration used to preconstrict arteries and veins (0.020 ± 0.008 mN mm^{-1} (ADM + L-NMMA, $n = 3$) *vs.* 0.002 ± 0.002 mN mm^{-1} (ADM + D-NMMA, $n = 3$), $P < 0.05$).

DISCUSSION

The results of this study are consistent with our proposal that ADM increases intrasplenic fluid extravasation by raising intrasplenic P_C, through differential vasoreactivity

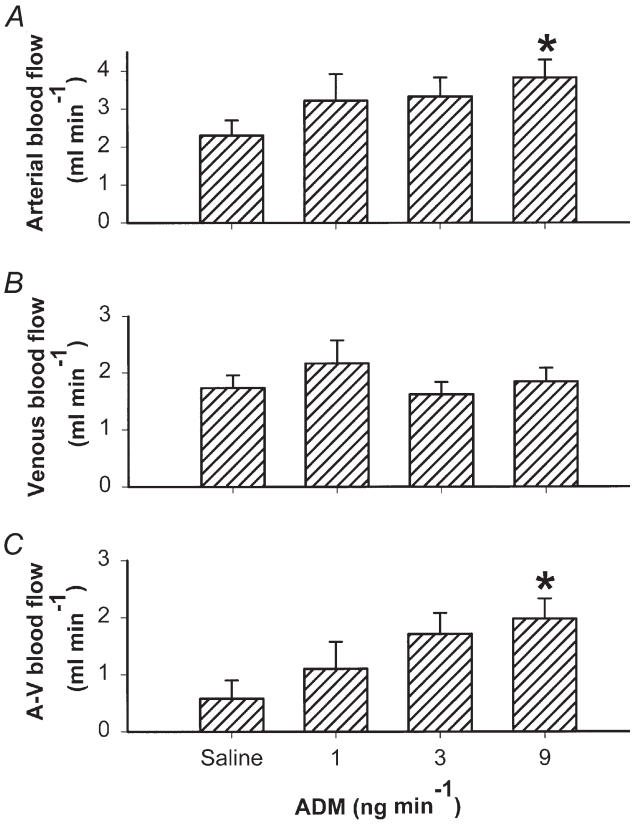

Figure 1. Effect of ADM on splenic arterial (*A*) and venous (*B*) blood flows, and fluid efflux (*C*) (arterial − venous blood flow) from the spleen

ADM was infused into the splenic artery at doses of 0 (saline), 1, 3 and 9 ng min^{-1} ($n = 11$). Error bars indicate S.E.M. * $P < 0.05$ compared with saline-infused control group.

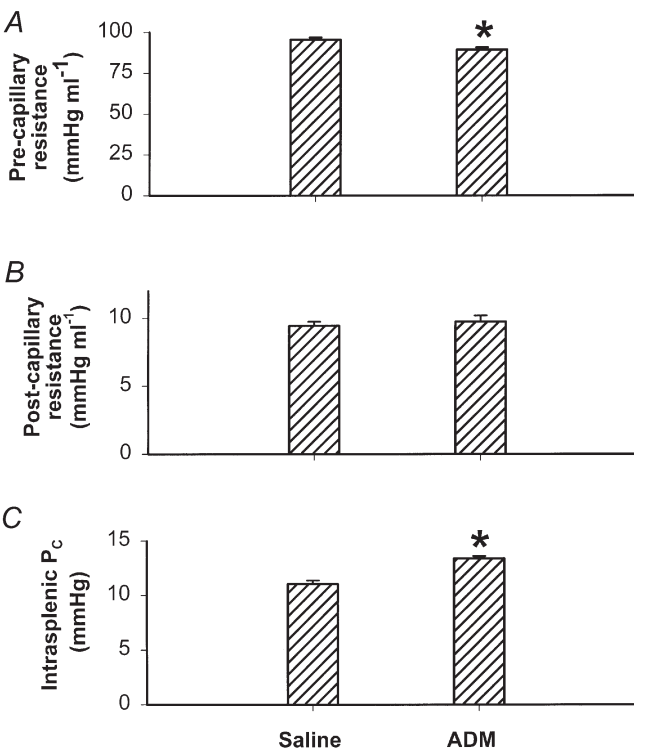

Figure 2. Effect of ADM on pre-capillary resistance (*A*), post-capillary resistance (*B*), and microvascular pressure (P_C, *C*) in the spleen

ADM was infused at a dose of 9 ng min^{-1}. Error bars indicate S.E.M. * $P < 0.05$, significant difference between saline-infused ($n = 5$) and ADM-infused ($n = 6$) groups.

of splenic resistance arteries and veins. When ADM was infused into the splenic artery (experiment A), it caused a dose-dependent increase in fluid extravasation from the splenic vasculature (Fig. 1*C*). This was achieved in the absence of a significant change in MAP. The ADM-induced increase in intrasplenic fluid efflux was due to a rise in splenic arterial flow (Fig. 1*A*) in the absence of any change in splenic venous flow (Fig. 1*B*). ADM caused an increase in intrasplenic microvascular pressure, due to a fall in pre-capillary resistance; there was no change in post-capillary tone (Fig. 2). We propose that it is the rise in intravascular hydraulic pressure that drives the increase in intrasplenic fluid extravasation. It may be calculated that, at a splenic blood flow of 1 ml min^{-1}, the plasma concentration of ADM was approximately 10^{-8} M. At this concentration *in vitro*, ADM-induced vasodilatation was significantly greater in the splenic resistance arteries than in the veins (Fig. 3).

Unlike most other vascular beds, the splenic vasculature has a discontinuous endothelium (Takubo *et al.* 1999) which enables protein-rich (iso-oncotic) fluid to cross readily from the intravascular compartment into extra-vascular spaces. Previous experiments have demonstrated that the extravasated fluid is not stored within the parenchyma of the spleen, but drains into the systemic lymphatic system through large splenic lymphatic ducts (Kaufman & Deng, 1993). This is consistent with the fact that the rat spleen is non-compliant and cannot acutely increase intrasplenic volume (Reilly, 1985; Kaufman & Deng, 1993; Chen & Kaufman, 1996). In the present study, the volume of fluid (approximately 10 ml) extravasated from the splenic circulation during the 5 min period of infusion of ADM (9 ng min^{-1}) was larger than the total volume capacity of the rat spleen. Moreover, there was no significant difference in wet splenic tissue weight between ADM- and saline-infused spleens ($P > 0.05$). This indicates that the splenic A − V flow differential must represent lymphatic drainage from the spleen. It should be recognized that it is the balance between such loss of fluid to the extravascular space and its rate of return to the blood, through, for example, the thoracic duct, that ultimately determines intravascular volume (Isbister, 1997).

Although it would have been preferable to measure lymph flow directly, rather than estimating it from the difference between splenic arterial inflow and venous outflow, we have not been able to do this in the rat. This is due to the fact that there are several branches of the splenic lymphatic duct that cannot be placed together in a flow probe without damaging them. We do, however, ensure vascular isolation of the spleen by tying off all vessels running to and from the surrounding tissues, and by infusing dye to confirm that the splenic artery and vein supply and drain only the spleen. Therefore, by the law of mass action, the difference between the volume of blood going into the spleen and the volume coming out must represent the fluid volume leaving the circulation.

We have shown that splenic resistance arteries are more sensitive than are the veins to the vasodilatory activity of ADM. *In vivo*, this raises intrasplenic P_C and causes increased fluid efflux into extravascular spaces. A parallel may be drawn with the effects of ADM on the renal vasculature. ADM increases glomerular filtration rate by differential vasorelaxation of the afferent arteriole compared with the efferent arteriole (Jougasaki *et al.* 1995). Although ADM receptors have been identified

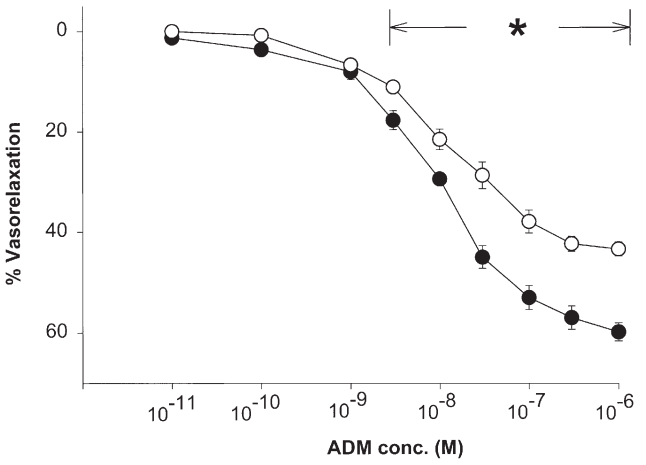

Figure 3. Effect of ADM on vasorelaxation of splenic resistance arteries (●, $n = 9$) and veins (○, $n = 8$)

The vessels were preconstricted with phenylephrine (EC_{80}). * $P < 0.05$, significant difference between relaxation of artery and vein.

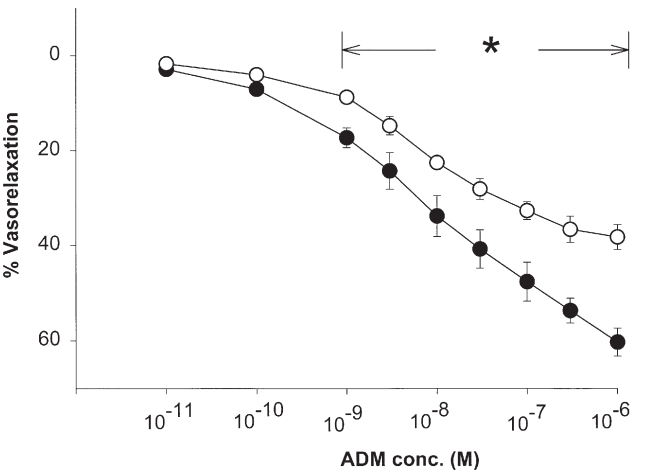

Figure 4. Effect of NO synthase inhibition on vasorelaxant activity of ADM on splenic resistance arteries

○, ADM plus L-NMMA (10^{-4} M). ●, ADM plus D-NMMA (10^{-4} M). The vessels were preconstricted with phenylephrine (EC_{80}). * $P < 0.05$, significant difference between L-NMMA- and D-NMMA-treated groups.

in the spleen (Kapas *et al.* 1995; Cameron & Fleming, 1998), no attempt has been made to study differential binding or receptor density in splenic resistance arteries or veins.

ADM may act at specific ADM receptors, or at non-specific (calcitonin gene-related peptide) receptors; these receptors have been found on endothelial cells and on vascular smooth muscle cells (Eguchi *et al.* 1994; Muff *et al.* 1995; McLatchie *et al.* 1998). There are two known mechanisms by which ADM causes vasodilatation: through cAMP and by increasing NO biosynthesis (Hirata *et al.* 1995; Ikeda *et al.* 1996; Minamino *et al.* 1998). In the kidney, ADM appears to act at specific ADM receptors rather than through the non-specific receptors for calcitonin gene-related peptide (Hjelmqvist *et al.* 1997); the ensuing vasodilatation is mediated, at least in part, through increased NO biosynthesis (Miura *et al.* 1995). Although we do not know the receptor type to which ADM binds in the spleen, our data suggest that the ensuing vasodilatation (at least in hilar arteries) is mediated, at least in part, through generation of NO. This is of interest considering our previous finding that NO increases fluid efflux from the splenic circulation of the rat (Andrew *et al.* 2001).

There has been only one other study on the effects of ADM on splenic haemodynamics; He and colleagues demonstrated that ADM increases splenic blood flow (He *et al.* 1995; Wang *et al.* 1998). Ours is thus the first report of ADM-induced changes in intrasplenic haemodynamics and in differential vasoactivity of the splenic resistance vessels. Furthermore, the dose of ADM used by He and colleagues (He *et al.* 1995; Wang *et al.* 1998), which was at least an order of magnitude higher than ours, caused a marked depression of mean arterial pressure. Since we used a low dose of ADM, and since we infused it into the splenic artery rather than systemically, our responses were not compromised by changes in splenic perfusion pressure.

In conclusion, we have shown that physiological/pathophysiological doses of ADM, which do not alter systemic blood pressure, increase fluid extravasation from the splenic vasculature by differentially reducing pre- *vs.* post-capillary resistance, thus raising intrasplenic P_C. The importance of this ADM-induced loss of fluid from the splenic circulation may be of significance during pathophysiological conditions such as septic shock, where ADM levels are greatly elevated (Nishio *et al.* 1997).

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Corresponding author

S. Jacobs-Kaufman: 475 Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2S2.

Email: susan.jacobs@ualberta.ca