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Vascular reactivity in rats with glycerol-induced acute renal failure

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1 The vascular reactivity of rats with glycerol-induced acute renal failure was assessed *in vitro* and *in vivo*.

2 The contractile responses to noradrenaline, angiotensin and potassium chloride of aortic strips and portal vein segments from uraemic rats were significantly smaller than the responses obtained in vessels from control animals.

3 The pressor responses to angiotensin were reduced significantly in rats with acute renal failure when compared to those in control rats.

4 The reduced vascular responses to a range of spasmogens suggests that in this model of renal failure there is a defect in some mechanism of vascular contraction common to all three constrictor agents.

Introduction

In a recent study of rats with glycerol-induced acute renal failure (ARF) (Bowmer *et al.*, 1983) we found decreased pressor responses to noradrenaline and elevated plasma noradrenaline concentrations. In addition the chronotropic responses to right cervical sympathetic and vagal stimulation were decreased. Rascher *et al.* (1982) also observed diminished vascular reactivity to noradrenaline in rats with chronic renal failure whilst the vascular responses to vasopressin in these uraemic animals were similar to those found in control rats. These workers concluded that the decreased vascular responses were specific for noradrenaline. By contrast, in an investigation of vascular reactivity in rats with ARF produced by bilateral ureter ligation, Ueda *et al.* (1981) showed that diminished pressor responses and decreased contractile responses of aortic strips occurred to both noradrenaline and angiotensin.

The aim of the present study was to assess vascular reactivity in ARF, both *in vitro* and *in vivo*, to a range of constrictor agents in order to determine any selectivity in the depression of vascular responses using the glycerol model of ARF.

Methods

Induction of acute renal failure

The method for the production of ARF has been described in detail previously (Bowmer *et al.*, 1983). Groups of male Wistar rats (250–350 g body weight), after being dehydrated for 24 h, were given an intramuscular injection of 50% v/v glycerol in sterile saline (0.9% w/v NaCl solution) (10 ml kg^{-1} body weight). Uraemic and control rats (injected with saline) were studied 48 h after their respective injections.

Isolated blood vessels

Rats were killed by a blow to the neck and a heparinised blood sample was removed immediately from the heart for subsequent determination of plasma urea concentration. The descending thoracic aorta and portal vein were exposed, cleared of adherent tissue and submerged in freshly prepared cold (5°C) Krebs-Ringer bicarbonate (KRB) solution of the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4 7H_2O$ 1.2, $NaHCO_3$ 25.0 and glucose 10.0. Helically cut aortic strips (1.4–1.6 mm in width by 25 mm in length) were set up in a 15 ml organ bath at a resting tension of 1.5 g for isometric contraction recordings. Segments of portal veins (10 to 12 mm long) were tied at both ends by inserting sutures through the wall of the vessel and similarly arranged under a resting tension of 0.5 g. The preparations were equilibrated for 1 h in KRB solution bubbled continuously with a mixture of 95% O_2 and 5% CO_2 and kept at 37°C. The loading tensions were maintained by periodic adjustment throughout the experiments. The incubation media

were routinely changed every 10 to 15 min to guard against accumulation of metabolites (Altura & Altura, 1970). Tissues were attached to a Dynamometer UFI force displacement transducer connected via a Devices preamplifier (3559) to a Devices pen recorder (MX216).

Contractions were recorded and concentration-response curves constructed to noradrenaline (5×10^{-11}–5×10^{-5} M) and angiotensin (1×10^{-10}–1×10^{-5} M), each added to the bath in single doses in order of increasing concentration. A dosing interval of 10 min was used for noradrenaline whilst doses of angiotensin were added every 20 min to avoid tachyphylaxis, concentration-response curves were also obtained to KCl (10^{-2}–10^{-1} M) by exposing the tissues to a series of KRB solutions in which the amount of KCl was increased in equimolar replacement for NaCl in order to maintain the tonicity of the bathing medium. The drugs and each high KCl solution were maintained in contact with the tissue until the maximum contractile response had developed and then removed by successive washings with normal KRB solution.

Since portal vein preparations exhibited spontaneous activity the contractile response to an agonist was determined as the difference between the mean peak spontaneous tension in the 1 min period before agonist application and the peak tension developed in the presence of the agonist. Contractile responses to agonists on the aortic strips were measured as the maximum increase in tension over the steady base line.

Only one agonist was used for each tissue. The agonists were prepared in KRB solution and their concentrations expressed as final molar concentration in the bath.

Measurement of pressor responses

Rats were anaesthetized with thiobutabarbitone (120–160 mg kg^{-1} i.p.), a tracheal cannula was inserted and artificial respiration was maintained with a Miniature Ideal Pump (BioScience) ventilation rate 80 strokes min^{-1} and stroke volume 10 ml kg^{-1}. Cannulae were also inserted into the right femoral artery and vein. The cannula in the right femoral artery was connected to a Bell and Howell pressure transducer (type 4-326-L212) and then to a Devices M2 pen recorder where the pressure wave was used to trigger a rate meter. Rectal temperature was maintained at 37°C by means of a heating lamp.

The peak responses of blood pressure and heart rate were recorded to a series of bolus intravenous injections of angiotensin (0.02–5.0 μg) dissolved in saline. The dose of angiotensin as a function of body weight (μg kg^{-1}) was determined and log dose-response lines constructed by linear regression.

At the end of the experiment a heparinized blood sample was taken for the measurement of plasma urea concentration.

Measurement of plasma urea

Plasma urea concentrations were measured by reaction with diacetyl monoxime using the reagents and procedure detailed in Sigma Technical Bulletin No. 535 (Sigma Chemical Co.).

Drugs

(−)-Noradrenaline bitartrate was obtained from Sigma Chemical Co. Angiotensin II amide (Hypertensin) was a kind gift from Ciba Laboratories. All doses of drug refer to the salt.

Statistical analysis

Results are expressed as mean ± s.d. and statistical comparisons were made using a non-paired Student's *t* test.

Results

Rats which were injected with glycerol had significantly elevated ($P < 0.001$) plasma urea concentra-

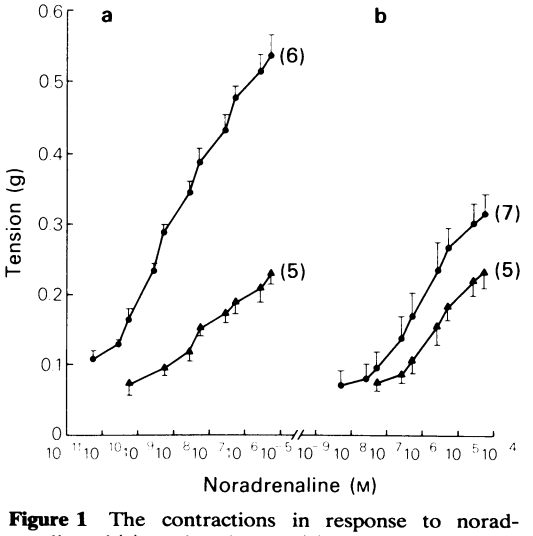

Figure 1 The contractions in response to noradrenaline of (a) aortic strips and (b) portal vein segments from control rats (●) and rats with acute renal failure (▲). Values shown are mean with the number of experiments shown in parentheses; s.d. indicated by vertical lines. The responses obtained in rats with acute renal failure were significantly different from control values at all concentrations ($P < 0.05$).

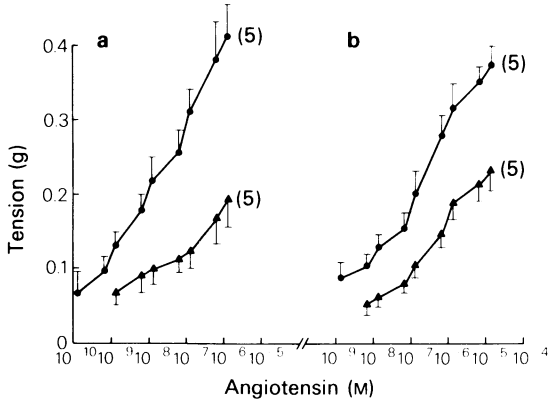

Figure 2 The contractions in response to angiotensin II amide of (a) aortic strips and (b) portal vein segments from control rats (●) and rats with acute renal failure (▲). Values shown are mean with the number of experiments shown in parentheses; s.d. indicated by vertical lines. The responses obtained in rats with acute renal failure were significantly different from control values at all concentrations ($P<0.001$).

tions (293 ± 132 mg $100\,ml^{-1}$, $n=23$) compared to control animals (35 ± 15 mg $100\,ml^{-1}$, $n=24$).

In vitro *studies*

After the equilibration period the rate and amplitude of the spontaneous contractions of the portal vein segment were measured over an interval of 5 min. Although the rates of contractions per min in control (5.4 ± 1.2, $n=17$) and uraemic rats (5.5 ± 2.3, $n=15$) were similar, the amplitude of these contractions in uraemic animals (0.06 ± 0.03 g, $n=15$) was significantly smaller ($P<0.001$) than that recorded in control animals (0.15 ± 0.05 g, $n=17$).

The contractions of the aortae and portal veins in control and uraemic animals in response to noradrenaline, angiotensin and KCl are shown in Figures 1, 2 and 3 respectively. In tissues from uraemic rats the dose-response curves to each spasmogen were shifted to the right and the sizes of the maximal responses were depressed. The contractions were significantly smaller than control responses at all concentrations of each constrictor agent in both vessels. Within the range of concentrations employed a greater depression of the maximum response to the spasmogens occurred in the aortic (50–60%) than in the portal vein preparations (20–45%).

In vivo *studies*

The mean arterial blood pressure and heart rate of uraemic animals (95 ± 22 mm Hg; 345 ± 25 beats min^{-1}, $n=8$) were significantly lower ($P<0.05$) than their control counterparts (122 ± 13 mmHg; 395 ± 19 beats min^{-1}, $n=7$). The pressor responses elicited by bolus intravenous injections of angiotensin were significantly smaller in uraemic rats than in control rats (Figure 4). All doses of angiotensin in both control and uraemic animals evoked a bradycardia ranging from 5–25 beats min^{-1}.

Discussion

In the present study we have clearly demonstrated reduced sensitivity of aortae and portal veins from

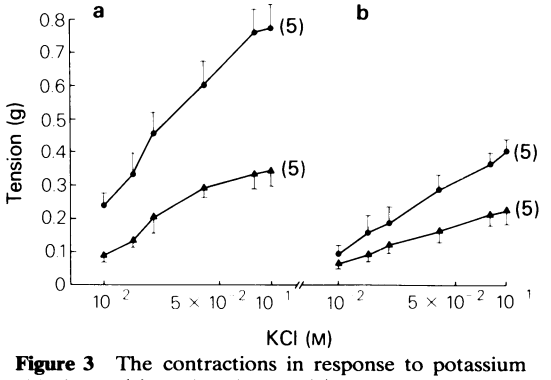

Figure 3 The contractions in response to potassium chloride of (a) aortic strips and (b) portal vein segments from control rats (●) and rats with acute renal failure (▲). Values shown are mean with the number of experiments shown in parentheses; s.d. indicated by vertical lines. The responses obtained in rats with acute renal failure were significantly different from control values at all concentrations ($P<0.05$).

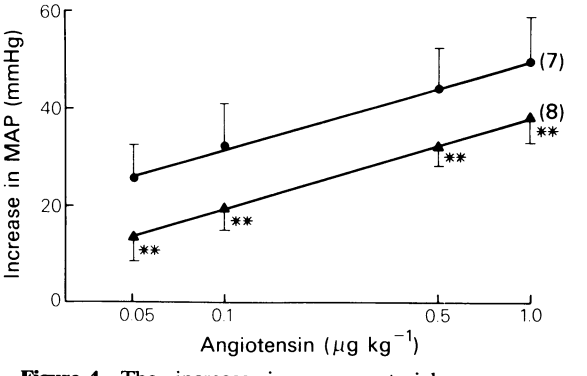

Figure 4 The increase in mean arterial pressure (MAP) in response to intravenous doses of angiotensin II amide in control rats (●) and rats with acute renal failure (▲). Values shown are mean with the number of rats shown in parentheses; s.d. indicated by vertical lines. Significantly different from control values: $^{**}P<0.01$.

ARF rats to a range of constrictor agents *in vitro*. The decreased vascular reactivity to noradrenaline supports our previous findings of reduced pressor responses to noradrenaline in uraemic rats (Bowmer *et al.*, 1983). Similarly diminished vascular responses to noradrenaline have been observed both in humans and rats with chronic renal failure (Campese *et al.*, 1981; Rascher *et al.*, 1982) and in rats with ARF produced by bilateral ureter ligation (Ueda *et al.*, 1981). In the study conducted by Rascher *et al.* (1982) the vascular responses to vasopressin in uraemic animals were unchanged whilst those to potassium chloride and barium chloride were increased. They concluded that the decreased response to noradrenaline in renal failure was due to down-regulation of α-receptors in response to elevated circulating noradrenaline levels. Whilst we too have found increased plasma noradrenaline concentrations in glycerol-induced ARF (Bowmer *et al.*, 1983) the decreased vascular responses to noradrenaline, angiotensin and potassium chloride observed in the present study suggest that, at least in this acute model of renal failure, the depression of vascular responses is less specific and not restricted to noradrenaline.

The decreased contractions of the isolated blood vessels from uraemic animals to angiotensin (Figure 2) is paralleled by the reduced pressor responses to the peptide *in vivo* (Figure 4) which supports similar findings observed in the investigation of ARF produced by ureter ligation (Ueda *et al.*, 1981). In the latter model of ARF, plasma renin activity was found to be elevated when compared to control animals and the authors suggested that since there is an inverse relationship between pressor responsiveness to angiotensin and the state of activation of the renin-angiotensin system, the elevated renin levels in ureter ligated rats may explain the diminished vascular response to exogenous angiotensin (Ueda *et al.*, 1981). However, it is doubtful whether this explanation would account for the reduced pressor response to angiotensin in the present study since plasma renin activity in the glycerol model of ARF after increasing at 12 h returns to control levels at 48 h (Semple *et al.*, 1976), which is the interval after glycerol injection when the present study was conducted.

Renal failure is normally associated with electrolyte imbalance and in the glycerol model of ARF various disturbances of electrolytes have been noted (Thiel *et al.*, 1967). It is possible that such an imbalance may affect the response of vascular smooth muscle to constrictor agents *in vivo*. However, it is unlikely that an electrolyte imbalance in the ARF rat would explain the reduced vascular responses observed in its isolated tissues after they had been equilibrated in KRB solution.

The amplitudes of the spontaneous contractions of portal veins in uraemic animals were significantly smaller than those recorded in control animals. Since these spontaneous contractions are coupled to functional voltage-dependent calcium channels (Johansson & Somlyo, 1980), their availability may have been reduced in ARF. Potassium chloride causes contraction by depolarization-induced calcium influx; the response to noradrenaline and angiotensin is a result of activation of receptor-coupled calcium channels (Bolton, 1979) and at high concentrations may involve intracellular calcium release (Bohr, 1973). Thus the decreased vascular responses to the range of constrictor agents used in the present study involves either a defect in both types of calcium channels or an alteration in some aspect of excitation-contraction coupling common to all three agents. This could be an accelerated rate of sequestration or efflux of calcium or possibly a result of abnormal cellular metabolism which has been shown to occur in uraemia (Knochel & Seldin, 1976). Some of these possibilities are the subject of further investigations.

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