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# Cardiovascular responses in rats with glycerol-induced acute renal failure

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**1** Autonomic and cardiovascular function were assessed in rats with glycerol-induced acute renal failure (ARF).

**2** Rats with ARF had significantly lower mean arterial blood pressures and heart rates and significantly elevated plasma noradrenaline concentrations.

**3** The chronotropic responses to right cervical sympathetic and vagal stimulation were diminished in rats with ARF.

**4** The pressor and depressor responses to noradrenaline and nitroprusside respectively when expressed as a change in mmHg pressure were significantly reduced in rats with ARF when compared to controls. However, when the depressor responses to nitroprusside were expressed as a percentage fall in basal mean arterial pressure, with the exception of the response to a dose of $10\,\mu g\,kg^{-1}$, there were no significant differences between control and uraemic rats.

**5** The present findings show that in the rat, changes in cardiovascular responsiveness occur after a brief period of uraemia which are similar to those observed in patients and rats with chronic renal failure.

## Introduction

A variety of abnormalities of autonomic function have been noted in patients with chronic renal failure. These include defective function of sweat glands (Hennessy & Siemsen, 1968), abnormal response to the Valsalva manoeuvre (Ewing & Winney, 1975; Campese, Romoff, Levitan, Lane & Massry, 1981), vagal neuropathy (Burgess, 1982; Endre, Perl, Kraegen, Charlesworth & Macdonald, 1982) and reduced pressor response to sustained hand grip exercise (Ewing & Winney, 1975; Campese *et al.*, 1981). Impairment of autonomic function has also been implicated in haemodialysis-induced hypotension (Kersh, Kronfield, Unger, Popper, Cantor & Cohn, 1974); although the importance of any defect in the autonomic nervous system in this disorder has been disputed (Naik, Mathias, Wilson, Reid & Warren, 1981).

In the study conducted by Campese *et al.* (1981) noradrenaline infusions produced smaller changes in mean arterial blood pressure and heart rate in undialysed than in dialysed patients with renal failure. In support of these clinical findings, diminished vascular response to noradrenaline has also been demonstrated in rats 35 days after induction of chronic renal failure (Rascher, Schömig, Kreye & Ritz, 1982).

The aim of the present investigation was to assess any changes in autonomic and cardiovascular function which may occur after a brief period of uraemia by studying rats with acute renal failure (ARF).

## Methods

### *Induction of acute renal failure*

Acute renal failure was produced by intramuscular injection of glycerol (Thiel, Wilson, Arce & Oken, 1967). Male Wistar albino rats (250–350 g) were deprived of drinking water for 24 h but allowed food *ad lib.* An intramuscular injection of 50% v/v glycerol in sterile saline (0.9% NaCl w/v), $10\,ml\,kg^{-1}$ body weight, was then administered, under ether anaesthesia, in divided doses in two sites in each of the hind limbs. Control rats were similarly dehydrated but injected with sterile saline only ($10\,ml\,kg^{-1}$ body weight). Both groups of rats were studied 48 h after the intramuscular injection of either saline or glycerol.

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*Experimental protocol*

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 Statham pressure transducer and then to a Grass Model 79 polygraph 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 right vagus and cervical sympathetic nerves were prepared for stimulation (Large, 1975) to assess cardiac chronotropic responses. The vagus in the neck was doubly ligated and sectioned. The right cervical sympathetic nerve was left intact and placed on shielded bipolar platinum electrodes immersed in liquid paraffin. The nerve was stimulated with rectangular pulses, 0.5 ms duration, supramaximal voltage (8–10V) and various frequencies. The frequency-response curves were obtained by initially stimulating at a low frequency (0.5 Hz) and, after a plateau appeared in the chronotropic responses, by progressively increasing the frequency to 1, 2, 5, 7.5, 10 and 15 Hz. Similar sequences of stimulation were applied to the cardiac end of the severed right vagus.

After the periods of nerve stimulation the response of blood pressure was recorded to a series of bolus i.v. injections of noradrenaline (0.1–5.0 μg $kg^{-1}$) and sodium nitroprusside (0.1–25.0 μg $kg^{-1}$). At the end of the experiment a heparinised blood sample was taken for the measurement of plasma urea concentration.

*Measurement of plasma urea and noradrenaline*

Plasma noradrenaline concentrations were measured in a separate series of experiments. Rats were anaesthetized with thiobutabarbitone (120–160 mg $kg^{-1}$ i.p.), a cannula placed in the right carotid artery and 45 min later a heparinised blood sample (0.6 ml) was taken. Plasma was separated from the blood samples and stored at −20°C. A further blood sample was taken for the determination of plasma urea concentration.

The plasma noradrenaline concentration was measured using the radio-enzymatic technique of Da Prada & Zürcher (1976). The assay is based upon the 3-*O*-methylation of noradrenaline by catechol-*O*-methyltransferase in the presence of the methyl donor, [$^3$H]-S-adenosyl methionine. The methoxy-derivative, labelled normetanephrine, was isolated by solvent extraction (diethyl ether followed by back extraction into 0.1 M HCl), freeze dried and separated by thin layer chromatography (Silica Gel 60 $GF_{254}$, Merck) using chloroform: ethanol: 70% ethylamine (16:3:2 by volume) as the mobile phase. Samples were counted in a Beckman LS-330 liquid scintillation counter.

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*

Sodium nitroprusside and (−)-noradrenaline bitartrate were obtained from Sigma Chemical Co. Drugs were dissolved in saline and all doses of drug refer to the salt.

*Statistical analysis*

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

## Results

Rats which were injected with glycerol had significantly elevated plasma urea concentrations when compared to control animals (Table 1). These uraemic animals had significantly lower basal systolic, diastolic and mean arterial blood pressures than controls and furthermore the heart rate of uraemic rats was significantly lower (Table 1).

The positive chronotopic response to right cervical sympathetic stimulation was diminished in uraemic

**Table 1** Blood pressure, heart rate and plasma urea concentrations in rats that had received an intramuscular injection of either glycerol or saline 48 h previously

| | *Saline injected* ($n=7$) | *Glycerol injected* ($n=7$) |
|---|---|---|
| Systolic pressure (mmHg) | 142 ± 5 | 100 ± 7*** |
| Diastolic pressure (mmHg) | 92 ± 4 | 53 ± 5*** |
| Mean pressure (mmHg) | 111 ± 4 | 72 ± 6*** |
| Heart rate (beats $min^{-1}$) | 367 ± 8 | 321 ± 9** |
| Plasma urea (mg 100 $ml^{-1}$) | 50 ± 9 | 377 ± 71*** |

Results are given as mean ± s.e.mean
**$P<0.01$; ***$P<0.001$, relative to respective control group.

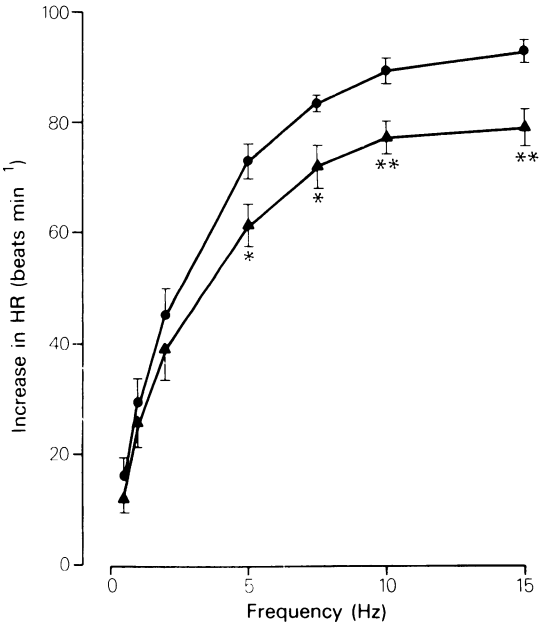


**Figure 1** The increase in heart rate (HR) with increasing frequency of right cervical sympathetic stimulation (8–10 V, 0.5 ms) in control rats (●) and rats with acute renal failure (▲). Values are mean ($n = 7$); s.e.mean indicated by vertical lines. Significantly different from control values: $*P < 0.05$; $**P < 0.01$.

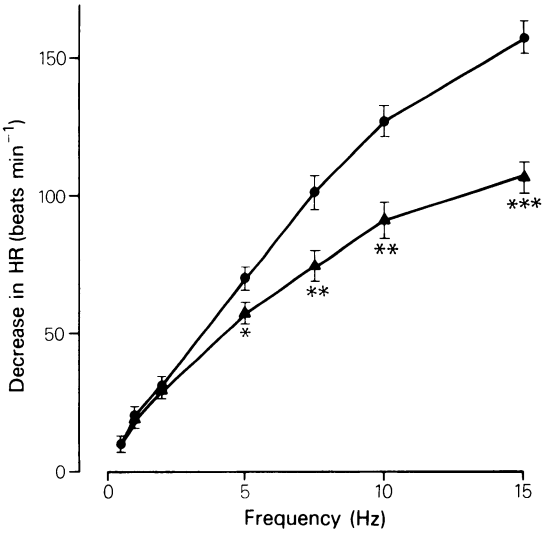


**Figure 2** The decrease in heart rate (HR) with increasing frequency of right vagal stimulation (8–10 V, 0.5 ms) in control rats (●) and rats with acute renal failure (▲). Values are mean ($n = 7$); s.e.mean indicated by vertical lines. Significantly different from control values: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

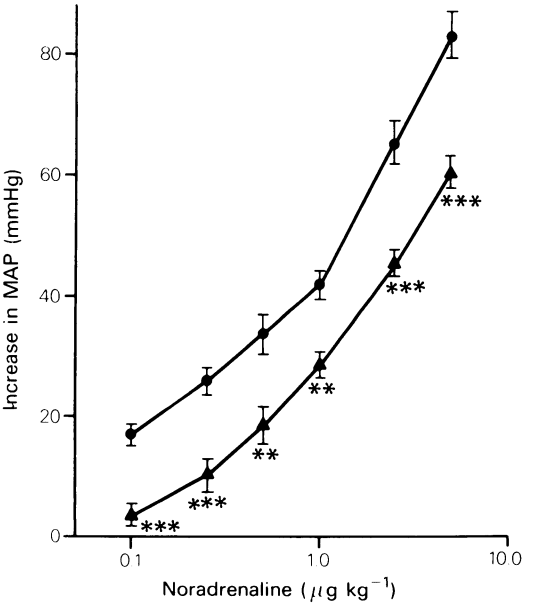


**Figure 3** The increase in mean arterial pressure (MAP) in response to intravenous doses of noradrenaline in control rats (●) and rats with acute renal failure (▲). Values are mean ($n = 7$); s.e.mean indicated by vertical lines. Significantly different from control values: $**P = 0.01$; $***P < 0.001$.

rats when compared to controls (Figure 1). This difference was statistically significant at the higher frequencies of stimulation (5–15 Hz). In addition, the negative chronotropic response to right vagal stimulation was also reduced in uraemic animals (Figure 2) with significant differences again occurring between 5 to 15 Hz.

The increase in mean arterial blood pressure in control and uraemic rats in response to injection of noradrenaline is shown in Figure 3, which clearly shows decreased pressor responses in uraemic animals at all doses of noradrenaline employed. The chronotropic reponses to noradrenaline were minimal such that a dose of 5 $\mu g\,kg^{-1}$ in both uraemic and control rats only elicited an increase in heart rate of 10–15 beats $min^{-1}$.

Uraemic rats exhibited significantly reduced depressor responses (mmHg) to nitroprusside in the dose range 1–25 $\mu g\,kg^{-1}$. (Figure 4a). These depressor responses were also expressed as a percentage fall in basal mean arterial blood pressure (Figure 4b) and when expressed in this manner the depressor response to nitroprusside was only significantly reduced at a dose of 10 $\mu g\,kg^{-1}$.

From a separate series of experiments in which blood samples only were taken, the animals which received glycerol had a mean plasma urea concentra-

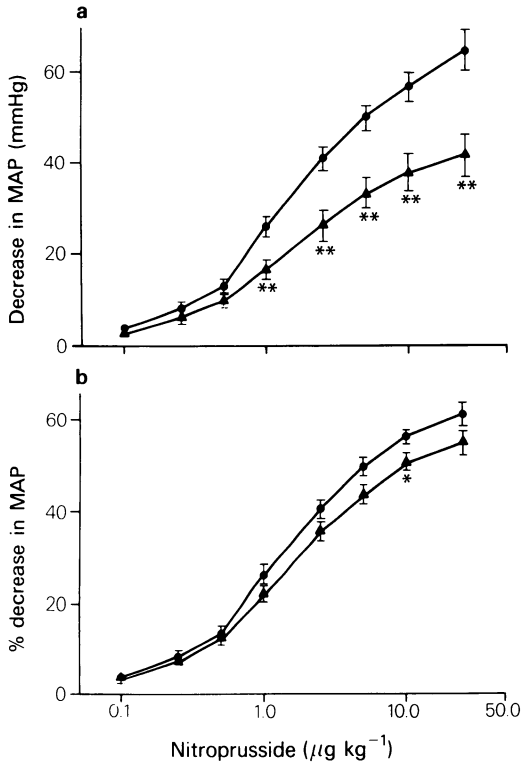


**Figure 4** The decrease in mean arterial pressure (MAP) in response to intravenous doses of nitroprusside expressed in (a) as mmHg fall in MAP and in (b) as a percentage fall in basal MAP in control rats (●) and rats with acute renal failure (▲). Values are mean ($n = 7$); s.e.mean indicated by vertical lines. Significantly different from control values: $^{*}P < 0.05$; $^{**}P < 0.01$.

tion ($282 \pm 51$ mg $100\,ml^{-1}$; $n = 6$) which was significantly greater ($P < 0.001$) than the control mean value ($44 \pm 2$ mg $100\,ml^{-1}$; $n = 6$). These uraemic rats had significantly elevated ($P < 0.05$) plasma noradrenaline concentrations ($504 \pm 78$ pg $ml^{-1}$; $n = 6$) which were about 2.5 times higher than control levels ($191 \pm 64$ pg $ml^{-1}$; $n = 6$).

## Discussion

The present investigation demonstrated that some abnormalities exist both in the status and reactivity of the cardiovascular system of rats with glycerol-induced ARF.

The diminished negative chronotropic response to vagal stimulation (Figure 2) supports certain clinical observations; for instance reduced cardiac beat-to-beat variation in uraemic patients which is indicative of vagal neuropathy (Burgess, 1982; Endre *et al.*, 1982). In addition, the abnormal response in heart rate during the release phase of the Valsalva manoeuvre in undialysed compared to dialysed patients also suggests some derangement in the vagal pathway in uraemia (Campese *et al.*, 1981). In the present study not only were responses to vagal stimulation reduced but also a diminished positive chronotropic response occurred to cervical sympathetic stimulation (Figure 1). Clinical evidence for reduced sympathetic function in uraemic patients is sparse although a reduced pressor response to handgrip exercise in undialysed compared to dialysed patients suggests some sympathetic dysfunction (Campese *et al.*, 1981).

It is not possible from the present findings to identify the mechanism responsible for reduced chronotropic responses to sympathetic and vagal stimulation. This effect may be a result of some defect in the response of the sinus node to autonomic transmitters. Desensitization of cardiac $\beta_1$-adrenoceptors may arise from the high circulating levels of noradrenaline we detected in uraemic rats. It would have been useful to study the cardiac reactivity of these rats to exogenous noradrenaline but unfortunately this could not be done because intravenous noradrenaline even in a high dose ($5\,\mu g\,kg^{-1}$) produced a minimal change in heart rate.

The finding of elevated circulating noradrenaline levels in rats with acute renal failure is in agreement with studies of both patients and rats with chronic renal failure (Ksiazek, 1979; Rascher *et al.*, 1982). The control plasma noradrenaline levels are similar to values from non-stressed conscious rats (Schömig, Dietz, Rascher, Lüth, Mann, Schmidt & Weber; 1978; Micalizzi & Pals, 1979). The elevated noradrenaline levels found in rats with chronic renal failure are considered to be a result of impaired neuronal uptake and metabolism of noradrenaline (Hennemann, Hevendehl, Reble & Heidland, 1973; Rascher *et al.*, 1982) and it is possible that a similar mechanism operates in rats with ARF.

The reduced pressor responses to noradrenaline (Figure 3) suggests a diminished vascular response which has also been shown to occur in aortic strips and isolated perfused hind limbs of rats with chronic renal failure (Rascher *et al.*, 1982). These investigators suggested that increased circulating levels of noradrenaline may account for the reduced vascular response to exogenous noradrenaline by inducing α-adrenoceptor down regulation. However, in the same study, paradoxically increased vascular responses to noradrenaline were observed in acute uraemia 48 h after bilateral nephrectomy. Increased sensitivity to various pressor agents has also been noted in the nephrectomized rat by other investigators (Mauz & Kreye, 1971). In contrast, decreased pressor responses and reduced contractions

of aortic strips to noradrenaline and angiotensin have been reported in acute renal failure produced by ureter ligation (Ueda, Ayano, Yano, Mutoh & Sakanashi, 1981). The discrepancy in the observations of vascular responsiveness between the present findings in glycerol-induced ARF plus those with ureter ligation (Ueda *et al.*, 1981) compared to the investigations with bilateral nephrectomy (Mauz & Kreye, 1971; Rascher *et al.*, 1982) may well be a consequence of the manner in which acute uraemia is induced.

The depressor responses to nitroprusside when expressed in mmHg were significantly reduced in uraemic rats compared to controls (Figure 4a). However, since uraemic rats had a lower mean arterial pressure blood pressure than control animals (Table 1), this may in itself reduce the response to a vasodilator drug so the depressor responses were also expressed as a percentage fall in basal mean arterial blood pressure (Figure 4b). When expressed in this manner the depressor responses to nitroprusside were in the main not significantly different from controls. These findings indicate that the response to a directly acting vasodilator is not appreciably affected in ARF.

The finding of a reduced basal heart rate in uraemic rats (Table 1) is supported by an earlier study in rats with the glycerol model of ARF (Hiley, Yates, Roberts and Bloom, 1980). However, in contrast to the present study, no reduction in blood pressure was noted although a lower level of mean arterial pressure has been observed in this model of ARF 24 h after glycerol injection (Hsu, Kurtz & Waldinger, 1977). An elevated cardiac output has been recorded in rats 24 h (Hsu *et al.*, 1977) and 48 h (Hiley *et al.*, 1980) after the induction of ARF, which suggests that for the maintenance of a lower blood pressure, peripheral resistance must be considerably reduced, perhaps by some vasodilator substance which is released in response to renal damage. A possible candidate in this respect is prostacyclin since increased prostacyclin-like activity has been demonstrated in uraemic patients (Remuzzi, Cavenaghi, Mecca, Donati & de Gaetano, 1977).

In conclusion, several abnormalities of cardiovascular responses have been demonstrated 48 h after the induction of ARF. These are similar to those occurring in patients and rats with chronic renal failure. The mechanisms for these abnormal responses are a subject of further studies.

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