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Cardiac reactivity in rats with acute renal failure

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Cardiac reactivity has been determined in rats with acute renal failure (ARF) induced by either bilateral nephrectomy or intramuscular glycerol injection. Rats with bilateral nephrectomy showed reduced chronotropic responses to cervical sympathetic stimulation but no appreciable alteration in the chronotropic responses to vagal stimulation. By contrast, we have previously noted that rats with glycerol-induced ARF show diminished chronotropic responses to stimulation of both nerves. The negative chronotropic and inotropic responses to carbachol in isolated atria from both nephrectomized and glycerol-injected rats were not significantly different from their respective controls. In both models of ARF the atrial positive chronotropic responses to isoprenaline were significantly decreased whilst positive inotropic responses were not significantly different from controls. The results indicate that the cause of the reduced chronotropic response to vagal stimulation observed in glycerol-injected rats is of presynaptic origin whilst the reduced chronotropic response to cervical sympathetic stimulation noted in both models of ARF may be due to an impaired postsynaptic response.

Cardiac function is usually depressed in patients with chronic renal failure (Prosser & Parsons 1975) but in experimental acute renal failure (ARF) the picture is somewhat confusing. For example, myocardial contractility has been shown to be increased in bilaterally nephrectomized rats (Nivatpumin et al 1975) and in dogs with short-term uraemia (Zebe et al 1976). However, in acute uraemia, myocardial end-organ resistance to catecholamines has been demonstrated, as studies of dogs with ARF have shown impaired contractile responses to the β-agonist orciprenaline whilst the heart rate responses were not significantly different from controls (Kreusser et al 1983). By contrast, rats that have undergone bilateral nephrectomy have diminished chronotropic responses to isoprenaline (Mann et al 1982).

Recent studies from our laboratory of anaesthetized rats with glycerol-induced ARF have shown diminished chronotropic responses to both vagal and cervical sympathetic stimulation (Bowmer et al 1983; Yates et al 1984). In an attempt to elucidate the mechanisms responsible for these changes we have extended our studies to rats with bilateral nephrectomy and employed both in-vivo and in-vitro experiments. The in-vitro experiments were conducted with isolated atria to assess end-organ response. A study of rats with bilateral nephrectomy permits an investigation of the effects of acute uraemia without the presence of damaged renal tissue.

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METHODS

Acute renal failure was induced in male Wistar rats, 300–350 g, by either bilateral nephrectomy or glycerol injection. Bilateral nephrectomy was performed under ether anaesthesia and control animals underwent a sham operation. These animals were studied 24 h after operation.

The detailed procedure for the induction of ARF with glycerol has been described previously (Bowmer et al 1983). Rats deprived of drinking water for 24 h were given an i.m. injection of 50% v/v glycerol in sterile saline (0.9% w/v NaCl solution) (10 ml kg^{-1}). Control animals received a saline injection (10 ml kg^{-1}). Both groups of rats were studied 48 h after injection.

Measurement of chronotropic responses in-vivo

Rats were anaesthetized with thiobutabarbitone (120–160 mg kg^{-1} i.p.) and a tracheal cannula inserted for artificial respiration. A cannula was also inserted into the right femoral artery which 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 to assess cardiac chronotropic responses as described previously (Bowmer et al 1983). At the end of the experiment a heparinized blood sample was taken for the measurement of plasma urea concentration.

Isolated atria

Rats were killed by a blow to the neck and a heparinized blood sample removed immediately from the left ventricle for subsequent plasma urea analysis. Both atria were removed and placed in an organ bath containing Krebs solution of the following composition (mM): NaCl 118·0, $NaHCO_3$ 25·0, glucose 11·0, KCl 4·7, KH_2PO_4 1·2, $MgSO_4 \cdot 7H_2O$ 1·2 and $CaCl_2$ 2·5. The solution was maintained at 35 °C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2. The spontaneously beating right atrium was connected to a Statham isometric transducer under a resting tension of 0·5 g. The rate of contraction was recorded on a Lectromed MX216 pen recorder via a preamplifier and instantaneous rate meter. The left atrium was paced at 2 Hz using rectangular pulses of 1 ms duration and 7 V amplitude (supramaximal). The left atrium was connected to an isometric transducer in manner similar to the right atrium and arranged for the recording of isometric contractions. Cumulative force and rate-concentration curves were obtained to carbachol and isoprenaline.

Measurement of plasma noradrenaline and urea

Plasma noradrenaline concentrations were measured in a separate series of experiments as described previously (Bowmer et al 1983). Rats were anaesthetized with thiobutabarbitone, a cannula placed in the right carotid artery and 45 min later a heparinized blood sample (about 0.6 ml) was taken. Plasma was separated from the blood samples and stored for 7 days at −20 °C. The plasma noradrenaline concentration was measured using the radioenzymatic technique of Da Prada & Zürcher (1976).

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. Carbachol chloride and (−)-isoprenaline bitartrate were obtained from the Sigma Chemical Co. and were dissolved in Krebs solution. All doses 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

In-vivo studies

Rats which underwent bilateral nephrectomy had significantly ($P < 0·001$) elevated plasma urea concentrations (337 ± 67 mg $100\,ml^{-1}$, n = 13) compared with sham-operated controls (30 ± 3 mg $100\,ml^{-1}$, n = 12). Bilaterally nephrectomized rats had a mean blood pressure of 87 ± 4 mm Hg which was significantly lower ($P < 0·01$) when compared with controls (112 ± 6 mm Hg). The heart rate of bilaterally nephrectomized animals (346 ± 12 beats min^{-1}) was lower than the values obtained in controls (372 ± 8 beats min^{-1}) but this difference was not statistically significant ($P > 0·05$).

The positive chronotropic response to right cervical sympathetic stimulation was diminished in bilaterally nephrectomized animals compared with controls (Fig. 1). This difference was statistically significant at all frequencies of stimulation (0·7–15 Hz). By contrast, the negative chronotropic response to right vagal stimulation (Fig. 2) was similar in bilaterally nephrectomized and control rats, with significant differences only occurring at the first two frequencies of stimulation (0·7 and 1·0 Hz).

From a separate series of experiments in which blood samples only were taken, bilaterally nephrectomized rats were found to have plasma noradrenaline concentrations (301 ± 66 pg ml^{-1}, n = 5) which were not significantly different to those obtained from sham-operated animals (333 ± 39 pg ml^{-1}, n = 6).

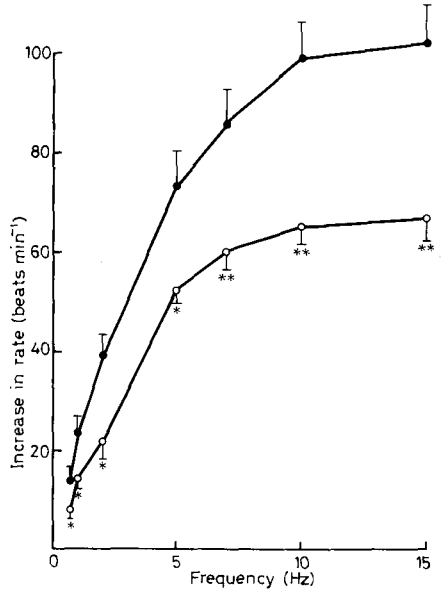

FIG. 1. The increase in heart rate with increasing frequency of right cervical sympathetic stimulation (8–10 V, 0·5 ms) in 7 sham-operated (●) and 8 bilaterally nephrectomized rats (○). Values are mean ± s.e.m. Significantly different from control values $*P < 0·05$, $**P < 0·01$.

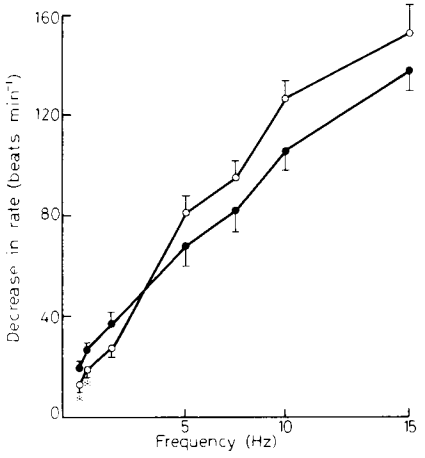

FIG. 2. The decrease in heart rate with increasing frequency of right vagal stimulation (8–10 V, 0·50 ms) in 7 sham-operated (●) and 7 bilaterally nephrectomized rats (○). Values are mean ± s.e.m. Significantly different from control values $^{*}P < 0{\cdot}05$.

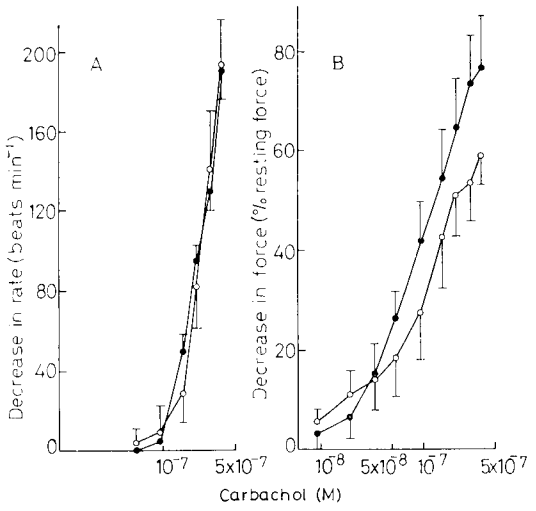

FIG. 3. The response of isolated atria to carbachol; (a) the decrease in rate of contraction of right atria and (b) the % decrease in force of contraction of paced left atria (7 V, 1 ms, 2 Hz) from 5 sham-operated (●) and 5 bilaterally nephrectomized rats (○). Values are mean ± s.e.m. The responses of atria from nephrectomized rats were not significantly different from controls.

In-vitro studies

Bilateral nephrectomy and glycerol injection produced similar elevations in plasma urea concentration (Table 1). The basal rate of contraction in right atria and basal force of contraction in left atria obtained from both groups of uraemic animals were not significantly different from their respective control values (Table 1).

There was no significant difference in the negative chronotropic and inotropic responses to carbachol of atria taken from bilaterally nephrectomized rats compared to controls (Fig. 3). However, the positive chronotropic responses to all concentrations of isoprenaline were significantly lower in right atria from nephrectomized rats (Fig. 4a) whilst the positive inotropic responses of the left atria were not significantly different from controls (Fig. 4b). The mean ED50% value for chronotropic responses to isoprenaline in atria from nephrectomized rats ($6{\cdot}1 \pm 0{\cdot}4 \times 10^{-10}$ M) was not significantly different from the control mean value ($6{\cdot}6 \pm 0{\cdot}9 \times 10^{-10}$ M).

Table 1. Plasma urea concentration, basal right atrial rate of contraction and basal force of contraction of the left atria from bilaterally nephrectomized and glycerol-injected rats.

	Plasma urea (mg 100 ml^{-1})	Right atrial rate (beats min^{-1})	Left atrial force (mg)
Sham-operated (n = 5)	35 ± 3	206 ± 6	220 ± 38
Bilaterally nephrectomized (n = 5)	227 ± 9***	216 ± 13	197 ± 17
Saline-injected (n = 6)	32 ± 3	205 ± 8	130 ± 20
Glycerol-injected (n = 7)	214 ± 41***	230 ± 16	158 ± 22

Results are given as mean ± s.e. mean.
*** $P < 0{\cdot}001$ relative to respective control group.

The responses of atria from rats with glycerol-induced ARF were similar to those obtained from bilaterally nephrectomized animals. There was no significant difference in the negative chronotropic and inotropic responses to carbachol or the positive inotropic responses to isoprenaline. The positive chronotropic responses to isoprenaline of the right atria from rats with glycerol-induced ARF were significantly lower at concentrations above $1{\cdot}3 \times 10^{-9}$ M when compared with saline-injected rats (Fig. 5). The sensitivity of atria from glycerol-injected rats to isoprenaline was unaltered since the mean ED50% ($1{\cdot}3 \pm 0{\cdot}4 \times 10^{-9}$ M) was not significantly different from the control mean values ($1{\cdot}5 \pm 0{\cdot}2 \times 10^{-9}$ M). However, the mean ED50% values from both glycerol and saline-injected rats were significantly higher ($P < 0{\cdot}001$) than those obtained in nephrectomized and sham-operated animals.

DISCUSSION

The present study has demonstrated changes in the cardiac reactivity of rats with bilateral nephrectomy and some of these are shared by rats with glycerol-induced ARF. Nephrectomized rats showed impaired chronotropic responses to cervical

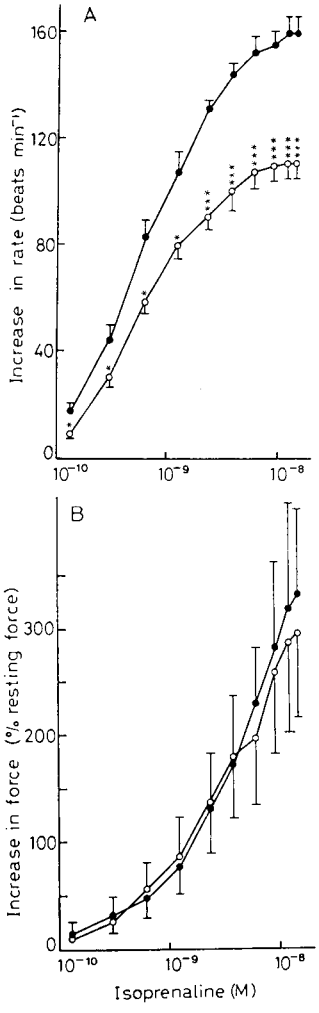

FIG. 4. The response of isolated atria to isoprenaline; (a) the increase in rate of contraction of right atria and (b) the % increase in force of contraction of paced left atria (7 V, 1 ms, 2 Hz) from 5 sham-operated (●) and 5 bilaterally nephrectomized rats (○). Values are mean ± s.e.m. Significantly different from control values $^{*}P < 0.05$, $^{***}P < 0.001$.

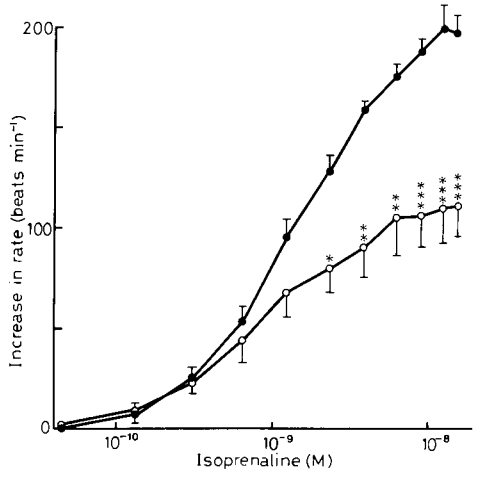

FIG. 5. The increase in rate of contraction in response to isoprenaline of isolated right atria from 6 saline-injected (●) and 7 glycerol-injected rats (○). Values are mean ± s.e.m. Significantly different from control values $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

sympathetic stimulation (Fig. 1) but no appreciable alteration in the response to vagal stimulation when compared with sham-operated animals (Fig. 2). By contrast, rats made acutely uraemic by glycerol injection showed diminished chronotropic responses to stimulation of both nerves (Bowmer et al 1983).

The mean blood pressure of nephrectomized rats was significantly lower than in controls which is in agreement with a previous study of bilaterally nephrectomized rats (Couture et al 1978). A reduction in blood pressure is also observed in rats with glycerol-induced ARF (Bowmer et al 1983). The resting heart rate of anaesthetized nephrectomized rats was lower than controls, although the difference was not statistically significant whereas we have noted a significantly lower heart rate in glycerol-injected rats (Bowmer et al 1983).

There was no evidence of overt cardiac dysfunction in rats with ARF produced by either method since atria from the uraemic animals had a basal rate and force of contraction which were not significantly different from their respective controls (Table 1). In addition, in both models of ARF the atrial negative chronotropic and inotropic responses to the muscarinic agonist carbachol were unaltered. However, by contrast to nephrectomized rats the chronotropic response to vagal stimulation is decreased in glycerol-induced ARF (Bowmer et al 1983). As the end-organ response appears normal in the glycerol model of ARF it would seem that the diminished response to vagal stimulation has a cause of presynaptic origin. These experiments do not identify the cause of this effect but it is interesting that it should only occur in the glycerol model. The significant difference between the two models of ARF is that in the glycerol model damaged renal tissue remains in-situ. It is possible that the damaged kidneys release a substance or substances which impair the response to vagal stimulation. A suitable candidate in this respect is prostaglandin E_2, levels of which are elevated in rats with glycerol-induced ARF (Torres et al 1974) and which may reduce the

release of acetylcholine elicited by vagal stimulation. (Feniuk & Large 1975).

The positive chronotropic response to the β-agonist isoprenaline was decreased in the right atria from both nephrectomized and glycerol-injected rats. This reduction in response to isoprenaline appears to be selective for rats since the inotropic responses of the left atria were not significantly reduced in either group of uraemic rat. These findings support observations of reduced chronotropic responses to isoprenaline in conscious rats 24 and 36 h after bilateral nephrectomy (Mann et al 1982; Hausen et al 1983). There was no change in the sensitivity of right atria from nephrectomized and glycerol-injected rats to isoprenaline as evidenced by no significant difference in the ED50% values between uraemic rats and their respective controls. However, the atria of bilaterally nephrectomized and sham-operated rats were more sensitive to the chronotropic effect of isoprenaline since the ED50% values were significantly lower than the values obtained from glycerol and saline-injected animals. There is no obvious explanation for this finding although it may be related to the stress of the surgery involved in bilateral nephrectomy and sham operation.

The diminished chronotropic responses to isoprenaline in ARF could be due to either a decrease in the number of cardiac β-receptors or a defect in receptor-response coupling. In this respect a recent study of rats bilaterally nephrectomized for 48 h has shown a reduction in cardiac β-adrenoceptor density and diminished activity of adenylate cyclase (Mann et al 1984). A reduction in β-adrenoceptor density may result from elevated catecholamine levels. We have detected increased plasma noradrenaline concentrations in rats with glycerol-induced ARF (Bowmer et al 1983) although in the present study we found no difference in plasma noradrenaline levels between nephrectomized and control rats. A decrease in cardiac β-adrenoceptor density in ARF should also produce a reduced inotropic response to isoprenaline although we could not detect this in isolated left atria. The reason for this selective depression of β-adrenoceptor mediated chronotropic responses is not apparent. Whatever its mechanism, the reduced chronotropic responses to isoprenaline of atria from nephrectomized and glycerol-injected rats suggest that the diminished chronotropic response to cervical sympathetic stimulation found in both models of ARF is mainly due to a reduction in the postsynaptic response to released noradrenaline. The elevated levels of prostaglandin E_2 in glycerol-injected rats (Torres et al 1974) may also contribute to the diminished response to cervical sympathetic stimulation in this model of ARF (Bowmer et al 1983) since prostaglandin E_2 has been shown to reduce the release of noradrenaline from cardiac noradrenergic nerve terminals (Hedqvist & Wennmalm 1971).

This study has clearly demonstrated changes in cardiac reactivity in both the bilateral nephrectomy and glycerol models of ARF. Although the exact mechanisms underlying these changes remain to be clearly defined the results point to the involvement of a presynaptic mechanism mediating the reduced chronotropic response to vagal stimulation in rats with glycerol-induced ARF. Whereas the reduced chronotropic response to cervical sympathetic stimulation observed in both models of ARF appears to result primarily from an impaired postsynaptic response.

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