J. Pharm. Pharmacol. 1987, 39: 711–716
Received January 30, 1987

Cardiovascular responses in rats with chronic renal failure

M. S. YATES, ELIZABETH A. ASKEY AND C. J. BOWMER*

Department of Pharmacology, Worsley Medical & Dental Building, The University of Leeds, Leeds LS2 9JT, UK

Cardiovascular responses were determined in rats with chronic renal failure (CRF) produced by five sixths nephrectomy and in sham-operated rats. The conscious systolic blood pressure of rats with CRF was significantly higher than the pressure in controls although, after anaesthesia, there were no significant differences in the mean arterial pressure between the two groups of rats. The pressor responses to noradrenaline in rats with CRF were not significantly different from those recorded in sham-operated controls. The bradycardia elicited by electrical stimulation of the vagus nerve was significantly diminished in rats with CRF. However, indomethacin treatment (1 mg kg^{-1} s.c. twice daily for 2 days) abolished the differences in response to vagal stimulation. Changes in heart rate in response to electrical stimulation of the cervical sympathetic nerve and to bolus i.v. injections of isoprenaline and carbachol were similar in rats with CRF and controls. The most notable disturbance of cardiovascular function in rats with CRF is the diminished cardiac chronotropic response to vagal stimulation which appears to be mediated by a presynaptic action of prostaglandins.

Several abnormalities of cardiovascular function have been noted in patients with either acute or chronic renal failure (CRF). These include: reduced Valsalva ratio, lower increments in blood pressure and heart rate in response to hand-grip exercise and reduced pressor responses to noradrenaline (Campese et al 1981; Levitan et al 1982). However, with regard to pressor responsiveness, Beretta-Piccoli et al (1982) have demonstrated an increased vasopressor response to noradrenaline in patients with mild chronic renal insufficiency.

In a series of studies of rats with glycerol-induced acute renal failure (ARF) we have observed reduced pressor responses to noradrenaline and angiotensin together with diminished cardiac chronotropic responses to electrical stimulation of the vagal and cervical sympathetic nerves and to intravenous isoprenaline administration (Bowmer et al 1983, 1984; Yates et al 1986). Reduced cardiac chronotropic responses to isoprenaline have also been observed in rats with ARF produced by bilateral nephrectomy (Mann et al 1986). There is, however, a paucity of data on cardiac chronotropic responses in rats with CRF. An investigation of rats with CRF has shown a diminished vascular response to noradrenaline in the isolated perfused hind-limb preparation (Rascher et al 1982), but by contrast, increased pressor responses to noradrenaline were found in rats with CRF induced by five sixths nephrectomy (Zimlichman et al 1984).

* Correspondence.

The aim of the present study was to investigate both cardiac reactivity and pressor responsiveness to noradrenaline in rats with chronic renal failure. This study should provide information on cardiac chronotropic responses in CRF in the rat and attempt to clarify the conflicting findings on vascular reactivity. In addition, the results will be compared with our previous observations on cardiovascular reactivity in ARF (Bowmer et al 1983, 1984).

METHODS

Induction of chronic renal failure

Chronic renal failure was induced in male Wistar rats (70–90 g) by five-sixths nephrectomy (Young et al 1973). Rats were anaesthetized with ether, then two-thirds of the right kidney were removed at the first operation and the left kidney was removed one week later. Sham operations, where the capsule was removed from the right kidney and the left kidney was exposed, were performed on a control group of rats. Blood samples (0·1 mL) for the subsequent measurement of plasma urea were removed from the tail vein at the time of the first operation and 7, 14 and 21 days after the second operation. In addition, systolic blood pressure was measured from the tail by means of a programmed electrosphygmomanometer (Narco Biosystems Inc) immediately before the first operation and 7, 14, 21 and 28 days after the operation to remove the left kidney.

Cardiovascular responses were recorded in anaesthetized rats 28 days after the second operation and

at the end of the experiment a final blood sample was taken from the femoral artery for plasma urea analysis.

Measurement of cardiovascular responses

Rats were anaesthetized with thiobutabarbitone (160–180 mg kg^{-1} i.p.). A dose of 160 mg kg^{-1} was given initially and if necessary smaller additional doses were given to achieve the depth of anaesthesia required for surgery. There was no significant difference ($P > 0{\cdot}3$) in the mean dose of anaesthetic given to rats with CRF (170 ± 1 mg kg^{-1}, n = 36) compared with controls (172 ± 1 mg kg^{-1}, n = 40). A tracheal cannula was inserted for artificial ventilation (ventilation rate 80 strokes min^{-1} and stroke volume 10 mL kg^{-1}) and cannulae were also placed in 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. Body (rectal) temperature was maintained at 37 °C with the aid of a heat lamp.

Chronotropic responses

The right vagus and cervical sympathetic nerves were prepared for stimulation as described by Bowmer et al (1983). Nerves were stimulated with rectangular pulses, 0·5 ms duration, supramaximal voltage (8–10 V) with frequencies of 0·5, 1, 2, 5, 7, 10 and 15 Hz. In a separate series of experiments chronotropic responses to vagal stimulation were recorded in rats that had been given indomethacin (1 mg kg^{-1} s.c.) twice daily on days 26–27 after left nephrectomy with a final injection of 1 mg kg^{-1} (s.c.) after anaesthesia (day 28).

The chronotropic responses to bolus i.v. injections of carbachol (1–5 μg kg^{-1}) were recorded in rats which had undergone bilateral vagotomy and received atenolol 0·1 mg kg^{-1} i.v. followed immediately by 0·1 mg kg^{-1} s.c. In other experiments chronotropic responses were recorded to bolus i.v. injections of isoprenaline (0·005–1·0 μg kg^{-1}) in acutely bilaterally vagotomized rats which had received pentolinium (2·5 mg kg^{-1} i.v.). In both groups of experiments the first dose of carbachol or isoprenaline was administered when the heart rate and blood pressure had stabilized after the injection of either atenolol or pentolinium.

Pressor responses

The peak responses of blood pressure were recorded to a series of bolus intravenous injections of noradrenaline (0·25–10·0 μg kg^{-1}).

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

Carbachol chloride, (−)-isoprenaline bitartrate, (−)-noradrenaline bitartrate and indomethacin were obtained from Sigma Chemical Co. Atenolol was obtained from ICI Pharmaceuticals and pentolinium tartrate from May & Baker Ltd. With the exception of indomethacin, which was dissolved in polyethylene glycol 400, all drugs were dissolved in saline.

Statistical analysis

Results were expressed as mean ± s.e. mean and statistical comparison was made using the non-paired Student's *t*-test.

RESULTS

At 7, 14, 21 and 28 days after the second operation, rats that had undergone partial nephrectomy had significantly elevated ($P < 0{\cdot}001$) plasma urea levels compared with sham-operated controls. The plasma urea concentration on day 28 in partially nephrectomized rats was 94 ± 5 mg dL^{-1} (n = 36) compared to 52 ± 3 mg dL^{-1} (n = 40) in controls. There was no significant difference ($P > 0{\cdot}05$) in conscious systolic blood pressure between partially nephrectomized rats and controls for up to 14 days after the second operation. On days 21 and 28, however, rats with CRF had conscious systolic blood pressures of 142 ± 5 and 150 ± 10 mmHg, respectively (n = 36), which were significantly higher ($P < 0{\cdot}05$) than those recorded in control rats, 122 ± 7 and 124 ± 4 mmHg, repectively (n = 40). By contrast, after general anaesthesia, which was given on day 28, the resting systolic and mean arterial blood pressures of rats with CRF were not significantly different ($P > 0{\cdot}6$) from values obtained in sham-operated controls. The mean arterial blood pressure in rats with CRF was 121 ± 5 mmHg compared with 116 ± 4 mmHg in controls. Furthermore, there was also no significant difference ($P > 0{\cdot}2$) between the basal heart rates of rats with CRF (394 ± 11 beats min^{-1}) and controls (376 ± 11 beats min^{-1}), after general anaesthesia.

Chronotropic responses

Chronotropic responses to electrical stimulation of the cervical sympathetic and vagal nerves in uraemic and control rats are shown in Fig. 1. Whilst the

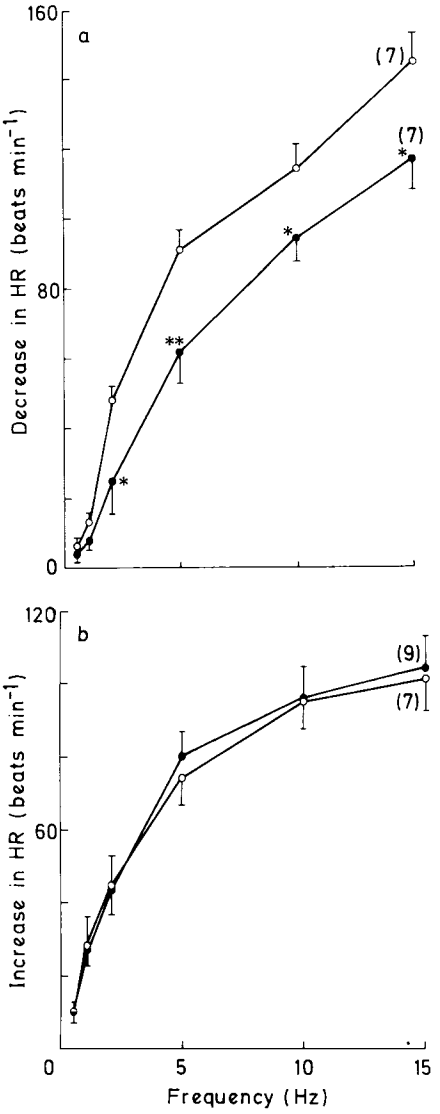

FIG. 1. The change in heart rate (HR) with increasing frequency of stimulation (8–10 V, 0·5 ms) of (a) the right vagus nerve and (b) the right cervical sympathetic nerve in sham-operated control rats (○) and rats with surgically-induced chronic renal failure (●). Values are given as mean ± s.e. mean with the number of experiments given in parentheses. Significantly different from control values: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

positive chronotropic responses to cervical sympathetic stimulation were similar, the negative chronotropic responses to vagal stimulation were significantly lower at frequencies of 2 Hz and above in rats with CRF when compared with controls. However, there were no significant differences in the responses to vagal stimulation between the two groups of rats when each had been treated with

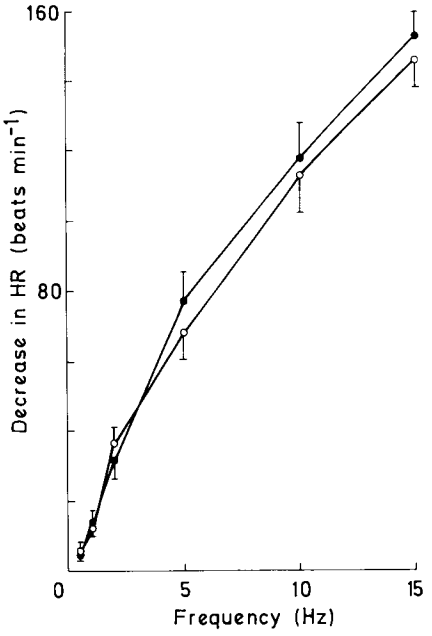

FIG. 2. The decrease in heart rate (HR) with increasing frequency of stimulation (8–10 V, 0·5 ms) of the right vagus in 6 sham-operated control rats (○) and 6 rats with surgically induced chronic renal failure (●). Both groups of rats received indomethacin (1 mg kg^{-1} twice daily s.c.) for 2 days prior to study and a single dose (1 mg kg^{-1} s.c.) before heart rate was measured. Values are mean ± s.e. mean.

indomethacin (1 mg kg^{-1} twice daily) for the immediate two days before study (Fig. 2).

Decreases in heart rate in response to carbachol were studied after the administration of atenolol. Atenolol administration produced similar falls in heart rate in rats with CRF (70 ± 6 beats min^{-1}, n = 10) and sham-operated controls (70 ± 7 beats min^{-1}, n = 10), although there was no change in mean arterial pressure in either group of rats. Bolus injections of carbachol in atenolol-treated rats resulted in falls in heart rate in the CRF group which were not significantly different from controls (Fig. 3a).

The positive chronotropic responses to isoprenaline were recorded after the administration of the ganglion blocker, pentolinium. Injection of pentolinium to uraemic rats produced falls in mean arterial blood pressure (75 ± 8 mmHg, n = 10) and heart rate (86 ± 7 beats min^{-1}) which were not significantly different ($P > 0{\cdot}5$) from the reductions noted in controls (84 ± 6 mmHg, 79 ± 8 beats min^{-1}, n = 8). Increases in heart rate elicited by bolus i.v. injections of isoprenaline in ganglion-blocked rats with CRF were not significantly different ($P > 0{\cdot}1$) from responses recorded in ganglion-blocked, sham-operated controls (Fig. 3b).

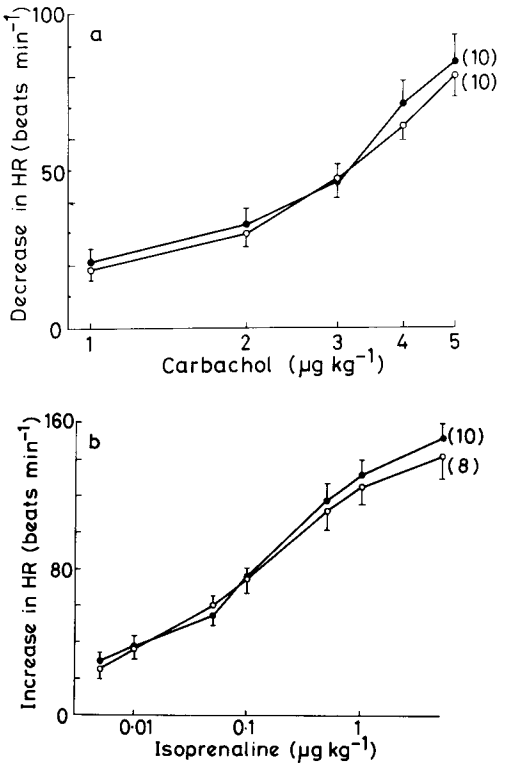

FIG. 3. The change in heart rate (HR) in response to bolus i.v. injections of (a) carbachol after pretreatment with atenolol (0·1 mg kg^{-1} i.v. and 0·1 mg kg^{-1} s.c.) and (b) isoprenaline after pretreatment with pentolinium (2·5 mg kg^{-1} i.v.) in sham-operated control rats (○) and rats with surgically induced chronic renal failure (●). Values are mean ± s.e. mean with the number of experiments given in parentheses.

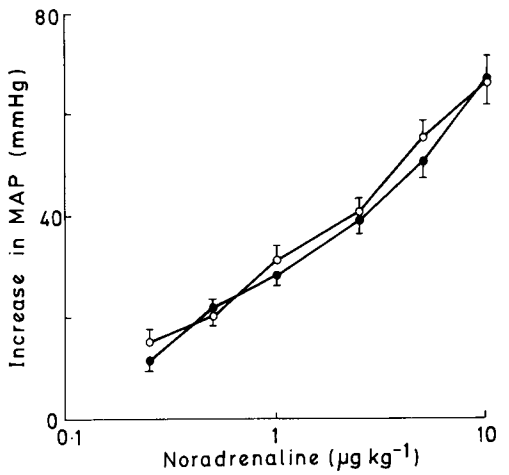

FIG. 4. The increase in mean arterial pressure (MAP) in response to bolus i.v. injections of noradrenaline in 14 sham-operated control rats (○) and 12 rats with chronic renal failure (●). Values are mean ± s.e. mean.

Pressor responses

Pressor responses to noradrenaline in rats with CRF were not significantly different ($P > 0·2$) from responses noted in controls (Fig. 4).

DISCUSSION

The most significant disturbance of cardiovascular function noted in rats with CRF was diminished chronotropic responses to vagal stimulation. We have reported similar results for rats with glycerol-induced ARF (Bowmer et al 1983) and furthermore, a derangement in the cardiac vagal pathway has also been noted in patients with CRF (Campese et al 1981; Burgess 1982). The postsynaptic response to cardiac muscarinic stimulation does not appear to be impaired since the negative chronotropic responses to carbachol were similar in rats with CRF and controls. This suggests that the cause of the reduced response to vagal stimulation is of presynaptic origin. It would appear to involve the production of prostaglandins since indomethacin administration abolished the differences in vagal response between control and CRF groups of rats. We have found a similar situation in our investigations of rats with glycerol-induced ARF (Yates et al 1986) and have suggested that increased production of prostaglandins may result in a reduction of acetylcholine release from the vagus. Increased renal levels of prostaglandin E_2 have been detected in glycerol-induced ARF (Torres et al 1974) and i.v. administration of this prostaglandin has been shown to reduce bradycardia produced by vagal stimulation but not bradycardia induced by i.v. injection of acetylcholine (Feniuk & Large 1975). A similar explanation may account for diminished vagal responses noted in the present study of rats with CRF, as increased renal levels of prostaglandin E_2 have been found in rats with reduced renal mass (Stahl et al 1986). However, no data are available on plasma prostaglandin levels in rats with CRF. In bilaterally nephrectomized rats where no damaged renal tissue is present to generate prostaglandins, cardiac chronotropic responses to vagal stimulation are normal (Yates et al 1985).

By contrast to vagal stimulation, responses of heart rate to cervical sympathetic stimulation were unimpaired in rats with CRF. Positive chronotropic responses to isoprenaline were also normal and this contrasts with the findings in ARF where positive chronotropic responses to both cervical sympathetic stimulation and isoprenaline administration are reduced in bilaterally nephrectomized rats and rats

with glycerol-induced ARF (Mann et al 1986; Yates et al 1985). In the former model of ARF the reduced response to cardiac β-adrenoceptor stimulation may be due, at least in part, to reduced activity of cardiac adenylate cyclase (Mann et al 1986). This difference in response to cardiac β-adrenoceptor stimulation between rats with ARF and CRF may be due to the duration and/or severity of renal failure. In the two models of ARF mentioned above plasma urea concentrations were 7–9 times greater than the urea concentrations found in the respective controls (Yates et al 1985; Mann et al 1986). However, in the present study, 28 days after surgery, partially nephrectomized rats had plasma urea concentrations which were slightly less than twice the concentrations in sham-operated controls. It is possible, therefore, that only severe renal failure results in a depression of responses to cardiac β-adrenoceptor stimulation. The presence of impaired responses to vagal stimulation but no detectable abnormality in cardiac response to sympathetic stimulation is supported in a clinical study by Malik et al (1986) which found that damage to cardiovascular autonomic reflexes is widespread in patients with CRF with cardiac vagal abnormalities being more striking than peripheral sympathetic involvement.

Pressor responses to noradrenaline in rats with CRF were similar to responses found in sham-operated animals. This is in contrast to depressed pressor responses found in a previous study of rats with glycerol-induced ARF (Bowmer et al 1983). The present findings are also in contrast to studies in rats with CRF in which Rascher et al (1982) found reduced constrictor responses to noradrenaline in the isolated perfused hind-limb preparation and Zimlichman et al (1984) reported enhanced pressor responses to noradrenaline. The difference in findings between these two investigations and the present one cannot be a result of differences in severity of uraemia which, as assessed by the percentage increase in either plasma urea or creatinine over controls, was similar in all three studies. Furthermore, the method of induction of CRF was the same, i.e. subtotal nephrectomy and the duration of CRF was similar (21–35 days).

In the present study, rats with CRF were hypertensive as indicated by an elevated conscious systolic blood pressure. They did not exhibit increased pressor responses to noradrenaline although hypertension is known to induce structural alterations in the vascular wall which produces increased reactivity to vasoconstrictor agents (Folkow 1978). Renal failure does depress the enhanced pressor responsiveness resulting from hypertension. We have found that spontaneously hypertensive rats with glycerol-induced ARF have decreased pressor responses to noradrenaline when compared with spontaneously hypertensive rats with normal renal function (Yates et al 1984). It is therefore possible that any vascular hyper reactivity, induced by hypertension in the rats with CRF, has been suppressed by the effect of uraemia resulting in no net change in pressor responsiveness to noradrenaline.

Immediately before general anaesthesia the conscious systolic blood pressure was significantly elevated in rats with CRF; but after general anaesthesia, the systolic and mean arterial pressures were not significantly different from values recorded in sham-operated controls. This suggests that the various systems involved in control of blood pressure in chronically uraemic rats are more sensitive to the depressant effects of barbiturate anaesthesia.

In conclusion, the most notable disturbance of cardiovascular function in rats with CRF was the diminished bradycardia elicited by vagal stimulation, a finding which has also been noted in rats with glycerol-induced ARF (Bowmer et al 1983). This may result from a presynaptic action of prostaglandins inhibiting the release of acetylcholine from the vagus.

Acknowledgement

This work was supported by the British Heart Foundation (Grant No. 82-23).

REFERENCES

Beretta-Piccoli, C., Weidmann, P., Schiffl, H., Cottier, C., Reubi, F. C. (1982) Kidney Int. 22: 297–303

Bowmer, C. J., Nichols, A. J., Warren, M., Yates, M. S. (1983) Br. J. Pharmacol. 79: 471–476

Bowmer, C. J., Clarke, C. A., Comer, M. B., Yates, M. S. (1984) Ibid. 81: 69–73

Burgess, E. D. (1982) Nephron 30: 228–230

Campese, V. M., Romoff, M. S., Levitan, D., Lane, K., Massry, S. G. (1981) Kidney Int. 20: 246–253

Feniuk, W., Large, B. J. (1975) Br. J. Pharmacol. 55: 47–49

Folkow, B. (1978) Clin. Sci. Mol. Med. 55: 3s–22s

Levitan, D., Massry, S. G., Romoff, M. S., Campese, V. M. (1982) Am. J. Nephrol. 2: 213–220

Malik, S., Winney, R. J., Ewing, D. J. (1986) Nephron 43: 191–195

Mann, J. F. E., Jakobs, K. H., Riedel, J., Ritz, E. (1986) Am. J. Physiol. 250: H846–H852

Rascher, W., Schömig, A., Kreye, V. A., Ritz, E. (1982) Kidney Int. 21: 20–27

Stahl, R. A. K., Kudelka, S., Paravicini, M., Schollmeyer, P. (1986) Nephron 42: 252–257

Torres, V. E., Romero, J. C., Strong, C. G., Wilson, D. M., Walker, V. R. (1974) Prostaglandins 8: 353–360

Yates, M. S., Bowmer, C. J., Brown, G. M. (1984) J. Pharm. Pharmacol. 36: 192–194

Yates, M. S., Critchley, M. A., Askey, E. A., Bowmer, C. J. (1985) Ibid. 37: 175–179

Yates, M. S., Askey, E. A., Wilmot, S. E., Bowmer, C. J. (1986) Ibid. 38: 59–62

Young, G. A., Anderson, C. K., Parsons, F. M. (1973) Br. J. Exp. Pathol. 54: 241–248

Zimlichman, R. R., Chaimovitz, C., Chaichenco, Y., Goligorsky, M., Rapoport, J. Kaplanski, J. (1984) Clin. Sci. 67: 161–166