J. Pharm. Pharmacol. 1985, 37: 486–490
Received January 14, 1985



# Vascular reactivity in experimental acute renal failure

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

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

Vascular reactivity in-vivo and in-vitro was examined in rats with acute renal failure produced by bilateral nephrectomy or intramuscular glycerol injection. Bilaterally nephrectomized rats displayed enhanced pressor responses to noradrenaline and angiotensin. However, the contractile responses to noradrenaline, angiotensin and potassium chloride of aortic rings and portal vein segments from nephrectomized rats were not significantly different from the responses obtained in vessels from sham-operated controls. Rats with glycerol-induced ARF which were pretreated with indomethacin had significantly lower pressor responses to noradrenaline and angiotensin than similarly treated control animals. Aortic rings from glycerol-injected rats produced significantly smaller contractions to noradrenaline than preparations from controls. This difference was not abolished by incubation of vessels with indomethacin. The findings suggest that the absence of kidneys or the presence of damaged renal tissue and not uraemia itself have pronounced but opposite effects on vascular reactivity. The depression of vascular reactivity in glycerol-induced ARF does not appear to be a result of increased production of prostaglandins.

In recent studies of rats with glycerol-induced acute renal failure (ARF) we have observed reduced pressor responses to noradrenaline and angiotensin and diminished contractions of isolated blood vessels to noradrenaline, angiotensin and potassium chloride (Bowmer et al 1983, 1984). But, in contrast, ARF produced by bilateral nephrectomy in rats (Mauz & Kreye 1971) and dogs (McCubbin & Page 1954) results in enhanced pressor responses to constrictor agents. The difference in vascular reactivity between the two models of ARF may be related to the presence of damaged renal tissue in the glycerol model. Damaged renal tissue may release substances which directly or indirectly are capable of depressing vascular reactivity and it is interesting that a diminished vascular response specific for noradrenaline and reversed by pretreatment with indomethacin has been noted in rats with chronic renal failure (Rascher et al 1982). This suggests a role for prostaglandins in the depressed vascular reactivity which occurs in models of renal failure where renal tissue is present.

In order to examine in greater detail the relationship between ARF and vascular reactivity we have studied both in-vitro and in-vivo, the vascular reactivity of bilaterally nephrectomized rats and rats with glycerol-induced ARF that have been treated with indomethacin.

* Correspondence.

## 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 procedure for the induction of ARF with glycerol has been described by 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 $kg^{-1}$) whilst control animals received a saline injection (10 ml $kg^{-1}$). The saline and glycerol injected rats that were destined for in-vivo experiments, received indomethacin (1 mg $kg^{-1}$ s.c.) dissolved in polyethylene glycol 400, twice daily for 2 days with a final injection of 1 mg $kg^{-1}$ after anaesthesia. Both in-vivo and in-vitro studies were performed 48 h after saline or glycerol injection.

### *Measurement of pressor responses*

Rats were anaesthetized with thiobutabarbitone (120–160 mg $kg^{-1}$ i.p.). A dose of 120 mg $kg^{-1}$ was given initially and smaller additional doses were administered as necessary to achieve the depth of anaesthesia required for surgery. There was no significant difference in the mean dose of anaesthetic given to nephrectomized rats compared to controls. A tracheal cannula was inserted for artificial respiration 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. Rectal temperature was maintained at 37 °C by means of a heating lamp.

The peak responses of blood pressure were recorded to a series of bolus intravenous injections of angiotensin (0·05–1·0 μg $kg^{-1}$) and noradrenaline (0·25–10·0 μg $kg^{-1}$). At the end of the experiment a heparinized blood sample was taken for the measurement of plasma urea concentration.

*Isolated blood vessels*

Rats were killed by a blow to the neck and a heparinized blood sample removed immediately from the heart for subsequent determination of plasma urea concentration. The descending thoracic aorta and portal vein were exposed, and submerged in freshly prepared 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, $MgSO_4.7H_2O$ 0·6, $NaHCO_3$ 25·0 and glucose 11·0. Aortic rings (2 mm in width) were set up in an organ bath (30 ml) at a resting tension of 1·5 g for isometric contraction recordings. Segments of portal veins (10–12 mm) 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 tension was maintained by periodic adjustment throughout the experiments. Tissues were attached to a Dynamometer UFI force displacement transducer connected via a Lectromed preamplifier (3559) to a Lectromed MX216 pen recorder.

Contractions of aortic rings and portal vein segments from nephrectomized rats were recorded and concentration-response curves constructed to noradrenaline ($5 \times 10^{-11}$–$5 \times 10^{-6}$ M), angiotensin ($5 \times 10^{-10}$–$5 \times 10^{-6}$ M) and KCl ($10^{-2}$–$10^{-1}$ M) as described by Bowmer et al (1984). The response to noradrenaline of aortic rings from saline and glycerol-injected rats were recorded in the absence and presence of indomethacin. Indomethacin was dissolved in $Na_2CO_3$ ($10^{-2}$ M) and then added to the KRB to give a final concentration of $10^{-5}$ M. When the effect of indomethacin on contractile responses was tested the medium was changed to the indomethacin-containing solution. The tissues were allowed to incubate in this medium for 1 h before further addition of noradrenaline.

*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 and indomethacin were obtained from Sigma Chemical Co. Angiotensin II amide (Hypertensin) was a kind gift from Ciba Laboratories.

*Statistical analysis*

Results are expressed as mean ± s.e.m. and statistical comparison was made using the non-paired Student's *t*-test or where appropriate two-way analysis of variance using repeated measurements (Winer 1971).

## RESULTS

*Bilateral nephrectomy*

Rats which underwent bilateral nephrectomy had significantly elevated ($P < 0·001$) plasma urea concentrations (261 ± 20 mg 100 $ml^{-1}$, n = 14) when compared with sham-operated animals (29 ± 2 mg 100 $ml^{-1}$, n = 14).

*In-vivo studies*

The mean arterial blood pressure of nephrectomized rats (86 ± 4 mm Hg, n = 6) was significantly lower ($P < 0·01$) than that of sham-operated rats (118 ± 7 mm Hg, n = 6) whilst the heart rate of nephrectomized rats (352 ± 11 beats $min^{-1}$) was not significantly different from control values (378 ± 12 beats $min^{-1}$). The pressor responses to noradrenaline were signifi-

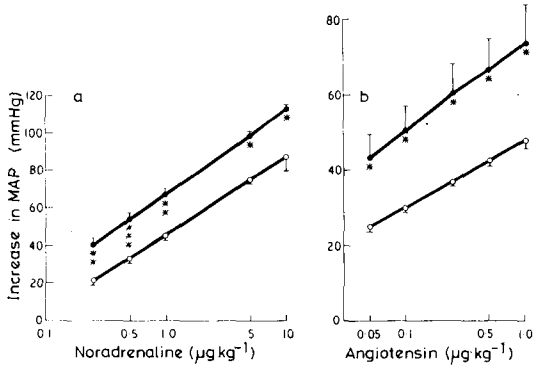


FIG. 1. The increase in mean arterial pressure (MAP) in response to (a) noradrenaline and (b) angiotensin II amide in 6 sham-operated (○) and 6 bilaterally nephrectomized rats (●). Values are mean ± s.e.m. Significantly different from control values: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

cantly greater in nephrectomized rats compared to controls at all doses tested (0·25–10·0 μg kg$^{-1}$) (Fig. 1a). Similarly, significantly larger pressor responses to angiotensin were observed in nephrectomized rats (Fig. 1b).

*In-vitro studies*

After equilibration the rate and amplitude of the spontaneous contractions of the portal vein segments were measured over an interval of 5 min. The rates of contraction per min in veins from nephrectomized rats (5·5 ± 0·3, n = 11) were very similar to the rates recorded in veins from sham-operated animals (5·4 ± 0·5; n = 10). However, the amplitude of these contractions in veins from nephrectomized rats (0·08 ± 0·02 g) was significantly smaller ($P < 0·05$) than that produced by veins of sham-operated controls (0·14 ± 0·02 g). There were no significant differences between sham-operated and nephrectomized rats in the size of contractions produced by noradrenaline in either portal vein segments or aortic rings (Fig. 2). Furthermore, neither vascular preparation showed any significant differences between the two groups of rats in the magnitude of the contractions produced by either angiotensin or KCl (data not shown).

*Glycerol-induced acute renal failure*

*In-vivo studies*

Glycerol-injected rats treated with indomethacin (1 mg kg$^{-1}$ twice daily) had a significantly elevated ($P < 0·001$) plasma urea concentration (323 ± 27 mg 100 ml$^{-1}$, n = 8) compared with similarly treated

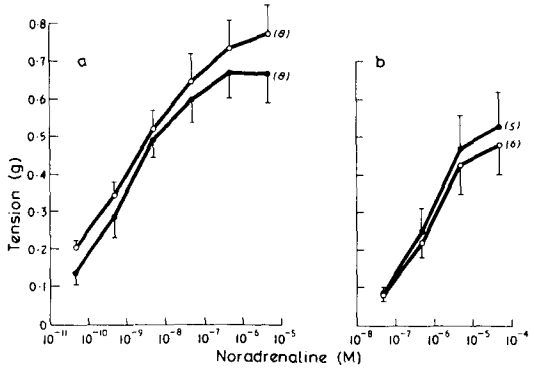


FIG. 2. The contractions in response to noradrenaline of (a) aortic rings and (b) portal vein segments from sham-operated rats (○) and bilaterally nephrectomized rats (●). Values are mean ± s.e.m. with the number of experiments in parentheses. The responses of vascular preparations from nephrectomized rats were not significantly different from controls.

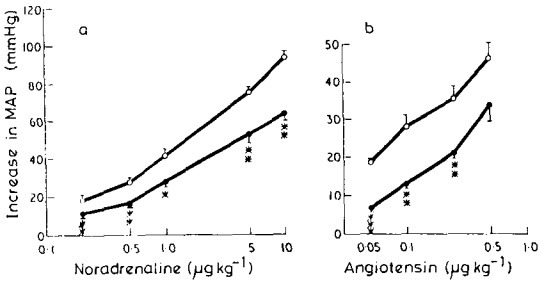


FIG. 3. The increase in mean arterial pressure (MAP) in response to (a) noradrenaline and (b) angiotensin II amide in 9 saline-injected (○) and 8 glycerol-injected rats (●) that were treated with indomethacin (1 mg kg$^{-1}$ twice daily). Values are mean ± s.e.m. Significantly different from control values: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

saline-injected animals (50 ± 9 mg 100 ml$^{-1}$, n = 9). The mean arterial blood pressure (88 ± 6 mm Hg) and heart rate (334 ± 14 beats min$^{-1}$) of these uraemic animals were significantly lower ($P < 0·05$) than the values noted in control rats (112 ± 5 mm Hg; 376 ± 9 beats min$^{-1}$). Fig. 3 shows that the pressor responses to noradrenaline and angiotensin in the glycerol-injected rats treated with indomethacin were significantly lower than those recorded in control animals.

*In-vitro studies*

Glycerol-injected rats used in in-vitro studies had a mean plasma urea concentration (272 ± 52 mg 100 ml$^{-1}$, n = 6) which was significantly ($P < 0·001$) greater than the mean concentration in saline-injected rats (32 ± 3 mg 100 ml$^{-1}$, n = 6) used in these experiments.

The effect of indomethacin on the response to noradrenaline of aortic rings from uraemic and control rats is shown in Fig. 4. In the absence of indomethacin the contractions produced by noradrenaline were significantly smaller ($P < 0·001$) in tissues from uraemic rats compared to controls. In aortic rings from both groups of animals indomethacin had no significant overall effect ($P > 0·05$) on the responses to noradrenaline. As a result, in the presence of indomethacin the responses to noradrenaline of tissues from uraemic animals remained significantly lower ($P < 0·001$) than the responses of preparations from control rats.

## DISCUSSION

The present study has shown that the pressor responses to noradrenaline and angiotensin are increased after bilateral nephrectomy as has been previously reported from studies in the dog (McCub-

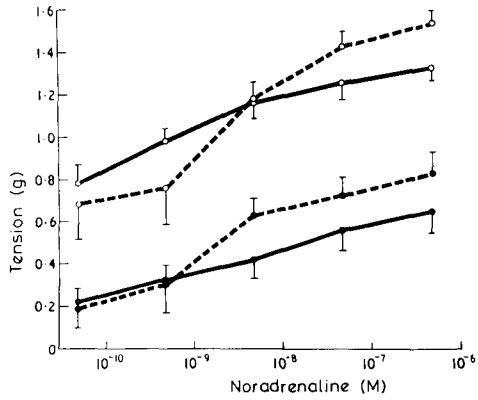


FIG. 4. The contractions in response to noradrenaline of aortic rings from 6 saline-injected (○) and 6 glycerol-injected rats (●) in the absence (——) and presence (– – –) of indomethacin ($10^{-5}$ M). Values are mean ± s.e.m. and statistical comparisons were made using two-way analysis of variance with repeated measurements: saline injected vs glycerol injected, $P < 0{\cdot}001$; saline-injected vs indomethacin saline-injected $P > 0{\cdot}05$; glycerol-injected vs indomethacin glycerol-injected, $P > 0{\cdot}05$ and indomethacin saline-injected vs indomethacin glycerol-injected, $P < 0{\cdot}001$.

bin & Page 1954) and rat (Mauz & Kreye 1971). However, in a more recent study of bilaterally nephrectomized rats, increased pressor responses to angiotensin but not to noradrenaline were observed 24 and 48 h after surgery (Couture et al 1978). These investigators found that aortic strips and portal veins removed from rats nephrectomized 24 h previously, showed no change in sensitivity to either angiotensin or noradrenaline. These in-vitro results are supported by the present findings and in addition we observed no differences in response to KCl between preparations from nephrectomized and sham-operated rats.

The amplitude of the spontaneous contractions of portal veins from nephrectomized rats was 43% lower than those recorded in veins from sham-operated controls. Similarly portal veins from rats with glycerol-induced ARF exhibit a 60% reduction in spontaneous contractions (Bowmer et al 1984). Since the spontaneous contractions of portal veins are coupled to functional voltage-dependent calcium channels (Johansson & Smolyo 1980) their availability may be reduced in ARF. However, in contrast to our findings in rats with glycerol-induced ARF (Bowmer et al 1984), any changes in these voltage-dependent channels in nephrectomized rats were not sufficient to impair the responses to potassium chloride which causes contraction by depolarization-induced calcium influx. Furthermore, in portal veins from rats with glycerol-induced ARF there is a reduction in response to angiotensin and noradrenaline (Bowmer et al 1984) which evoke contractions by activation of receptor-coupled calcium channels (Bolton 1979).

Bilateral nephrectomy removes the major source of renin and plasma renin activity is undetectable in the rat 24 h after nephrectomy (Vollmer et al 1984). In the presence of low levels of circulating angiotensin, increased pressor responses to exogenous peptide may be due to an increase in number of angiotensin receptors (Devynck & Meyer 1976) or decreased receptor occupancy (Thurston 1976). The former explanation should produce an increase in response of isolated blood vessels from nephrectomized rats, which we did not observe, whereas in the latter case receptor occupancy should be similar in isolated tissues incubated in KRB solution. However neither explanation accounts for the increased pressor responses to noradrenaline. Supersensitivity to noradrenaline could result from reduced levels of circulating adrenaline due to damage to the adrenal glands incurred at nephrectomy. However, during the operation care was taken to avoid damage to the adrenal gland or its blood supply. Furthermore, in a recent study we could detect no significant difference in the plasma adrenaline concentrations between sham-operated and nephrectomized rats (Yates et al unpublished observations). It is possible that the absence of renomedullary depressor substances after nephrectomy could account for enhanced pressor responsiveness to noradrenaline and angiotensin since these agents have a direct inhibitory action on blood vessels (Prewitt et al 1979). There may also be a central component to the increased vascular reactivity in-vivo since pithed nephrectomized rats exhibit decreased pressor responses to noradrenaline compared to sham-operated controls (Vollmer et al 1984). In spite of increased pressor responsiveness in bilaterally nephrectomized rats, the blood pressure of these animals was significantly lower than controls which is in agreement with the study of Couture et al (1978). This may result from the absence of plasma renin activity or may be a consequence of the acidosis associated with acute renal failure (Kerr 1979) which can produce vasodilation (Mellander & Johansson 1968).

In contrast to increased pressor responses in ARF produced by bilateral nephrectomy, pressor responses to noradrenaline and angiotensin are diminished in glycerol-induced ARF (Bowmer et al 1983, 1984). After pretreatment with indomethacin (1 mg $kg^{-1}$ twice daily) the pressor responses to both constrictor agents in glycerol-injected and control

rats were similar to the respective responses obtained in the absence of indomethacin (Bowmer et al 1983, 1984). Consequently the pressor responses in glycerol-injected rats were still significantly lower than controls after administration of indomethacin. The failure of cyclo-oxygenase inhibition to abolish the reduced pressor responsiveness in glycerol-induced ARF is supported by the present in-vitro findings. In these experiments the response to noradrenaline of aortic rings from glycerol-injected rats were still significantly less than controls after incubation with indomethacin. The effect of indomethacin on portal vein responses to noradrenaline was not investigated in detail since indomethacin has been shown to reduce contractile responses in this vascular preparation (Altura & Altura 1976). We confirmed these observations in preliminary studies in which indomethacin ($10^{-5}$ M) reduced the responses to noradrenaline by approximately 20% in portal veins from both glycerol and saline-injected rats (Yates et al unpublished observations). Our findings with indomethacin are in contrast to experiments in isolated perfused hind-limbs of rats with chronic renal failure (Rascher et al 1982). In this study a depression of vascular response to noradrenaline was abolished by pretreatment of animals with indomethacin at the same dose as used in the present study (1 mg $kg^{-1}$ twice daily). However, there is some disagreement concerning the nature of vascular responsiveness in experimental chronic renal failure since in another study vascular hyperreactivity to noradrenaline has been noted in rats that have undergone sub-total nephrectomy (Zimlichman et al 1984).

In conclusion the enhanced pressor responses in ARF produced by nephrectomy and decreased responses in the glycerol model of ARF suggest that the absence of kidneys or the presence of damaged renal tissue, and not uraemia itself, have pronounced, but opposite effects, on vascular reactivity. Furthermore, the decreased vascular reactivity in glycerol-induced ARF does not appear to be the result of increased production of prostaglandins.

*Acknowledgement*

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

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