J. Pharm. Pharmacol. 1986, 38: 59–62
Communicated June 21, 1985

The effect of glycerol-induced acute renal failure upon cardiac reactivity in the rat: influence of indomethacin treatment and renal pedicle ligation

M. S. YATES*, ELIZABETH A. ASKEY, SUSAN E. WILMOT, C. J. BOWMER, *Department of Pharmacology, Worsley Medical and Dental Building, The University, Leeds LS2 9JT, UK*

Cardiac chronotropic responses to isoprenaline, carbachol and electrical stimulation of the cervical sympathetic and vagal nerves were recorded in rats with glycerol-induced acute renal failure (ARF) and control rats. The experiments involving electrical stimulation of cardiac nerves were performed in rats which either had been pretreated with indomethacin (1 mg kg^{-1} twice daily for 2 days) or had undergone acute bilateral renal pedicle ligation. The findings from this investigation indicate that the reduced chronotropic responses to vagal stimulation in rats with glycerol-induced ARF are due to a reduction in acetylcholine release mediated by prostaglandins possibly originating from the damaged kidneys. The diminished response to cervical sympathetic stimulation results from a decreased postsynaptic response to β-adrenoceptor stimulation.

In studies of rats with glycerol-induced acute renal failure (ARF) we observed reduced pressor responses to noradrenaline and angiotensin and diminished cardiac chronotropic responses to vagal and sympathetic electrical stimulation (Bowmer et al 1983, 1984). In contrast to rats with glycerol-induced ARF, bilaterally nephrectomized rats showed impaired chronotropic responses to cervical sympathetic but not to vagal stimulation (Yates et al 1985a). Negative chronotropic responses to carbachol in isolated atria from both nephrectomized and glycerol-injected rats were not significantly different from their respective controls. However, the positive chronotropic responses to isoprenaline were significantly reduced in atria from both models of ARF (Yates et al 1985a).

The results so far indicate that the reduced chronotropic responses to sympathetic stimulation noted in both glycerol-injected and nephrectomized rats are due to an impaired postsynaptic response whilst the cause of the diminished chronotropic response to vagal stimulation, observed only in rats with glycerol-induced ARF, is of presynaptic origin. It is possible that the damaged renal tissues present in the glycerol model of ARF, release a substance or substances which attenuate responses 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 reduces the release of acetylcholine elicited by vagal stimulation (Feniuk & Large 1975).

* Correspondence.

In the present study we have further investigated cardiac reactivity in the glycerol model of ARF. The chronotropic responses to isoprenaline and carbachol were recorded in anaesthetized animals in order to determine whether the postsynaptic responses to these agents are modified in-vivo by acute uraemia. In other experiments, the chronotropic responses to vagal and sympathetic stimulation were measured in rats that were either pretreated with indomethacin or had undergone acute renal pedicle ligation. This latter procedure should prevent the release from the kidney of any substances which modify cardiac reactivity.

Methods

Acute renal failure was induced in male Wistar rats, 300–350 g, by i.m. injection of glycerol, the details of which have been described (Bowmer et al 1983). Control rats received an i.m. injection of saline (0·9% NaCl). In one series of experiments saline and glycerol-injected rats 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.

Measurement of chronotropic responses. Forty-eight hours after saline or glycerol injection, rats were anaesthetized with thiobutabarbitone (120–160 mg kg^{-1} i.p., Yates et al 1985b), a tracheal cannula was inserted for artifical 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. Body temperature (rectal) was maintained at 37 °C by means of a heating lamp.

(a) *Response to i.v. administration of drugs.* In one series of experiments the chronotropic responses to bolus i.v. injections of carbachol (1–5 μg kg^{-1}) were recorded in rats which had undergone acute bilateral vagotomy and received atenolol, 0·1 mg kg^{-1} i.v., followed immediately by 0·1 mg kg^{-1} s.c. In another group of 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.

(b) *Response to electrical stimulation.* In rats that had been pretreated with indomethacin, the right vagus and cervical sympathetic nerves were prepared for stimulation to assess cardiac chronotropic responses as described by Bowmer et al (1983). The nerves were stimulated with rectangular pulses, 0·5 ms duration, supramaximal voltage 8–10 V with frequencies of 1, 2, 5, 7, 10 and 15 Hz.

In a separate series of experiments the kidneys of rats that had received no pretreatment were exposed by a midline abdominal incision and a loose ligature placed around the ureter, renal artery and vein on each side as close as possible to the renal pedicle. The vagus was exposed and stimulated as described above but with selected frequencies of 1, 5, 10 and 15 Hz. This was done immediately before and 5, 15 and 30 min after bilateral ligation of the renal pedicles.

Measurement of plasma urea. At the end of each experiment a heparinized blood sample was taken from the femoral artery for the determination of plasma urea which was 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 and indomethacin were obtained from the Sigma Chemical Co. Atenolol was obtained from ICI 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 are expressed as mean ± s.e. mean and statistical comparison was made using the non-paired Student's *t*-test and where appropriate analysis of covariance.

Results

Intramuscular injection of glycerol resulted in a uraemic state characterized by at least a fourfold increase in plasma urea concentration. The basal mean arterial blood pressure and heart rate of uraemic rats (86 ± 4 mmHg; 348 ± 7 beats min^{-1}, n = 35) were significantly lower ($P < 0·01$) than the values recorded in control animals (111 ± 2 mm Hg; 378 ± 8 beats min^{-1}, n = 38). These results are similar to those previously obtained in our laboratory (Bowmer et al 1983, 1984; Yates et al 1985a).

Chronotropic responses to carbachol and isoprenaline. Administration of atenolol produced a fall in heart rate in uraemic rats (85 ± 15 beats min^{-1}, n = 7) which was not significantly different ($P > 0·05$) from controls (96 ± 12 beats min^{-1}, n = 6). The response of mean arterial blood pressure to atenolol in uraemic rats was variable: in three rats there was no change whilst in four animals a fall of 5–10 mm Hg occurred. In control animals atenolol always produced a fall in blood pressure (28 ± 6 mm Hg). Bolus injections of carbachol to atenolol-treated rats resulted in negative chronotropic responses in uraemic rats which were not significantly different from controls (Fig. 1a).

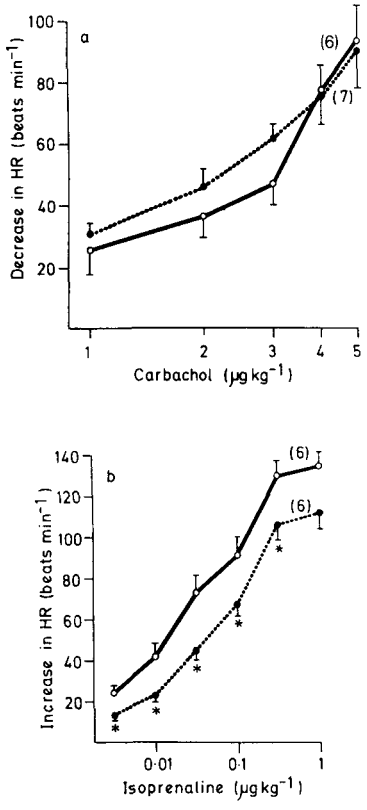

FIG. 1. The change in heart rate (HR) in response to bolus i.v. injections of (a) carbachol after pretreatment with atenolol (0·1 mg kg^{-1} i.v. and 0·1 mg kg^{-1} s.c.) and (b) isoprenaline after pretreatment with pentolinium (2·5 mg kg^{-1} i.v.) in control rats (○) and rats with glycerol-induced acute renal failure (●). Values are mean ± s.e. mean with the number of experiments given in parentheses. Significantly different from control values: $^*P < 0·05$.

Ganglion blockade induced by pentolinium produced a significantly greater ($P < 0·05$) fall in mean arterial blood pressure in control animals (81 ± 9 mm Hg, n = 6) compared with uraemic rats (46 ± 7 mm Hg, n = 6). However, the fall in heart rate produced by ganglion blockade in control rats (67 ± 14 beats min^{-1}) was not

significantly different ($P > 0.05$) from the decrease noted in uraemic animals (78 ± 13 beats min^{-1}). Increases in heart rate elicited by bolus i.v. injections of isoprenaline in ganglion blocked uraemic rats were significantly lower than the corresponding responses recorded in control rats with the exception of the chronotropic response to 1·0 μg kg^{-1} (Fig. 1b).

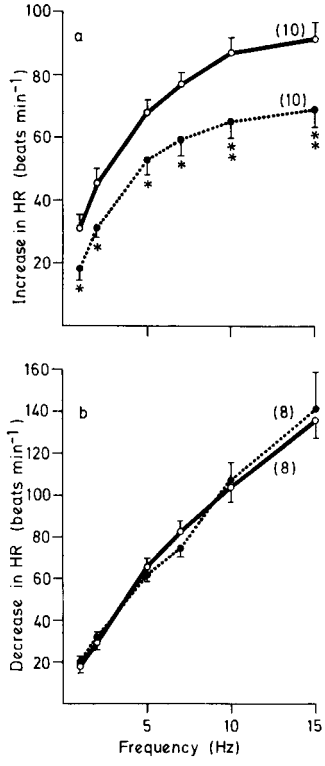

FIG. 2. The change in heart rate (HR) with increasing frequency of stimulation (8–10 V, 0·5 ms) of (a) the right cervical sympathetic nerve and (b) the right vagus nerve in control rats (○) and rats with glycerol-induced acute renal failure (●) that were treated with indomethacin (1 mg kg^{-1} twice daily). Values are mean ± s.e. mean with the number of experiments given in parentheses. Significantly different from control values: $^{*}P < 0.05$, $^{**}P < 0.01$.

Chronotropic responses to electrical stimulation. The basal heart rate and mean arterial blood pressure of uraemic rats treated with indomethacin (355 ± 6 beats min^{-1}; 82 ± 6 mmHg, n = 10) were significantly lower ($P < 0.01$) when compared with similarly treated control rats (388 ± 8 beats min^{-1}; 109 ± 5 mmHg, n = 10). Chronotropic responses to electrical stimulation of the cervical sympathetic and vagal nerves in indomethacin-treated rats are shown in Fig. 2. Positive chronotropic responses to sympathetic stimulation were significantly lower in the uraemic rats (Fig. 2a), but there were no significant differences between uraemic and control animals with respect to negative chronotropic responses elicited by vagal stimulation (Fig. 2b).

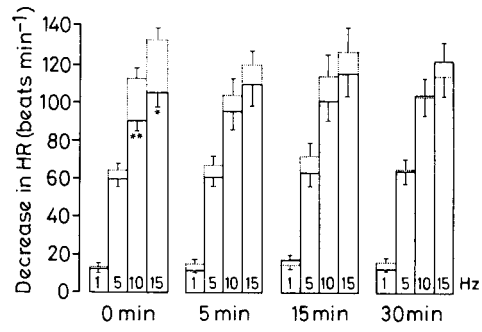

FIG. 3. The decrease in heart rate (HR) with increasing frequency of stimulation (8–10 V, 0·5 ms) of the right vagus nerve in control rats (– – –) and rats with glycerol-induced acute renal failure (——) before (0 min) and 5, 15 and 30 min after bilateral renal pedicle ligation. Values are mean ± s.e. mean (n = 12–15). Significantly different from the control values at each time period: $^{*}P < 0.05$, $^{**}P < 0.01$.

In separate experiments the negative chronotropic responses to selected frequencies of vagal stimulation were recorded before and after bilateral renal pedicle ligation (Fig. 3). In both uraemic and control rats, renal pedicle ligation resulted in either no change or small transient decreases in blood pressure and heart rate of up to 25 mmHg and 30 beats min^{-1}, respectively. The chronotropic responses to vagal stimulation at 10 and 15 Hz in uraemic rats were significantly lower than controls before ligation, whereas 5, 15 and 30 min after ligation there were no significant differences at these frequencies between uraemics and their corresponding controls. Analysis of covariance revealed that the responses to various frequencies of vagal stimulation showed no significant correlation ($P > 0.05$) with time in either control or uraemic groups.

Discussion

The present findings have shown that, in anaesthetized rats with glycerol-induced ARF, the negative chronotropic responses to carbachol were not significantly different from controls, which supports our previous findings in isolated atria (Yates et al 1985a). Thus the postsynaptic response to cardiac muscarinic stimulation is not affected by any circulating factor present in acute uraemia, although the response to vagal stimulation is reduced in the glycerol model of ARF (Bowmer et al 1983). This provides further evidence that the cause of reduced responses to vagal stimulation is of presynaptic origin. A diminished response to stimulation of the vagus nerve could result from either neuropathy which has been reported to occur in motor nerves in rats with ARF (Tegner & Brismar 1984) or inhibition of acetylcholine release. The latter explanation is more likely because treatment with the cyclo-oxygenase inhibitor indomethacin resulted in no significant differences in negative chronotropic responses to vagal stimulation between control and uraemic rats. Thus a product of

cyclo-oxygenase appears to inhibit acetylcholine release from the vagus and, of the ones tested, prostaglandins E_1 and E_2 have been shown to possess this activity (Feniuk & Large 1975). The source of the prostaglandins could be either cardiac tissues or the kidney since increased renal levels of prostaglandin E_2 have been detected in glycerol-induced ARF in the rat (Torres et al 1974), however, no data are available on plasma prostaglandin levels in ARF. Experiments involving ligation of the renal pedicles would tentatively suggest that it is the kidney since negative chronotropic responses to higher frequencies of vagal stimulation were significantly lower in uraemic animals compared with controls before ligation, but 5, 15 and 30 min post-ligation there was no significant difference. However, although the responses to higher frequencies of stimulation in uraemic animals showed an increase with time after ligation, this relationship was not statistically significant. In contrast, the corresponding control responses showed an overall, but not significant, tendency to decrease with time.

The decreased positive chronotropic response to sympathetic stimulation in rats with glycerol-induced ARF appears to be mediated by a diminished postsynaptic response to β-adrenoceptor stimulation as evidenced by decreased chronotropic responses to isoprenaline noted here in-vivo and observed in-vitro in our previous study (Yates et al 1985a). Prostaglandins E_1 and E_2 have been shown to reduce the release of noradrenaline from cardiac noradrenergic nerve terminals (Hedqvist & Wennmalm 1971); but by contrast to the results with vagal stimulation, indomethacin treatment failed to abolish the significant differences between uraemic and control rats in their chronotropic responses to sympathetic stimulation. This indicates that any increased production of prostaglandins in this model of ARF has no significant role in mediating these diminished responses.

Reduced chronotropic responses to isoprenaline have been noted in conscious rats with ARF produced by bilateral nephrectomy (Mann et al 1982; Hausen et al 1983). In support of these observations we have shown that isolated atria from bilaterally nephrectomized rats also have diminished chronotropic responses to isoprenaline (Yates et al 1985a). These changes in nephrectomized rats have been attributed to a reduction in cardiac β-adrenoceptor density as shown by decreased binding of the ligand [^{3}H]dihydroalprenolol to cardiac sarcolemmal preparations (Mann et al 1984). In addition, Mann et al (1984) found that in-vivo responses of heart rate to forskolin were blunted and adenylate cyclase activity in heart homogenates from nephrectomized rats was also diminished. Together these observations may account for the reduction in response to isoprenaline and sympathetic stimulation in rats with glycerol-induced ARF since increased plasma levels of noradrenaline have been detected in these rats (Bowmer et al 1983) which may produce down-regulation of β-adrenoceptors. It is also possible that diminished responses to cardiac β-adrenoceptor stimulation are due to increased activity of the parathyroid gland which has been noted in rats with ARF (Mazzocchi et al 1967) since in patients with chronic renal failure and secondary hyperparathyroidism, parathyroidectomy significantly increases the heart rate response to isoprenaline (Ulmann et al 1977).

In conclusion, the findings of this study indicate that the reduced chronotropic responses to vagal stimulation in rats with glycerol-induced ARF are due to a reduction in acetylcholine release mediated by prostaglandins possibly originating from the damaged kidneys. The diminished response to cervical sympathetic stimulation results from a decreased postsynaptic response to β-adrenoceptor stimulation.

This work was supported by the British Heart Foundation (Grant No. 82-23). We wish to thank Dr T. Pullan, Department of Statistics, Leeds University, for his advice.

REFERENCES

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

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

Hausen, M., Mann, J. F. E., Ritz, E. (1983) Kidney Int. 23: 150

Hedqvist, P., Wennmalm, A. (1971) Acta Physiol. Scan. 83: 156–162

Mann, J. F. E., Hausen, M., Kutter, A., Sudhoff, R., Ritz, E. (1982) Proc. Eur. Dial. Transplant Assoc. 19: 788–789

Mann, J. F. E., Jakobs, K.-H., Nagel, W., Schick, M., Hausen, M., Ritz, E. (1984) Kidney Int. 25: 249

Mazzocchi, G., Meneghelli, V., Serafini, M. T. (1967) Acta Anat. 68: 550–566

Tegner, R., Brismar, T. (1984) J. Neurological Sci. 65: 29–36

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

Ulmann, A., Drüeke, T., Zingraff, J., Crosnier, J. (1977) Clin. Nephrol. 7: 58–60

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

Yates, M. S., Askey, E. A., Wilmot, S. E., Bowmer, C. J. (1985b) Ibid. 37: 486–490