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Vascular Smooth Muscle Function in Rats with Acute Renal Failure

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Abstract—Vascular smooth muscle function in rats with glycerol-induced acute renal failure (ARF) was investigated by recording both the contractile responses of portal vein segments and the relaxant responses of aortic ring preparations. The portal veins from rats with ARF showed depressed contractile responses to the α_1-agonist methoxamine, the α_2-agonist B-HT 920 and to the calcium agonist BAY K 8644 when compared with controls. Both isoprenaline and nitroprusside produced 100% reversal of KCl induced tone in control aortic rings but in rings from rats with ARF complete reversal of tone could not be obtained with either dilator drug. It is suggested that a defect in mitochondrial function and hence energy supply may account for the diminished ability of vascular preparations from rats with ARF to constrict and relax.

Patients with acute or chronic renal failure have impaired cardiovascular responses, which include reduced responses of heart rate and blood pressure during hand-grip exercise and diminished pressor responses to infusions of noradrenaline (Campese et al 1981; Levitan et al 1982). Studies in rats have also demonstrated, both in-vivo and in-vitro, impaired cardiac and vascular reactivity associated with renal failure (Rascher et al 1982; Bowmer et al 1983, 1984; Mann et al 1986; Yates et al 1987). The study of Rascher et al (1982) noted a diminished constrictor response, specific for noradrenaline, in the perfused hind limb of rats with chronic renal failure. In our study of rats with acute renal failure (ARF) we observed reduced contractile responses of aortic strips and portal vein segments to noradrenaline, angiotensin and potassium chloride (Bowmer et al 1984).

As an extension to our earlier work we have further investigated the range of vascular dysfunction in rats with ARF by examining in-vitro the contractile responses of the portal vein to selective α_1 and α_2-adrenoceptor agonists, methoxamine and B-HT 920 (van Meel et al 1981), respectively, and to the 'calcium agonist' BAY K 8644 (Schramm et al 1983). In addition, we have investigated the influence of ARF on the ability of blood vessels to relax, which has not been previously studied, by examining the response of aortic rings to vasodilators.

Materials and Methods

Materials

(−)-Isoprenaline bitartrate, (−)-noradrenaline bitartrate, acetylcholine iodide, and sodium nitroprusside were obtained from the Sigma Chemical Co. BAY K 8644 (methyl 1,4-dihydro-2, 6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl) pyridine-5-carboxylate) and B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo [4,5-d] azepindihydrochloride) were gifts from Bayer AG and Boehringer Ingelheim Ltd, respectively. Methoxamine hydrochloride was obtained from Wellcome PLC.

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Induction of acute renal failure

ARF was induced according to the method developed by Thiel et al (1967). Male Wistar albino rats (250–300 g) were deprived of drinking water for 24 h and ARF was produced by intramuscular injection of 50% v/v glycerol in sterile saline (0·9% w/v NaCl), 10 mL kg^{-1}. Control rats were injected with saline (10 mL kg^{-1} i.m.) and both groups of rats were studied 48 h after injection.

Measurement of vascular responses

Rats were killed by a blow to the neck, the thorax and abdomen opened and a blood sample (0·5 mL) was removed from the heart for subsequent determination of plasma urea concentration. The descending thoracic aorta and portal vein were cleared of adherent tissue, excised and submerged in freshly prepared Krebs-Ringer bicarbonate (KRB) solution (5°C) of the following composition (mM): NaCl 118·0, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ $7H_2O$ 1·2, $NaHCO_3$ 25·0 and glucose 10·0. A ring of aorta (2–3 mm wide) was cut from the proximal end of the vessel and the interior rubbed gently with a wooden cocktail stick in order to remove the endothelium. The aortic ring was suspended between two parallel stainless steel hooks in a 20 mL organ bath at a resting tension of 1·0 g. A segment of portal vein (8–10 mm long) was tied at both ends, by inserting sutures through the wall of the vessel, and placed 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. Loading tensions were maintained by periodic adjustment throughout the experiments and incubation media routinely changed every 10–15 min to guard against accumulation of metabolites (Altura & Altura 1970). Tissues were attached to a Dynamometer UF1 Force displacement transducer connected to a Lectromed MX216 pen recorder via a Lectromed (3559) pre-amplifier. One hour after attachment of the aortic ring to the transducer a contraction was elicited with noradrenaline (1·0 μM). When the noradrenaline contraction had reached its maximum, the tissue response to 1·0 μM acetylcholine was noted. Following washing the tissues were allowed to equilibrate for a further period of 1 h. After this time the tissue was contracted by the

addition of sufficient KCl to the bath to raise the concentration to 15 mM. When the contraction to KCl had reached a steady state the relaxant responses were recorded to the cumulative addition of either isoprenaline or nitroprusside. The relaxant responses were expressed as % reversal of the induced tone.

The portal vein preparations were allowed to equilibrate for 1 h after which the contractile responses were recorded to cumulative additions of either methoxamine, B-HT 920 or BAY K 8644. The experiments with BAY K 8644 were conducted under sodium light. Since the portal vein preparations exhibited spontaneous activity, the contractile response to an agonist was determined as the difference between the mean peak spontaneous tension in the 1 min period before agonist application and the peak tension developed in the presence of the agonist. At the end of the experiment the tissue was weighed and the increase in tension produced by the drug expressed as mg mg^{-1} tissue.

From each dose-response curve the concentration producing 50% of the maximum response achieved in that particular experiment was determined and then expressed as the negative log (pD_2). The difference between tissues from control and glycerol-injected animals in their ability to contract or relax to a particular drug was expressed as the ratio of the mean maximum responses obtained in the group with ARF compared to the control group.

All drugs were dissolved in KRB with the exception of BAY K 8644 which was initially dissolved in dimethyl sulphoxide and subsequently this solution was slowly diluted with KRB to avoid precipitation.

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).

Statistical analyses

Results are expressed as mean ± s.e. mean and statistical comparisons were made using either a non-paired Student's *t*-test or a Mann-Whitney test.

Results

The plasma urea concentrations detected in glycerol-injected rats (167 ± 18 mg dL^{-1}; n = 17) were approximately 5-fold higher ($P < 0.001$) than the concentrations found in saline-injected rats (34 ± 1 mg dL^{-1}; n = 17). This showed that the glycerol-injected rats were severely uraemic.

After the 1 h equilibration, the frequency of spontaneous contractions of portal veins from uraemic rats (12 ± 1 min^{-1}; n = 17) was found to be less than the rate observed in portal veins from control rats (18 ± 4 min^{-1}; n = 17) although this difference was not statistically significant ($P > 0.05$). The mean amplitude of the spontaneous contractions was significantly smaller ($P < 0.05$) in portal veins removed from uraemic rats (18 ± 3 mg mg^{-1} tissue; n = 17) when compared with the amplitude measured in tissues removed from control animals (37 ± 8 mg mg^{-1} tissue; n = 17).

The constrictor responses of portal vein segments from control and uraemic animals to methoxamine, B-HT 920 and BAY K 8644 are shown in Fig. 1. With all three drugs the tissues from uraemic animals showed significantly depressed responses when compared to controls. This is further illustrated by the observation that ratios of the mean maximum responses were all less than 1·0 (Table 1). By contrast, pD_2 values obtained from these experiments showed no significant differences between control and uraemic groups (Table 1).

Aortic rings contracted with noradrenaline (1·0 μM) produced a further small contraction when acetylcholine (1·0 μM) was added to the bath. This indicated that the endothelium had been successfully removed from the arterial rings (Furchgott 1983). When the tissues were exposed to KCl (15 mM) the degree of tone induced in aortic rings from uraemic rats (47 ± 11 mg mg^{-1} tissue, n = 11) was significantly smaller ($P < 0.01$) than the tone produced by preparations from control rats (107 ± 14 mg mg^{-1} tissue). Fig. 2 shows the effects of the vasodilators isoprenaline and nitroprusside on the aortic rings contracted with 15 mM KCl. In control preparations both vasodilators produced 100% reversal of induced tone whereas in rings from uraemic rats complete reversal could not be achieved. The ratios of the mean

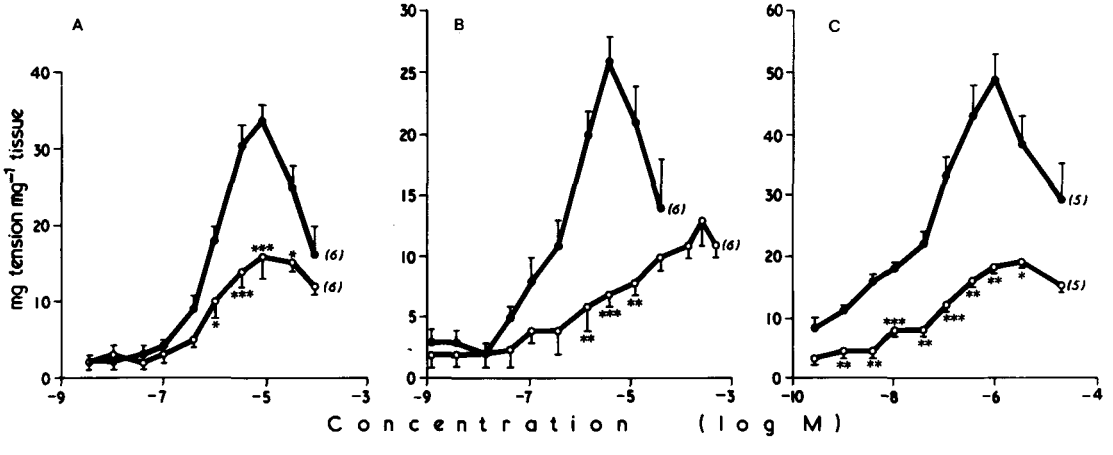

FIG. 1. The increase in tension developed by isolated portal vein preparations from control rats (●) and rats with acute renal failure (○) in response to (A) methoxamine, (B) B-HT 920 and (C) BAY K 8644. Values are given as mean with vertical lines showing s.e. mean. The number of experiments are shown in parentheses. Significantly different from control values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (*t*-test).

Table 1. pD_2 values and ratios of mean maximum responses for methoxamine, B-HT 920, BAY K 8644, isoprenaline and nitroprusside on isolated vascular preparations from control rats and rats with acute renal failure.

	pD_2		
	Control group	Uraemic group	Ratio max.*
Methoxamine†	5·95 ± 0·06 (6)	5·79 ± 0·24 (6)	0·49
B-HT 920†	6·24 ± 0·15 (6)	5·79 ± 0·43 (6)	0·50
BAY K 8644†	7·35 ± 0·08 (5)	7·34 ± 0·15 (5)	0·38
Isoprenaline	7·10 ± 0·28 (6)	7·28 ± 0·14 (6)	0·57
Nitroprusside	9·13 ± 0·22 (5)	8·54 ± 0·16 (5)	0·42

pD_2 values are given as mean ± s.e. mean with the number of experiments given in parentheses.

* Ratio max. is the ratio of the mean maximum response obtained in tissues from rats with acute renal failure compared to the mean maximum response observed in controls.

† Experiments with these drugs were performed on portal vein segments whilst experiments with the remaining two drugs were carried out on aortic rings.

maximum responses are shown in Table 1. Despite the impaired ability of tissues from uraemic animals to relax, the pD_2 values for the uraemic group were not significantly different from those obtained from controls (Table 1).

Discussion

In the present study we have clearly demonstrated that isolated blood vessels from rats with acute renal failure have an impairment both in their ability to dilate and constrict. In addition the mean amplitude of the spontaneous contractions of portal veins from uraemic animals was significantly smaller than that obtained for preparations from control animals. Since these spontaneous contractions are coupled to functional voltage-dependent calcium channels (Johanson & Somlyo 1980), their availability or duration of opening may be reduced in ARF. This is supported by the reduced contractile response of portal veins from uraemic rats to the calcium agonist BAY K 8644 which binds to the dihydropyridine receptor and prolongs the open times of voltage-dependent calcium channels (see Bechem et al 1988). In a previous study (Bowmer et al 1984) we have noted diminished contractile responses of both portal veins and aortae from rats with ARF to angiotensin and noradrenaline. Furthermore, in the present study we have found reduced contractions of the portal vein of uraemic rats to the α_1-agonist methoxamine and to the α_2-agonist B-HT 920. This shows that diminished vascular contraction in ARF occurs when receptor-coupled or voltage-dependent calcium channels are activated and that the reduced response to α-adrenoceptor stimulation is not confined to a particular subtype of α-receptor.

It has been demonstrated that in the rat portal vein the response to activation of α_2-receptors shows a greater dependency on extracellular calcium than the response to α_1-stimulation (Hicks 1983). The present study showed that the maximum response to methoxamine and B-HT 920 of portal veins from rats with ARF was depressed to the same extent (Table 1). This indicates that the depression of the contractile response in ARF, whilst not restricted to activation of one type of calcium channel, is also not a consequence of impaired calcium entry into vascular smooth muscle.

It would appear likely that in ARF there is a defect in some aspect of excitation-contraction coupling common to all the agents described above. These findings are in contrast to the observations of Rascher et al (1982) who found a diminished vascular response, specific for noradrenaline, in a study of rats with chronic renal failure. This discrepancy in the specificity of vascular depression in renal failure is possibly related to the severity and rate of development of renal dysfunction. In the investigation of Rascher et al (1982) plasma creatinine levels were elevated 2-fold after 5 weeks whereas in our study plasma urea concentrations were increased 5-fold after two days.

Aortic rings contracted with KCl showed relaxant responses to both isoprenaline and nitroprusside. The ring preparations were submaximally contracted with KCl (15 mM) since it has been shown that aortic rings maximally contracted with KCl do not relax to isoprenaline (O'Donnell & Wanstall 1987), a finding we confirmed in preliminary studies. By contrast to the responses of aortic rings from

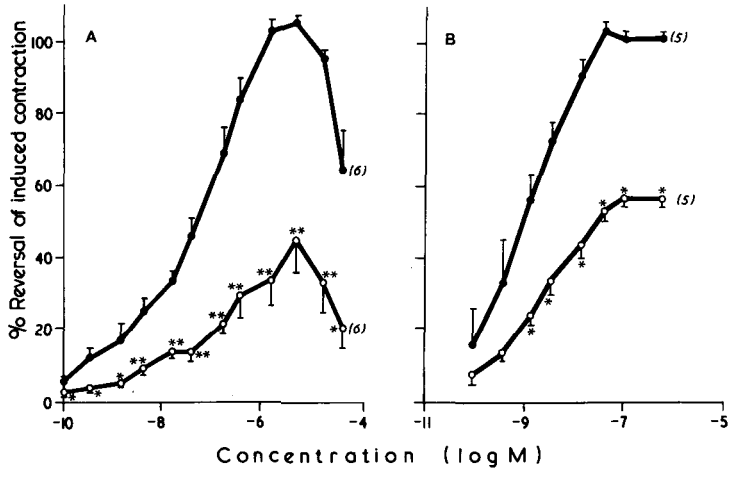

FIG. 2. The percentage reversal of potassium chloride (15 mM)-induced tone in isolated aortic ring preparations from control rats (●) and rats with acute renal failure (○) in response to (A) isoprenaline and (B) nitroprusside. Values are given as mean with vertical lines showing s.e. mean. The number of experiments are shown in parentheses. Significantly different from control values: * $P < 0.05$; ** $P < 0.01$ (Mann–Whitney test).

control rats, administration of increasing concentrations of isoprenaline or nitroprusside could not completely reverse the tone induced by KCl in aortic rings from uraemic animals. This unusual effect occurred in spite of the fact that the degree of KCl induced tone in tissue preparations from uraemic animals was approximately 50% of the tone generated in control tissues. Isoprenaline initiates relaxation by β-adrenoreceptor mediated increases in cyclic AMP (Namm 1982) whereas nitroprusside reduces vascular smooth muscle tone via the formation of nitric oxide which activates guanylate cyclase (Rapoport et al 1983). There is no clear explanation from the present findings as to why there is an impairment in the ability to relax to either vasodilator drug. However, the mechanism probably involves a process activated by both agents.

The concentration ranges of both constrictor and relaxant drugs which were effective in tissues from uraemic and control animals differed little, as indicated by the similarity of pD_2 values. However, as discussed above, there were marked differences between tissues from control and uraemic animals in absolute constrictor and dilator responses. Since both contraction and relaxation are energy dependent processes, one explanation for this non-specific depression of vascular function is an impaired production of ATP. It is interesting to note that a decrease in electron transport and oxidative phosphorylation have been found in mitochondria isolated from the intestinal mucosa of rats with chronic renal failure (Russell & Avioli 1974). A similar defect of mitochondrial function may occur in vascular smooth muscle during ARF. This could lead to a decrease in ATP available for contraction and relaxation and account for the depressed vascular responses.

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