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Cardiac Function in Rats with Acute Renal Failure

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Abstract—Inotropic responses of isolated cardiac preparations from rats with glycerol-induced acute renal failure (ARF) were recorded, following a range of cardiac stimulants. Left atria of rats with ARF showed diminished inotropic responses only to the calcium agonist Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate) whilst right ventricular strips exhibited reduced responses to isoprenaline, 3-isobutyl-1-methylxanthine, Ca^{2+} and Bay K 8644. Investigations of cardiac mitochondrial respiration indicated that there is a site-unspecific 'pseudo' uncoupling of oxidative phosphorylation in ARF but that electron transport is unaffected. This uncoupling of oxidative phosphorylation did not have any detectable effect on either levels of total adenine nucleotides and creatine phosphate or cellular energy charge. Measurements were also made of the activity of pyruvate dehydrogenase which provides an index of mitochondrial Ca^{2+} levels. The proportion of pyruvate dehydrogenase in its active form was threefold higher following isoprenaline injection in hearts of rats with ARF compared with controls. The results suggest that in hearts of rats with ARF there is a change in the number, affinity, efficacy or coupling of the dihydropyridine receptor on the L-type calcium channel. Moreover, in the ventricle, a defect in cellular Ca^{2+} control, resulting in an increase in mitochondrial Ca^{2+} uptake, may contribute to the depression of inotropic response to the range of cardiac stimulants tested.

Investigations of rats with acute renal failure (ARF) have demonstrated abnormalities in the sympathetic influence on cardiac function. For example, depressed chronotropic responses in-vivo to cervical sympathetic stimulation and isoprenaline have been noted (Bowmer et al 1983; Yates et al 1985, 1986; Mann et al 1986) and isolated right atria from rats with ARF exhibit reduced chronotropic responses to isoprenaline (Yates et al 1985). In addition to an impairment in cardiac chronotropic responses, we have observed that right ventricular strips removed from rats with ARF display reduced inotropic responses to a variety of cardiac stimulants, including isoprenaline, 3-isobutyl-1-methylxanthine and Ca^{2+}. These results were reported in a preliminary communication (Robinson et al 1989). A reduction in the inotropic responses to a range of cardiac stimulants indicates a defect within the myocardium at or beyond the entry of Ca^{2+} into the myocyte. Such a defect could result from a derangement of cellular Ca^{2+} control, or from abnormal cellular energy production. The aim of this study was to characterize further the effect of ARF on cardiac inotropic responses and to investigate possible mechanisms for the impairment in inotropic response. The responses of both atrial and ventricular tissues were recorded to a range of cardiac stimulants acting at various levels within the myocardium. Biochemical studies were also conducted to examine mitochondrial respiration and the levels of adenine nucleotides and creatine phosphate in cardiac tissue. Furthermore, activity of the Ca^{2+}-sensitive enzyme pyruvate dehydrogenase (PDH) was determined in order to assess cellular control of Ca^{2+} in the myocardium.

Materials and Methods

Materials

(−)-Isoprenaline sulphate, 3-isobutyl-1-methylxanthine (IBMX) and all substrates and inhibitors used in experiments with mitochondria were purchased from Sigma Chemical Co., UK. Thiobutabarbitone was purchased from BYK (Germany) and Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate) was a kind gift from Bayer, Germany. *p*-(*p*-Aminophenyl) azobenzene sulphonic acid (AABS) was obtained from Pfaltz & Bauer Inc., USA, and arylamine acetyltransferase and pyruvate dehydrogenase phosphate phosphatase were prepared according to the methods of Tabor et al (1953) and Severson et al (1974), respectively. Tetrabutylammonium hydroxide, Freon (1,1,2-trichlorotrifluoroethane) and tri-octylamine were obtained from Aldrich Chemical Co., UK. All other chemicals were purchased from either Sigma Chemical Co. or BDH Chemicals, UK.

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Induction of ARF

Male albino Wistar rats, 250–350 g, denied water for 24 h, were injected intramuscularly with 10 mL kg^{-1} of 50% (v/v) glycerol in saline (0·9% w/v NaCl) as described by Bowmer et al (1983). Control animals were similarly dehydrated but received an intramuscular injection of saline (10 mL kg^{-1}). All rats were studied 48 h following injection.

Isolated cardiac tissues

Rats were killed by cervical dislocation and the heart excised, from which the left atria and a longitudinal strip of right ventricular muscle (about 2 × 5 mm) were removed and placed in organ baths containing Krebs solution, 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, $CaCl_2$ 2·5. This solution was maintained at 35°C and was gassed continuously with 95% O_2–5% CO_2. The left atrium and right ventricular strip were mounted on tissue holders and connected to isometric transducers (Pioden Controls) under resting tensions of 0·5 and 2·0 g, respectively. Transducers were connected via

preamplifiers to a Lectromed MX216 pen recorder. The left atrium was paced at 2 Hz with rectangular pulses of 1 ms duration and 6 V amplitude (supramaximal) (Yates et al 1985) whereas the right ventricular strip was paced at 1·6 Hz with rectangular pulses of 5 ms duration and 5 V amplitude (supramaximal) (Ramanadham & Tenner 1987). For each tissue, cumulative inotropic concentration-response curves were constructed to isoprenaline, IBMX, Bay K 8644 or Ca^{2+}. The responses to one stimulant only were determined in each tissue. All experiments involving Bay K 8644 were carried out under the light from a sodium lamp. Responses to Ca^{2+} were obtained by first decreasing the concentration of Ca^{2+} in the bathing medium from 2·5 to 0·025 mM. With the exception of experiments involving Bay K 8644, four concentration-response curves were determined for each inotrope, with a 30 min interval between curves. The first curve was discarded and the response for each stimulant was taken as the mean of the subsequent three curves. Due to the tissue binding properties of Bay K 8644 (MacLeod 1987), only one curve was constructed for this drug in each tissue. At the end of each experiment the wet weight of cardiac tissue was recorded.

Measurement of PDH activity

Rats which were deprived of food overnight were anaesthetized with thiobutabarbitone and hearts freeze-clamped 60 s following intravenous injection of either saline (1 mL kg^{-1}) or isoprenaline (1 $\mu g\ kg^{-1}$). PDH was extracted according to the method of McCormack & Denton (1981). Briefly, heart tissue was ground to a powder under liquid nitrogen and ice-cold extraction buffer, composition (mM): potassium phosphate 100·0, EDTA 2·0, dithiothreitol 1·0, 0·1% Triton-X-100 (pH 7·3) and 50 $\mu L\ mL^{-1}$ rat plasma was added to a portion of tissue (50–100 mg) to give a final concentration of 50 mg mL^{-1}. Tissue was homogenized in ice-cold buffer using a Polytron homogenizer set at mark 5 for 20 s. The homogenate was allowed to stand for 5 min before being centrifuged for 1 min at 7500 *g*. PDH activity was determined for the supernatant.

PDH activity was assayed spectrophotometrically at 30°C by monitoring the change in absorption that occurs at 460 nm when AABS is acetylated by acetyl CoA in the presence of arylamine transferase (Coore et al 1971). Acetyl CoA is synthesized from pyruvate, a reaction catalysed by PDH. For the assay of PDH in its active form (PDH_a), a sample (25 μL) of heart extract was added to a mixture of 1·5 mL of incubation buffer, 20 μL of substrate solution and 15 μL of arylamine transferase (0·1 units mL^{-1}). The incubation buffer contained (mM): Tris (pH 7·8) 100·0, EDTA 0·5, $MgSO_4$ 1·0, 2-mercaptoethanol 5·0 and 20 $\mu g\ mL^{-1}$ AABS and the substrate solution contained (mM): thiamine pyrophosphate chloride (cocarboxylase) 80·0, NAD^+ 35·0, pyruvate 90·0 and coenzyme A 10·0. For measurement of total PDH activity, 25 μL of heart extract were incubated with 20 μL of pig heart PDH phosphate phosphatase (1·25 units mL^{-1}) and 5 μL of a solution containing 250 mM $MgCl_2$ and 10 mM $CaCl_2$ at 30°C for 20 min in order to convert all PDH to its active form (Whitehouse et al 1974). The incubate (25 μL) was used instead of heart extract in the assay of enzyme activity. Total PDH activity is expressed in units of enzyme activity (g wet tissue wt)$^{-1}$ where one unit of activity is the amount of PDH which converts 1 μmol pyruvate to acetyl CoA min^{-1} at 30°C. PDH_a activity is expressed as a percentage of total enzyme activity in the sample.

Measurement of mitochondrial respiration

Mitochondria were isolated from rat heart according to the method of Kerbey et al (1976). Animals were killed by cervical dislocation and hearts were excised and placed in 8 mL of ice-cold buffer composition (mM): sucrose 250, Tris (pH 7·4) 20·0, EGTA 2·0 and 1% (w/v) defatted bovine albumin. Hearts were homogenized using an Ystral homogenizer (3 × 2 s, mark 8). Mitochondria were separated by differential centrifugation (1000 and 10000 *g*) at 4°C. The final pellet obtained was suspended in 350 μL of buffer without albumin to give a final mitochondrial protein concentration of approximately 60 mg mL^{-1}.

Oxygen consumption of isolated mitochondria was measured polarographically at 30°C with an oxygen electrode (Model 781, Strathkelvin Instruments). The incubation buffer contained 125 mM KCl, 20 mM Tris (pH 7·3), 6 mM KH_2PO_4 and 1 mM EGTA. A 1 mL volume of buffer was added to the electrode chamber and endogenous oxygen consumption was recorded for 2 min following addition of 20 μL of mitochondrial suspension. State 4 (excess substrate, no ADP) and state 3 (substrate and ADP) respiration rates were measured with either 5 mM glutamate and 5 mM malate or 10 mM succinate and 5 μM rotenone as electron donors. State 3 respiration was initiated by addition of 200 nmol of ADP to the electrode chamber. Uncoupled respiration rates, for both electron donors, were measured in the presence of 10 μM dinitrophenol (DNP). All respiratory rates are expressed as nmol O_2 (mg mitochondrial protein)$^{-1}$ min^{-1}. The respiratory control ratio was obtained from the ratio of state 3 to state 4 respiration rates and the ADP:O ratio was calculated from mol ADP added divided by mol oxygen consumed.

Measurement of adenine nucleotides and creatine phosphate

Adenine nucleotides and creatine phosphate were measured by HPLC according to the method of Harmsen et al (1982). Rats were anaesthetized with 15% (w/v) urethane in saline (1 mL/100 g, intraperitoneally) and tail vein cannulae were inserted. In this study, urethane was used in place of thiobutabarbitone as it has no detectable UV absorbance at 214 nm which could interfere with the nucleotide peaks on the chromatogram. Rats received intravenous injections of either saline (1 mL kg^{-1}) or isoprenaline (1 $\mu g\ kg^{-1}$) and 1 min later, hearts were excised and freeze-clamped in liquid nitrogen. One hundred and fifty microlitres of 0·6 M perchloric acid was added to a portion (50–100 mg) of frozen heart powder and the tube was frozen in liquid nitrogen. The tissue was allowed to thaw for 1–2 min and then was simultaneously homogenized and acid-extracted with 10 passes of a motor-driven microhomogenizer pestle. The tissue was refrozen at intervals throughout the homogenization to ensure that it never totally thawed. The microhomogenizing vessel, pestle and contents were centrifuged at 13000 rev min^{-1} for 1 min in an MSE Microcentaur centrifuge. An aliquot (100 μL) of the acid extract was removed, neutralized by vortexing for 30 s with 400 μL of a 4:1 (v/v) solution of Freon/tri-octylamine and centrifuged for a further 1 min at 13000 rev min^{-1}. A sample (10 μL) of the clear upper

aqueous layer was injected immediately onto the HPLC column.

The HPLC system (Waters Associates, Hartford, UK) consisted of a Model 510 pump with a U6K injector connected to a Model 481 detector ($\lambda = 214$ nm). Chromatography was performed using a Novapak C18 5 μm reverse phase radial compression column (100 × 8 mm). The analytical column was protected by a pre-column module containing a Guard-Pak cyanopropyl cartridge. A flow rate of 0·8 mL min^{-1} was used. The mobile phase used consisted of 10% methanol and 90% 0·2 M NaH_2PO_4 which contained 0·025 M tetrabutylammonium hydroxide (pH 6·0). Levels of adenine nucleotides and creatine phosphate are expressed as nmol (mg myocardial protein)$^{-1}$. The energy charge for each heart sample was also calculated (Atkinson 1968).

Urea and protein assay

A blood sample was taken from each animal from either the exposed abdominal aorta or the tail vein for determination of plasma urea. This was measured by reaction with diacetyl monoxime using the procedure outlined in the Sigma Technical Bulletin No. 353. Myocardial and mitochondrial protein was measured by the biuret method using bovine serum albumin as the protein standard (Gornall et al 1949).

Statistical analysis

Inotropic responses were characterized by the maximum increase in force of contraction expressed as mg increase in force and percent increase on basal force and pD_2 (−log EC50). All results are expressed as mean ± s.e.m. Statistical comparisons were performed using either the non-paired Student's *t*-test or two way analysis of variance with the least significant difference test used to compare group means.

Results

The intramuscular injection of glycerol resulted in a 10-fold increase in plasma urea concentration compared with saline-injected animals in all the experiments conducted. For example, in the series of experiments which determined the inotropic response of isolated cardiac tissues, plasma urea levels in glycerol-injected rats were 267 ± 24 mg dL^{-1} (n = 32) which were significantly greater ($P < 0.001$) than the levels found in saline-injected rats (24 ± 1 mg dL^{-1}, n = 32).

Isolated cardiac tissues

There was no significant difference in the wet weight of tissues removed from rats with ARF and controls. For instance, in experiments with isoprenaline the left atria weight was 25 ± 2 mg (n = 8) in controls and 25 ± 3 mg (n = 8) in rats with ARF whilst right ventricular strips weighed 27 ± 2 mg (n = 8) in controls and 28 ± 2 mg (n = 8) in rats with ARF. The basal force, pD_2, and maximum response (expressed either as mg increase in force or as percent increase on basal force) to isoprenaline, IBMX, Bay K 8644 and Ca^{2+} of left atrial preparations are shown in Table 1. There was no significant difference in basal force between tissues from rats with ARF and controls when statistical comparisons were made between data generated in experiments involving individual inotropes. Furthermore, when the combined basal forces from experiments which used isoprenaline, IBMX and Bay K 8644 were compared, i.e. experiments with a constant Ca^{2+} concentration (2·5 mM), basal forces were very similar in control tissues (203 ± 36 mg, n = 24) and in tissues from rats with ARF (202 ± 29 mg, n = 24). The only significant difference between left atria from the two groups of rats was a marked reduction in the inotropic response of tissues from rats with renal failure to Bay K 8644 (Table 1). This was apparent when the inotropic response was expressed as either mg increase in force or percent increase on basal force. The responses to a range of doses of Bay K 8644 expressed in the latter manner are shown in Fig. 1 which illustrates the pronounced depression in inotropic response.

Table 2 shows the basal force, pD_2 values and maximum inotropic responses of right ventricular strips to the range of cardiac stimulants tested. Basal force in preparations from rats with ARF was greater than controls in all series of experiments, the percent difference from controls ranging from 11 to 57%. However, due to the large variation in basal force, none of these differences were statistically significant ($P > 0.05$). A comparison of the pooled recordings of basal force from experiments using isoprenaline, IBMX and Bay K 8644 revealed that basal force in tissues from rats with ARF (278 ± 42 mg, n = 24) was 31% greater than the basal force in controls (212 ± 32 mg, n = 24) although this difference did not attain statistical significance. The maximum % increase on basal force produced by all the inotropic agents in ventricular strips from rats with ARF was significantly reduced compared with responses in tissues from controls (Table 2). The full concentration response curves for isoprenaline and Bay K 8644 are shown in Fig. 2. When maximum responses were expressed as mg increase in force, responses in tissues from rats with ARF were still considerably smaller than controls although only the maximum increase in force to Bay K 8644 was significantly diminished ($P < 0.05$) (Table

Table 1. Basal force, maximum inotropic responses and pD_2 values in isolated left atria from rats with glycerol-induced acute renal failure (ARF) and controls in response to isoprenaline, IBMX, Bay K 8644 and Ca^{2+}.

	Basal force (mg)		Max increase in force (mg)		Max % increase on basal force		pD_2	
	Control	ARF	Control	ARF	Control	ARF	Control	ARF
Isoprenaline	127 ± 24	136 ± 37	167 ± 36	215 ± 80	130 ± 11	144 ± 16	7·7 ± 0·1	7·8 ± 0·1
IBMX	264 ± 69	302 ± 60	148 ± 28	152 ± 45	70 ± 15	61 ± 14	5·3 ± 0·1	5·4 ± 0·2
Bay K 8644	218 ± 80	167 ± 32	172 ± 44	42 ± 11*	95 ± 25	24 ± 7*	7·4 ± 0·3	7·3 ± 0·3
Ca^{2+}	57 ± 10	54 ± 8	296 ± 53	216 ± 44	525 ± 37	418 ± 51	2·8 ± 0·1	2·9 ± 0·1

Values are mean ± s.e.m. (n = 8). *$P < 0.05$ relative to respective control group (Student's *t*-test).

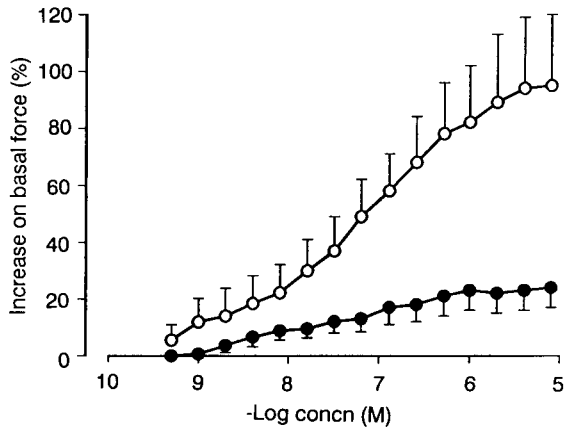

FIG. 1. Inotropic responses to Bay K 8644 in left atria of rats with glycerol-induced acute renal failure (●) and controls (○). Values are given as mean with bars indicating s.e.m. (n = 8).

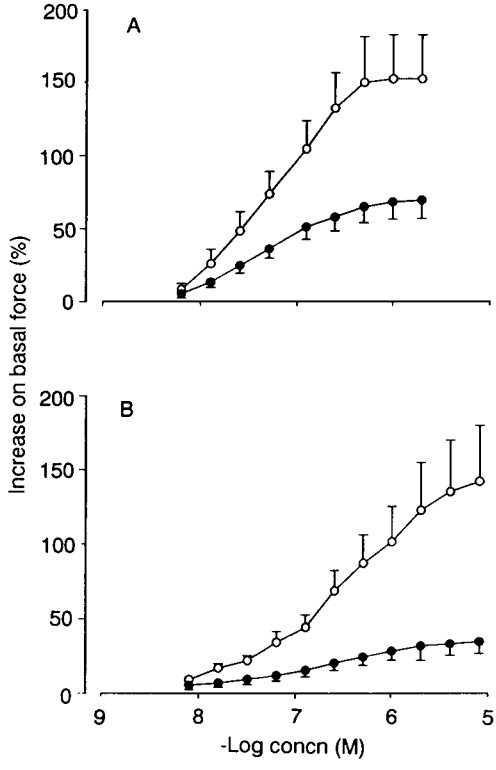

FIG. 2. Inotropic responses to isoprenaline (A) and Bay K 8644 (B) in right ventricular strips from rats with glycerol-induced acute renal failure (●) and controls (○). Values are given as mean with bars indicating s.e.m. (n = 8).

2). The mean pD_2 values for Ca^{2+} determined in preparations from rats with renal failure was significantly higher ($P < 0.01$) than the value in controls. However, the pD_2 values for the remaining stimulants showed no significant differences.

Myocardial pyruvate dehydrogenase activity in ARF

Fig. 3A shows the total activity of PDH in heart extracts from rats with ARF and controls following injection of saline or isoprenaline. There was no significant difference ($P > 0.05$) in the total activity of PDH between rats with ARF and controls following administration of either saline or isoprenaline. Furthermore, injection of isoprenaline produced no change in total PDH activity in either group of rats. Fig. 3B shows the percent of PDH in the active form (PDH_a) in the hearts of both groups of rats following injection of saline or isoprenaline. PDH_a levels were 40% higher in the hearts of rats with ARF compared with controls after saline injection, but this change was not statistically significant ($P > 0.05$). Isoprenaline caused an increase in the activity of PDH_a in both control and ARF rats. In controls, the activity of PDH_a was increased by 1·6 times following isoprenaline compared with the activity obtained after saline injection, although this difference was not significant ($P > 0.05$). By contrast, in rats with glycerol-induced ARF, PDH_a activity was increased 4-fold ($P < 0.001$) in response to isoprenaline compared with the activity obtained after saline injection. This level of activity was also significantly greater ($P < 0.001$) than that recorded in control rats given isoprenaline.

Mitochondrial respiration in ARF

Table 3 shows the respiratory parameters measured in mitochondria isolated from rats with ARF and controls with either glutamate plus malate or succinate as electron donors. For both substrates, state 3, state 4, and uncoupled rates of respiration were similar in mitochondria from rats with ARF and controls ($P > 0.05$). There was also no significant difference ($P > 0.05$) in respiratory control ratios between the two groups of rats. However, with both glutamate plus malate and succinate, ADP:O ratios were reduced by

Table 2. Basal force, maximum inotropic responses and pD_2 values in isolated right ventricular strips from rats with glycerol-induced acute renal failure (ARF) and controls in response to isoprenaline, IBMX, Bay K 8644 and Ca^{2+}.

	Basal force (mg)		Max increase in force (mg)		Max % increase on basal force		pD_2	
	Control	ARF	Control	ARF	Control	ARF	Control	ARF
Isoprenaline	168±31	194±44	243±63	161±49	152±30	69±13*	7·3±0·2	7·4±0·1
IBMX	267±75	420±72	191±48	141±58	80±13	32±8*	4·9±0·1	5·1±0·1
Bay K 8644	200±49	221±76	247±62	66±19*	142±37	35±8*	6·6±0·1	6·7±0·1
Ca^{2+}	74±18	108±27	430±94	235±90	611±41	187±29***	2·7±0·1	3·1±0·1**

Values are mean±s.e.m. (n = 8). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ relative to respective control group (Student's t-test).

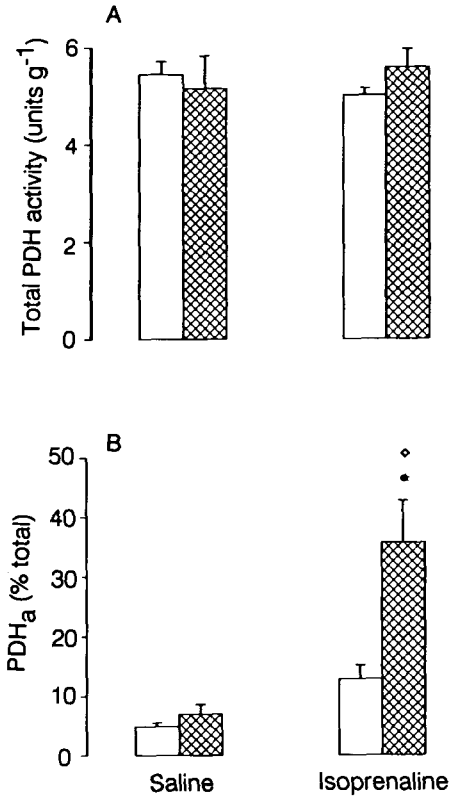

FIG. 3. A. Total activity of pyruvate dehydrogenase (PDH). B. The percent of pyruvate dehydrogenase in the active form (PDH_a) in hearts from rats with glycerol-induced acute renal failure (hatched bars) and controls (open bars) 1 min following intravenous injection of either saline (1 mL kg^{-1}) or isoprenaline (1 μg kg^{-1}). Columns represent means and vertical bars s.e.m. (n = 8). *$P < 0.001$ relative to enzyme activity in control rats following isoprenaline injection; $^{\diamond}P < 0.001$ relative to enzyme activity in rats with acute renal failure following saline injection (two-way analysis of variance).

approximately 20% ($P < 0.05$) in mitochondria isolated from rats with ARF (Table 3).

Adenine nucleotide and creatine phosphate levels in ARF

Recoveries from the extraction procedure were (%): AMP 88 ± 3, ADP 92 ± 4, ATP 95 ± 1 and creatine phosphate 89 ± 3, n = 8; all values given are corrected for these recoveries. Levels of AMP, ADP, ATP and creatine phosphate following injection of saline or isoprenaline in both groups of rats are illustrated in Fig. 4. Following saline injection there was no significant difference ($P > 0.05$) in levels of any nucleotide or creatine phosphate between rats with ARF and controls. As a consequence cellular energy charge in animals with renal failure (0·87 ± 0·01, n = 8) was not significantly different ($P > 0.05$) from the charge in controls (0·89 ± 0·01, n = 8). In addition, total adenine nucleotide levels in the hearts of rats with ARF (24 ± 3 nmol (mg myocardial protein)$^{-1}$, n = 8) were not significantly different ($P > 0.05$) from the levels recorded in controls (20 ± 4 nmol (mg myocardial protein)$^{-1}$, n = 8). Isoprenaline caused a significant increase ($P < 0.05$) in levels of AMP and a significant decrease ($P < 0.01$) in levels of creatine phosphate in both groups of rats. By contrast, isoprenaline had no significant

Table 3. Respiratory parameters of mitochondria isolated from hearts of rats with glycerol-induced ARF and controls.

	5 mM glutamate plus 5 mM malate		10 mM succinate	
	Control	ARF	Control	ARF
State 3	79 ± 4	83 ± 4	110 ± 7	116 ± 6
State 4	29 ± 1	33 ± 2	64 ± 4	72 ± 4
DNP-stimulated	74 ± 4	77 ± 5	110 ± 5	122 ± 7
Respiratory control ratio	2·8 ± 0·1	2·6 ± 0·2	1·7 ± 0·1	1·7 ± 0·1
ADP:O	2·2 ± 0·1	1·7 ± 0·2*	1·3 ± 0·1	1·0 ± 0·1*

State 3, state 4 and DNP-stimulated respiratory rates are expressed as nmol O_2 (mg mitochondrial protein)$^{-1}$ min^{-1}. Experiments with succinate as substrate were performed in the presence of 5 μM rotenone. All values are mean ± s.e.m. (n = 7). *$P < 0.05$ relative to respective control group.

effect on levels of ATP or ADP. There was no significant difference ($P > 0.05$) in levels of ADP, ATP, or creatine phosphate after isoprenaline between rats with ARF and controls. However, in rats with ARF, AMP was significantly higher ($P < 0.05$) following isoprenaline compared with controls. The cellular energy charge following isoprenaline injection was 0·81 ± 0·01 (n = 8) in rats with ARF and 0·83 ± 0·01 (n = 8) in controls. This difference was not statistically significant ($P > 0.05$) but in both groups of rats the energy charge after isoprenaline was significantly lower ($P < 0.001$) than the values obtained after saline. Total adenine nucleotide levels after isoprenaline were similar ($P > 0.05$) in rats with renal failure (22 ± 2 nmol (mg myocardial protein)$^{-1}$, n = 8) and controls (19 ± 3 nmol (mg myocardial protein)$^{-1}$, n = 8). In neither case were these levels significantly different ($P > 0.05$) from those noted in the corresponding group following saline injection.

Discussion

The results from isolated tissue experiments showed that rats with ARF have impaired cardiac inotropic responses. Left atria from rats with ARF exhibited normal responses to isoprenaline, IBMX and Ca^{2+} but a marked depression of the response to Bay K 8644. This dihydropyridine compound appears to exert its inotropic effect by prolonging the mean opening time of the L-type calcium channel within the sarcolemma (Hess et al 1984; Hofmann et al 1988). The selective depression of the responsiveness to Bay K 8644 in left atria indicates there is a change in the number, affinity, efficacy or coupling of the dihydropyridine receptor on the L-type calcium channel in left atria of rats with ARF. The inotropic response to Bay K 8644 was also greatly reduced in right ventricular preparations removed from rats with ARF. However, in this tissue the inotropic responses to isoprenaline, IBMX and Ca^{2+} when expressed as percent increase on basal tension were also markedly diminished. When the results were expressed as mg increase in tension the responses of all four inotropes were lower in tissues from animals with ARF but because of the variation in these data, only the response to Bay K 8644 was statistically significant (Table 2). Indeed, of the four inotropes examined on the ventricular preparation, the diminution in inotropic response was most pronounced with Bay K 8644 which indicates that, as with

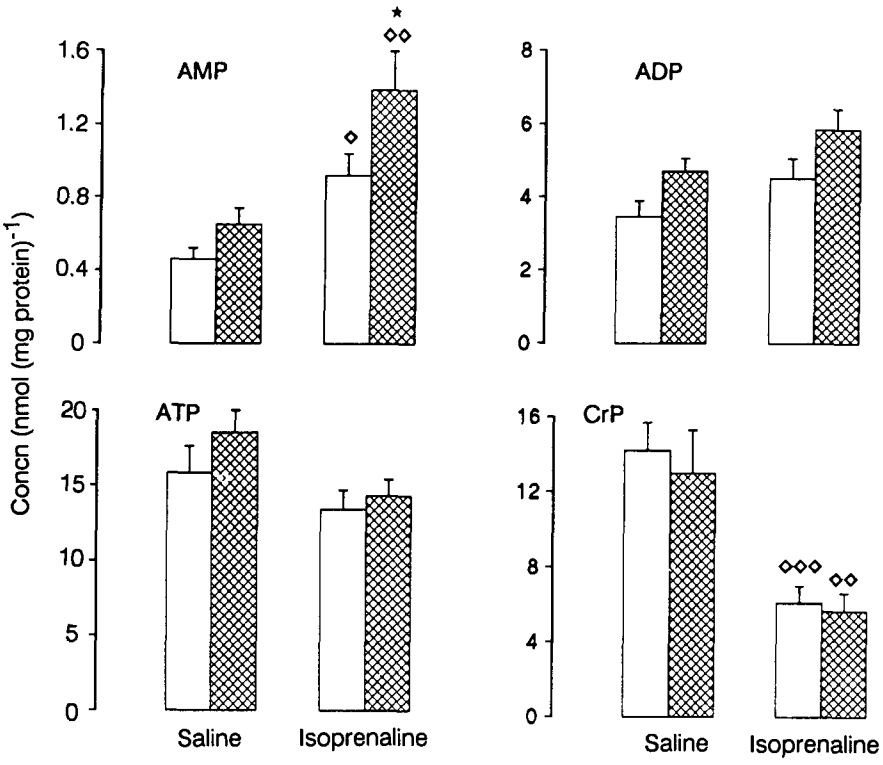

FIG. 4. Concentrations of AMP, ADP, ATP and creatine phosphate (CrP) in hearts from rats with glycerol-induced acute renal failure (hatched bars) and controls (open bars) 1 min following intravenous injection of either saline (1 mL kg^{-1}) or isoprenaline (1 μg kg^{-1}). Columns represent means and vertical bars s.e.m. (n=8). $^{*}P<0.05$ compared with the concentration in control rats following isoprenaline injection; $^{\diamond}P<0.05$, $^{\diamond\diamond}P<0.01$, $^{\diamond\diamond\diamond}P<0.001$ compared with concentrations following saline injection in the same group (two-way analysis of variance).

the left atria, there is a change in the dihydropyridine receptor on the L-type calcium channel.

It is possible that an abnormality of the calcium channel could account for depressed responses to Ca^{2+}. Furthermore, such a defect might also be responsible for the reduced inotropic responses to isoprenaline and IBMX, since both compounds, via an elevation in cAMP, produce much of their inotropic effect by phosphorylation of the calcium channel (Evans 1986). The difference between atria and ventricle, in response to isoprenaline and IBMX in ARF, could be a reflection of tissue differences in the contribution of flow through this channel to the inotropic actions of compounds which elevate cAMP. However, this explanation cannot account for differences between atrial and ventricular tissues in respect to the inotropic response to Ca^{2+} in ARF. An alternative explanation for the reduced response to the range of inotropes tested on the right ventricle is that in ventricular tissue there is a further defect in events which mediate excitation-contraction coupling in addition to a change in the dihydropyridine receptor. This could result from an alteration in cellular Ca^{2+} handling or a depression of myocardial energy production.

PDH_a activity was higher following both saline and isoprenaline injection in rats with ARF compared with controls although only in the latter case was this statistically significant ($P<0.05$). PDH_a activity provides an index of mitochondrial Ca^{2+} levels (Denton & McCormack 1985). The finding of increased PDH_a activity in rats with ARF suggests there may be a change in cellular Ca^{2+} control. This could be related to a restriction in Ca^{2+} sequestration by the sarcoplasmic reticulum in the myocardium of rats with ARF. A depression of Ca^{2+} transport of fragmented isolated sarcoplasmic reticulum has been observed in the hearts of bilaterally nephrectomized rats (Penpargkul et al 1976). If such a defect occurred in rats with glycerol-induced ARF the resulting increase in cytosolic Ca^{2+} may be buffered by mitochondria thus increasing PDH_a activity. In the heart, most of the Ca^{2+} that activates contraction is released from the sarcoplasmic reticulum (Wier et al 1988). Thus a reduction in Ca^{2+} sequestration by the sarcoplasmic reticulum would reduce intra-cellular stores available for Ca^{2+}-induced Ca^{2+} release and therefore could contribute to the depressed responses to the range of inotropes noted in the right ventricle of rats with ARF. If such a defect in Ca^{2+} sequestration of the sarcoplasmic reticulum occurs in glycerol-induced ARF, it is restricted to the ventricle since the left atria only showed an impaired inotropic response to Bay K 8644.

A further reason for an overall reduction in inotropic responsiveness in the right ventricle is that there is an impairment in myocardial energy production. Indeed accumulation of Ca^{2+} within the mitochondria in rats with ARF, suggested by enhanced PDH_a activity, may restrict cell energy production since Ca^{2+} uptake takes place as an alternative to oxidative phosphorylation (Carafoli 1986). However, there appeared to be no overt disruption of

myocardial energy production in rats with ARF on the basis of studies on mitochondrial respiration and measurement of levels of high energy phosphates. Mitochondria from animals with ARF had significantly lower ADP:O ratios with either glutamate plus malate or succinate as electron donors. These substrates feed electrons in at site 1 and site 2, respectively, of the respiratory chain. As all other respiratory parameters were comparable between the two groups of rats for all substrates used, this suggests that there is a site-unspecific 'pseudo' uncoupling of oxidative phosphorylation in ARF (Kessler et al 1976), but that electron transport is unaffected. These findings are in contrast with those of Yamada et al (1969) and El-Belbessi et al (1986) who observed aberrations of both electron transport and the coupling of oxidative phosphorylation in hepatic mitochondria and cardiac mitochondria from rats with ARF and chronic renal failure, respectively.

The uncoupling of oxidative phosphorylation did not have any appreciable effect on either levels of adenine nucleotides and creatine phosphate or cellular energy charge. The only difference that achieved statistical significance was the increase in AMP levels which was more pronounced in the rats with ARF following isoprenaline injection. No studies have measured adenine nucleotides in rats with renal failure following β-adrenoceptor stimulation. However, under basal conditions, Penpargkul et al (1976) observed normal levels of ATP and creatine phosphate in hearts of rats with either ARF or chronic renal failure, data which are consistent with the levels we noted following saline injection. By contrast, El-Belbessi et al (1986) and Krog et al (1986) found reductions in basal levels of ATP and creatine phosphate in the hearts of rats with chronic renal failure. The pattern of changes in adenine nucleotide and creatine phosphate levels in response to isoprenaline was similar in rats with ARF and controls. Under increased energy utilization, necessarily imposed by β-adrenoceptor stimulation, isoprenaline caused increased hydrolysis of ATP to ADP and AMP and therefore a reduction in energy charge in the hearts of both groups of rats. As creatine phosphate provides an intra-cellular buffering system, increased hydrolysis of ATP did not result in a fall in ATP levels but creatine phosphate levels fell by 50%, 1 min after isoprenaline injection. These results are in agreement with those of Unitt et al (1989) who observed little change in ATP but a marked fall in creatine phosphate concentrations in rat isolated heart perfused with 10^{-7} M isoprenaline.

A change at the level of the contractile elements could also account for the reduced inotropic responsiveness observed in the ventricle in ARF. For example, in experimental models of diabetes, hypertension and cardiomyopathy, a shift in the myosin isozyme pattern from predominantly V_1 (with high Ca^{2+}-ATPase activity) to V_3 (with low Ca^{2+}-ATPase activity) has been noted and in all cases was associated with reduced cardiac contractility (Dillman 1980; Malhotra et al 1985; Morano et al 1988). Such changes have been detected at periods ranging from 2 weeks to several months after induction of the disease state. However, in the present study, ventricular tissue was used 48 h after the induction of ARF. Thus there may be insufficient time for a significant shift in myosin isozymes to occur.

In conclusion, the present study has demonstrated that both left atria and right ventricles of rats with glycerol-induced ARF have an impaired inotropic response to the calcium agonist Bay K 8644. This suggests some defect in the dihydropyridine receptor on the L-type calcium channel. In addition, the ventricular preparation also displayed a depression of inotropic responses to isoprenaline, IBMX and Ca^{2+} which may also result from an abnormality in the calcium channel and/or from a defect in cellular Ca^{2+} control resulting in an increase in mitochondrial Ca^{2+} uptake. Although a site-unspecific 'pseudo' uncoupling of oxidative phosphorylation in mitochondria from hearts of rats with ARF has been observed, this appears to have no detectable impact on cellular energy balance. Thus, if the impairment in cellular Ca^{2+} control contributes to the reduced ventricular responsiveness, it is a result of this change itself and not a consequent disruption of myocardial energy production.

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