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Mibefradil is more effective than verapamil for restoring post-ischemic function of isolated hearts of guinea pigs with acute renal failure

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Abstract

The deleterious intracellular Ca^{2+} overload in the ischemic–reperfusion injury of the heart can be even more expressed in subjects with acute renal failure in whom maintenance of intracellular Ca^{2+} has already been disturbed in normoxia. To study the influence of acute renal failure in ischemic–reperfusion injury on the heart, we used isolated Langendorff's hearts of guinea pigs with gentamicin-induced acute renal failure. We examined arrhythmias, heart contractility and myocardial cell damage during reperfusion. Two specific Ca^{2+} channel antagonists, mibefradil (0.1 and 1 μM) and verapamil (0.1 μM), were used to test the possible involvement of T-type and L-type Ca^{2+} channels in these processes. We exposed hearts to 50 min of zero-flow global ischemia and 60 min of reperfusion. During reperfusion, unrecoverable ventricular fibrillation appeared more often in hearts of animals with acute renal failure than in control hearts (80% vs. 0%, respectively). Mibefradil, but not verapamil, applied either pre- or post-ischemically, terminated ventricular fibrillation in all hearts of animals with acute renal failure. Mibefradil (0.1 μM only) improved contractility in hearts of animals with acute renal failure during reperfusion by 30%. During reperfusion, lactate dehydrogenase (LDH) release rate increased less in hearts of guinea pigs with acute renal failure than in control hearts and only verapamil decreased it additionally. Thus, our results suggest a more important role of T- than of L-type Ca^{2+} channels in ischemic–reperfusion injury in isolated guinea pig hearts with acute renal failure.

Keywords: Ischemic–reperfusion injury; Acute renal failure; Mibefradil; Verapamil; isolated, guinea pig

1. Introduction

Ischemic–reperfusion injury of the heart is manifested during reperfusion by ventricular arrhythmias, depressed myocardial contractility and myocardial cell necrosis (Opie and Phil, 1989). In the pathogenesis of ischemic–reperfusion injury, intracellular Ca^{2+} overload (Opie and Phil, 1989) and especially intramitochondrial overload (Miller and Tormey, 1995; Halestrap et al., 1998; Lemasters et al., 1998) play an important role. Calcium overload results from a diminished or reversed function of many ionic pumps and exchangers pumping Ca^{2+} ions out of the cell or exchanging them for sodium, hydrogen and other ions (Halestrap et al., 1998). Many authors (Bersohn and Shine, 1983; Nayler et al., 1987; Budihna et al., 1995) reported a decreased ischemic–reperfusion injury of the heart on use of L-type Ca^{2+} channel antagonists. Under some pathologic conditions, e.g. acute and chronic renal failure, Ca^{2+} equilibrium in myocardial cells is already disturbed under normoxic conditions (Robinson et al., 1992; Raine et al., 1993). In our study, we assumed that acute renal failure might aggravate ischemic–reperfusion injury of the heart, and that certain Ca^{2+} antagonists might reduce this injury. To investigate this assumption, we used isolated hearts of guinea pigs with gentamicin-induced acute renal failure and rendered them ischemic. The heart rhythm and contractility during reperfusion were studied. The extent of myocardial cell damage was assessed from the lactate dehydrogenase (LDH) release rate. To study the possible involvement of T- and L-type Ca^{2+} channels in these processes, we perfused the hearts of animals who had acute renal failure and the control hearts with two specific Ca^{2+} channel antagonists, either mibefradil (a T- and L-type antagonist) or verapamil (an L-type antagonist), either pre- or post-ischemically. Mibefradil in a lower concentration (0.1 μM) affects only T-type Ca^{2+}

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channels and, at higher concentrations, both T- and L-type Ca^{2+} channels (Abernethy, 1997).

2. Materials and methods

2.1. Experimental procedure

Guinea pigs of either sex (300–500 g) were s.c. injected with gentamicin sulphate 80 mg/kg/day (10 mg/ml, Lek, Slovenia) once a day for eight consecutive days to induce acute renal failure. Control animals were s.c. injected with an equivalent volume of saline (8 ml/kg/day of 0.9% NaCl). On the 9th day, the guinea pigs were anaesthetized with 20% urethane 6 ml/kg i.p. Having opened the thorax, we inserted a cannula into the ascending aorta and collected venous blood samples for the determination of urea and creatinine serum concentrations. The heart was isolated and perfused retrogradely under constant pressure (60 cm H_2O) on the Langendorff's apparatus. The perfusion solution (modified Krebs–Henseleit solution, in mmol/l: 118 NaCl, 11.1 glucose, 25 $NaHCO_3$, 4.7 KCl, 1.2 $NaH_2PO_4 \cdot 2H_2O$, 1.2 $MgSO_4 \cdot 7H_2O$, 1.4 $CaCl_2 \cdot 2H_2O$) was kept at 38.5 °C and gassed with 95% O_2 and 5% CO_2.

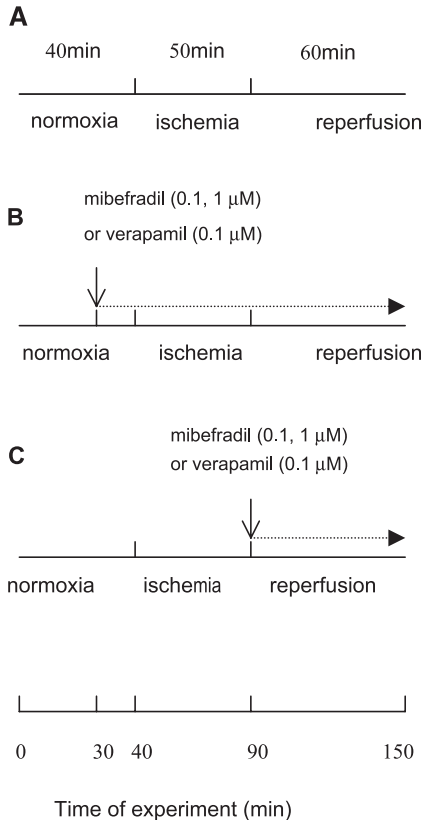

Fig. 1. Experimental protocol with ischemia in the isolated hearts of guinea pigs. (A) After 40 min of equilibration (normoxia), the perfusion of hearts was stopped for 50 min (ischemia); then 60 min of normoxic perfusion followed (reperfusion). (B) As in (A), but 10 min before the ischemia till the end of experiment, either mibefradil or verapamil was added to the perfusion solution (pre-ischemic group). (C) As in (A), but at the beginning of reperfusion till the end of experiment, either mibefradil or verapamil was added to the perfusion solution (post-ischemic group).

Table 1
Serum concentrations of urea and creatinine before the beginning of experiments in control guinea pigs and in those with acute renal failure

Parameter	Control	Animals with acute renal failure
Serum urea (mmol/l)	7.7 ± 1.1	24.1 ± 3.1[a]
Serum creatinine (μmol/l/100 g)	3.0 ± 1.0	24.7 ± 12.0[b]
Heart rate (beats/min)	198.0 ± 20.0	204.0 ± 30.0
LVP (mm Hg)	33.1 ± 1.6	32.8 ± 2.6
Coronary flow (ml/g min)	7.4 ± 0.4	10.7 ± 1.1[b]
LDH release rate (μkat/g min)	2.5 ± 0.7	3.9 ± 0.6

Heart rate, left ventricular pressure (LVP), coronary flow and lactate dehydrogenase (LDH) release rate in the isolated guinea pig hearts of control and of animals with acute renal failure at the 30th min of experiment (end of equilibration time).
Values are mean ± S.E., $n = 7–15$.
[a] $P < 0.01$ vs. control.
[b] $P < 0.05$.

Experiments with 50 min of zero-flow ischemia and 60 min of reperfusion were carried out on the hearts of animals with acute renal failure and the control hearts as shown in Fig. 1A ('ischemic no-drug' groups). Calcium channel antagonists, mibefradil (0.1 or 1 μM, Hoffman La Roche, Suisse) or verapamil (0.1 μM, Lek), were being added to the perfusion solution either 10 min before ischemia and till the end of experiments (pre-ischemic group) or during reperfusion only (post-ischemic group) (Fig. 1B and C).

Control experiments with 150-min-lasting oxygenated perfusion were carried out with the normoxic hearts of animals with acute renal failure and the control hearts ('no-drug' groups).

In the isolated Langendorff's hearts, electrocardiogram (ECG) and left ventricular pressure (LVP) were recorded continuously. We measured ECG using two epicardial silver electrodes and LVP (mm Hg) with an isovolumetric pressure recorder (ISOTEC, HSE, Germany). The heart rate (beats/min) and arrhythmias were obtained from ECG signals. The Lambeth Convention (Walker et al., 1988) was used for the definition of arrhythmias. The incidence and duration of ventricular tachycardias and ventricular fibrillations were analysed. LDH activity (μkat/ml; 1 katal corresponds to the conversion of 1 M of substrate in 1 s; Dybkaer, 2002) in coronary effluents (collected at 2-, 5- or 10-min intervals) was measured spectrophotometrically by the modified method of Wroblewski and LaDue (1955). LDH release rate (μkat/g min) was calculated from LDH activity (μkat/ml), multiplied by coronary flow (ml/min) and divided by heart weight (g) (Budihna et al., 1995), and was used to assess the extent of myocardial cell damage.

All experiments were carried out according to the Guidelines for Animal Experiments and were approved by the Medical Ethics Committee of the Faculty of Medicine in Ljubljana.

Table 2
Effects of mibefradil (MIB) and verapamil (VER) on the incidence and the duration of post-ischemic ventricular tachycardia and ventricular fibrillation during reperfusion period in the isolated hearts of control guinea pigs (Co) and of those with acute renal failure (ARF)

Group	VTI (*n*/*N*)	VTD (s)	VFI (*n*/*N*)	VFD (s)	VFS (*n*/*N*)
(A) Ischemic—no drug					
(A1) Co – no drug	7/8	107.1 ± 86.5	7/8	463.4 ± 244.5	0/8
(A2) ARF – no drug	10/10	34.3 ± 10.8	9/10	2856.1 ± 474.1[a]	8/10[b]
(B) Pre-ischemic					
(B1) Co + 0.1 μM MIB	5/5	6.8 ± 2.5	5/5	779.4 ± 10.41	1/5
Co + 1 μM MIB	4/4	11.5 ± 2.5	3/4	28.8 ± 12.4	0/4
Co + 0.1 μM VER	4/4	8.3 ± 1.6	4/4	1035.0 ± 98.5	1/4
(B2) ARF + 0.1 μM MIB	3/5[c]	8.2 ± 4.5	4/5	46.0 ± 22.8[d]	0/5[e]
ARF + 1 μM MIB	1/5[c]	6.4 ± 0.0	1/5[c]	4.0 ± 0	0/5[e]
ARF + 0.1 μM VER	5/7	9.0 ± 6.1	7/7	3121.6 ± 468.5	2/7
(C) Post-ischemic					
(C1) Co + 0.1 μM MIB	5/5	13.8 ± 2.5	5/5	829.6 ± 687.9	1/5
Co + 1 μM MIB	5/5	12.8 ± 4.1	5/5	28.8 ± 12.0	0/5
Co + 0.1 μM VER	5/5	9.3 ± 5.3	5/5	1569.6 ± 882.9	2/5
(C2) ARF + 0.1 μM MIB	4/5	9.6 ± 3.7	4/5	123.8 ± 57.9[d]	0/5[e]
ARF + 1 μM MIB	5/5	15.4 ± 2.9	5/5	80.2 ± 36.1	0/5[e]
ARF + 0.1 μM VER	5/5	14.6 ± 3.3	5/5	2950.4 ± 632.6	4/5

MIB or VER were added to the perfusion solution either 10 min before ischemia (pre-ischemic group) or at the beginning of reperfusion (post-ischemic group). VTI—incidence of ventricular tachycardia; VTD—duration of ventricular tachycardia; VFI—incidence of ventricular fibrillation; VFD—duration of ventricular fibrillation; VFS—incidence of sustained ventricular fibrillation; *n*/*N*–number of fibrillating hearts (*n*) against all hearts (*N*) in respective group. Values are means ± S.E., $n = 4–10$.

[a] $P < 0.05$ vs. 'Co - no drug' group.
[b] $P < 0.01$ vs. 'Co - no drug' group.
[c] $P < 0.05$ vs. 'ARF - no drug' group.
[d] $P < 0.001$ vs. 'ARF - no drug' group.
[e] $P < 0.01$ vs. 'ARF - no drug' group.

Table 3
Heart rate in the isolated hearts of control guinea pigs (Co) and of guinea pigs with acute renal failure (ARF) at different times of experiments

Group	Heart rate (%)					
	40th min	95th min	100th min	110th min	120th min	150th min
(A) Ischemic—no drug						
Control – no drug	98.9 ± 1.4	144.5 ± 27.0	155 ± 31.7	119.9 ± 4.8	122.1 ± 14.9	115.9 ± 5.8
ARF – no drug	88.1 ± 12.1	97.1 ± 15.6[a]	86.9 ± 17.7	83.4 ± 20.6[a]	137.2 ± 30.8	99.8 ± 1.0
(B) Pre-ischemic						
(B1) Co + 0.1 μM MIB	98.1 ± 1.5	112.2 ± 2.0	112.3 ± 5.8	98.2 ± 3.7[b,c]	95.8 ± 4.5[c]	85.4 ± 9.9[b,d]
Co + 1 μM MIB	91.9 ± 4.7	87.4 ± 8.7[b]	85.0 ± 9.5	69.1 ± 6.7[e]	62.0 ± 7.5	59.3 ± 9.5[e]
Co + 0.1 μM VER	96.6 ± 2.7	89.3 ± 7.8	84.7 ± 0.1	86.3 ± 4.9[b]	87.1 ± 3.1	88.3 ± 3.3[f]
(B2) ARF + 0.1 μM MIB	96.5 ± 2.9	117.6 ± 4.4[g]	111.1 ± 3.3[g]	101.9 ± 1.9[g]	92.9 ± 8.6[c]	73.8 ± 12.7
ARF + 1 μM MIB	90.0 ± 3.4[b]	94.3 ± 4.0	79.8 ± 3.7	74.8 ± 3.0	72.2 ± 3.5[b]	65.2 ± 3.5[b]
ARF + 0.1 μM VER	92.1 ± 2.8	75.1 ± 15.3	81.3 ± 1.9	81.3 ± 1.9	80.7 ± 1.2	81.7 ± 2.2
(C) Post-ischemic						
(C1) Co + 0.1 μM MIB	100.4 ± 0.9	115.5 ± 5.3[b,d]	114.1 ± 6.1	103.4 ± 2.3[b,c]	101.9 ± 2.4	97.7 ± 1.8[b,c]
Co + 1 μM MIB	102.2 ± 2.3	103.5 ± 5.7[b]	102.8 ± 4.7	87.2 ± 5.6[d]	82.2 ± 6.7[b]	68.9 ± 4.8[f]
Co + 0.1 μM VER	95.5 ± 4.1	108.9 ± 15.8	108.8 ± 15.8	91.1 ± 14.6[b]	84.4 ± 15.9	69.2 ± 1.0[e]
(C2) ARF + 0.1 μM MIB	98.7 ± 1.7	109.5 ± 4.8	111.2 ± 3.4	104.0 ± 3.2	102.3 ± 4.2[d]	95.9 ± 2.4[g]
ARF + 1 μM MIB	107.2 ± 7.5	110.7 ± 8.6	97.2 ± 9.8	88.7 ± 7.2	81.6 ± 4.4[b]	68.2 ± 3.3[e]
ARF + 0.1 μM VER	113.9 ± 16.9	90.1	65.2	98.8	93.3	101.2

The zero-flow ischemia lasted from the 40th to the 90th min of experiments. Mibefradil (MIB) or verapamil (VER) was added to the perfusion solution either 10 min before ischemia (pre-ischemic group) or at the beginning of reperfusion (post-ischemic group). The values are percentages of the 30th min value (end-equilibration time). Values are means ± S.E., $n = 5–10$.

[a] $P < 0.05$ vs. control 'no drug' group.
[b] $P < 0.05$ vs. respective 'no drug' group.
[c] $P < 0.05$ vs. 1 μM mibefradil.
[d] $P < 0.01$ vs. 1 μM mibefradil.
[e] $P < 0.01$ vs. respective 'no-drug' group.
[f] $P < 0.001$ vs. respective 'no-drug' group.
[g] $P < 0.001$ vs. 1 μM mibefradil.

2.2. Statistics

The percentage incidence of reperfusion arrhythmias was calculated and was compared using Fisher's exact probability test. The heart rate and LVP during reperfusion are shown as the percentage of the 30th min value (end-equilibration time) and expressed as mean ± S.E. The LDH release rate (μkat/g min) was expressed either relative to the value at the 30th min or as absolute value. Student's *t*-test was used to test the differences between groups. $P \leq 0.05$ was considered indicative of a statistically significant difference between values.

3. Results

Serum concentrations of both urea and creatinine were respectively three and eight times higher in the guinea pigs with acute renal failure than in the controls ($P < 0.05$) (Table 1). Histologically, in the kidneys of gentamicin-treated animals, epithelial degeneration and urine cylinders in the proximal tubules, as well as interstitial mononuclear infiltration, were found. The most remarkable finding in all preparations was the proximal tubular epithelial cell degeneration with apoptosis. During normoxia, the coronary flow was about 30% higher in the isolated hearts of animals with acute renal failure than in the control hearts (Grašič Kuhar and Budihna, 2000). However, the heart rate and the LVP were similar in both groups (Table 1).

3.1. Arrhythmias

3.1.1. Reperfusion arrhythmias in isolated hearts of guinea pigs with acute renal failure and in control hearts

At the end of ischemia, all hearts, both from guinea pigs with acute renal failure and the controls, had supraventricular rhythm (narrow QRS complexes). In the control hearts, this rhythm continued for the first few seconds of reperfusion. In the first seconds of reperfusion, wide bizarre QRS complexes arose in all hearts, followed by short periods of ventricular tachycardia, which finally degenerated into ventricular fibrillation. The incidence of ventricular tachycardia and of ventricular fibrillation as well as the duration of ventricular tachycardia did not differ between the two groups (Table 2, A1 and A2). Eight out of 10 fibrillating hearts of animals with acute renal failure did not recover sinus rhythm during the whole reperfusion (sustained ventricular fibrillation), whereas it recovered in all control hearts ($P < 0.01$; Table 2, A1 and A2). As a consequence, the ventricular fibrillation was longer-lasted in the hearts of animals with acute renal failure than in the control hearts ($P < 0.05$; Table 2, A1 and A2).

3.1.2. Effects of mibefradil and verapamil on reperfusion arrhythmias

Mibefradil (0.1 and 1 μM), when added to the perfusion solution of the isolated hearts of animals with acute renal failure 10 min before ischemia or during reperfusion only, prevented the occurrence of sustained ventricular fibrillation during reperfusion ($P < 0.01$; Table 2, B2 and C2). In the hearts of animals with acute renal failure, 1 μM mibefradil, if added pre-ischemically, decreased ($P < 0.05$) the incidence of ventricular tachycardia and ventricular fibrillation (Table 2, B2). Verapamil, however, had no influence on any of the observed arrhythmias in the hearts of animals with acute renal failure (Table 2, B2 and C2).

In the control hearts, perfusion with mibefradil or verapamil did not affect any of the observed post-ischemic arrhythmias (Table 2, B1 and C1).

3.2. Heart rate in nonfibrillating periods

3.2.1. Heart rate during reperfusion in isolated hearts of guinea pigs with acute renal failure and in control hearts

In the hearts of animals with acute renal failure without sustained ventricular fibrillation during reperfusion (2 out of 10 hearts), the heart rate, as compared with the last pre-ischemic value, was not accelerated in the first 20 min of reperfusion as it was in the control hearts (Table 3, A).

3.2.2. Effects of mibefradil and verapamil on heart rate during reperfusion

In the hearts of animals with acute renal failure, 1 μM mibefradil and verapamil decreased the heart rate by about 10% (Fig. 2, B2 and Table 3, B2; $P < 0.05$) pre-ischemically. However, neither drug had this effect in the control hearts (Fig. 2, B1).

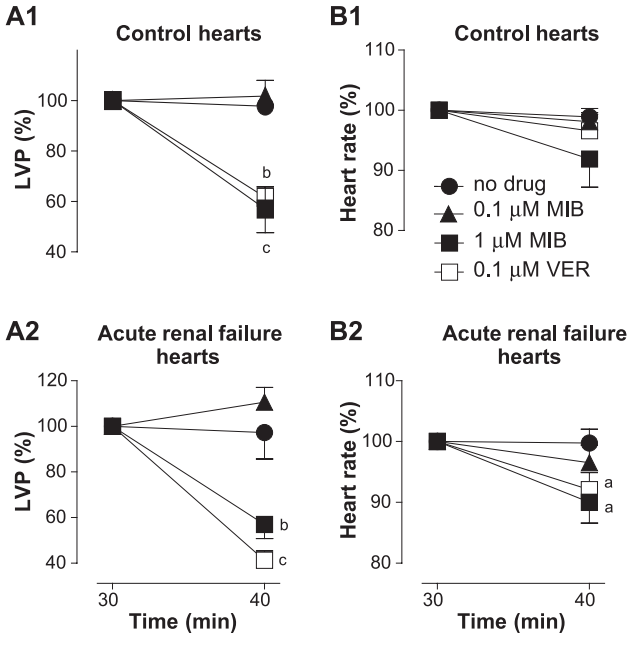

Fig. 2. The effects of mibefradil (MIB) and verapamil (VER) on left ventricular pressure (left side, A) and heart rate (right side, B) during normoxia in the isolated hearts of control guinea pigs and of those with acute renal failure (acute renal failure hearts). LVP—left ventricular pressure. Each dot represents means ± S.E., $n = 5–10$, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ vs. 'no drug' group.

In the hearts of animals with acute renal failure, mibefradil (1 μM only), applied either pre- or post-ischemically, decreased the heart rate during reperfusion (from 120th to 150th min; Table 3, B2 and C2); $P<0.05$–$P<0.01$). However, in the control hearts, verapamil and mibefradil in both concentrations, applied either pre- or post-ischemically, decreased the heart rate during the whole reperfusion period (Table 3, B1 and C1; $P<0.05$–$P<0.001$). The heart rate during reperfusion was significantly lower at 1 μM than at 0.1 μM mibefradil in both groups of hearts, those of animals with acute renal failure and the control ones ($P<0.005$–$P<0.001$; Table 3, B and C).

3.3. Left ventricular pressure

3.3.1. LVP during reperfusion

In 8 out of 10 hearts of animals with acute renal failure, a sustained ventricular fibrillation developed during reperfusion. As a result, the LVP decreased to 0–6% of the pre-ischemic value. In the nonfibrillating hearts of animals with acute renal failure during reperfusion, the LVP recovered to 47–76% and, in the control hearts, to 32–41% of the pre-ischemic value (Table 4, A).

3.3.2. Effects of mibefradil and verapamil on LVP during reperfusion

In the hearts of guinea pigs with acute renal failure, the LVP decreased by about 40–50% ($P<0.01$–$P<0.001$) when mibefradil (1 μM, but not 0.1 μM) or verapamil was applied pre-ischemically (Fig. 2, A2, Table 4, B2). Similar effects were seen in the control hearts (Fig. 2, A1; Table 4, B1).

In the hearts of animals with acute renal failure, 1 μM mibefradil and verapamil, when applied either before or after ischemia, significantly ($P<0.05$–$P<0.001$; Table 4, B2 and C2) decreased LVP in the first 20 min of reperfusion compared with the 'no-drug' group of hearts with acute renal failure. In the pre-ischemic group, the perfusion with 0.1 μM mibefradil did not decrease LVP, but increased it ($P<0.05$) instead, with respect to the 'ischemic no-drug' group in the 30th min of reperfusion (120th min of experiment, Table 4, B2). In the pre-ischemic group of the control hearts, neither mibefradil nor verapamil influenced LVP during reperfusion (Table 4, B1), whereas in the post-ischemic group, mibefradil in both concentrations, but not verapamil, increased LVP ($P<0.05$) during reperfusion if compared with the 'ischemic no-drug' group (Table 4, C1). In both groups of hearts, those of animals with acute renal failure and the control ones, 0.1 μM mibefradil, applied pre- or post-ischemically, restored LVP to significantly higher values than did 1 μM mibefradil ($P<0.05$–$P<0.001$; Table 4, B and C).

3.4. Lactate dehydrogenase release rate

3.4.1. LDH release rate during reperfusion

In the hearts of animals with acute renal failure, the LDH release rate increased almost twofold at the beginning of reperfusion in comparison to the end-equilibration value (in

Table 4
Left ventricular pressure in the isolated control hearts of guinea pigs (Co) and of guinea pigs with acute renal failure (ARF) at the different times of experiments

Group	Left ventricular pressure (%)					
	40th min	95th min	100th min	110th min	120th min	150th min
(A) No drug						
Control + no drug	97.8 ± 1.4	33.0 ± 10.1	32.6 ± 6.5	38.6 ± 15.8	41.2 ± 16.8	39.8 ± 16.3
ARF + no drug	97.3 ± 1.6	98.7 ± 3.1	76.8 ± 27.3	65.5 ± 31.2	46.4 ± 22.8	47.1 ± 34.7
(B) Pre-ischemic						
(B1) Co + 0.1 μM MIB	101.8 ± 6.2[a]	45.1 ± 8.4	50.9 ± 9.9[a]	53.3 ± 10.4[a]	50.2 ± 10.6[a]	38.0 ± 9.0[a]
Co + 1 μM MIB	57.0 ± 8.8[b]	32.1 ± 3.7	26.0 ± 2.5	27.7 ± 1.4	24.9 ± 1.4	14.9 ± 0.9
Co + 0.1 μM VER	61.7 ± 14.0[c]	30.4 ± 2.2	30.3 ± 8.5	40.5 ± 10.0	46.9 ± 10.3	26.1 ± 7.0
(B2) ARF + 0.1 μM MIB	110.6 ± 6.6[a]	63.7 ± 12.4[d]	63.3 ± 6.4[d]	65.3 ± 5.5[e]	56.1 ± 3.6[e,f]	37.3 ± 2.8[d]
ARF + 1 μM MIB	57.0 ± 6.2[c]	28.6 ± 4.1[c]	30.6 ± 2.2[b]	29.6 ± 3.3	21.6 ± 2.5	12.5 ± 2.8
ARF + 0.1 μM VER	41.3 ± 4.1[b]	16.2 ± 1.4[b]	20.4 ± 2.2[b]	25.1 ± 3.6	29.5 ± 4.9	27.4 ± 5.5
(C) Post-ischemic						
(C1) Co + 0.1 μM MIB	97.8 ± 2.1	64.8 ± 11.9[f]	65.6 ± 11.3[f]	66.1 ± 10.8[f]	62.4 ± 10.3	43.3 ± 6.6[a]
Co + 1 μM MIB	98.0 ± 2.2	84.9 ± 20.0	64.0 ± 10.8[f]	51.6 ± 9.5	48.7 ± 9.1	23.3 ± 7.3
Co + 0.1 μM VER	103.3 ± 0.9	33.3 ± 10.3	33.8 ± 18.5	16.3 ± 4.7	18.3 ± 6.8	26.7 ± 1.0
(C2) ARF + 0.1 μM MIB	102.0 ± 2.5	60.8 ± 18.4[a]	67.4 ± 11.9[a]	68.2 ± 6.1[e,f]	78.8 ± 15.9[e,e]	51.9 ± 9.3[e]
ARF + 1 μM MIB	106.4 ± 5.3	32.7 ± 6.0[f]	29.7 ± 1.4[b]	28.9 ± 2.2	20.7 ± 1.9	17.0 ± 3.0
ARF + 0.1 μM VER	111.1 ± 10.2	28.3 ± 34.6	12.9 ± 4.0	16.7	15.7	15.4

Mibefradil (MIB) or verapamil (VER) was added to the perfusion solution either 10 min before ischemia (pre-ischemic group) or at the beginning of reperfusion (post-ischemic group). The values are percentages of the 30th min value (end-equilibration time). Values are means ± S.E., $n=5$–10.

[a] $P<0.05$ vs. 1 μM mibefradil.
[b] $P<0.001$ vs. respective 'no-drug' group.
[c] $P<0.01$ vs. respective 'no-drug' group.
[d] $P<0.01$ vs. 1 μM mibefradil.
[e] $P<0.001$ vs. 1 μM mibefradil.
[f] $P<0.05$ vs. respective 'no-drug' group.

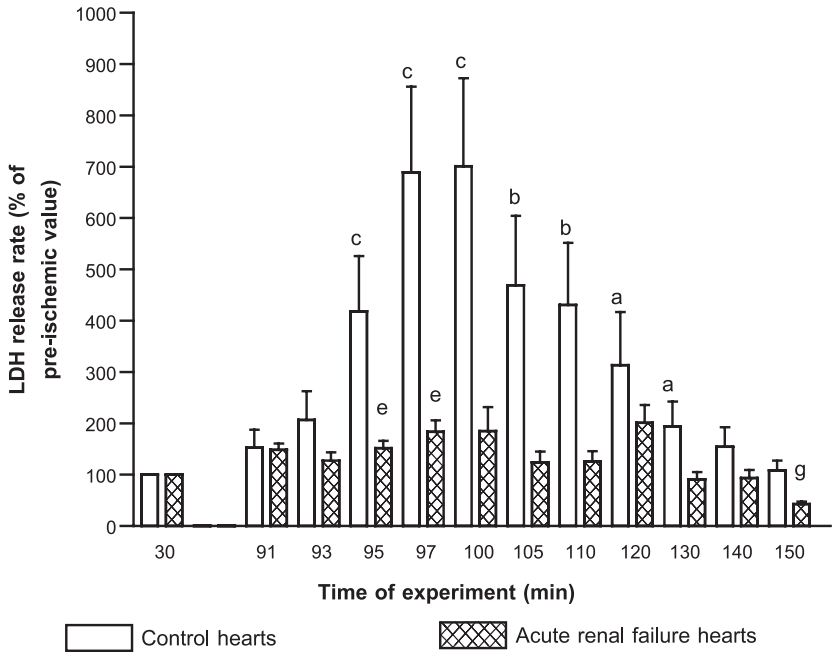

Fig. 3. Lactate dehydrogenase (LDH) release rate (% of the end-equilibration value) during reperfusion time in the isolated hearts of control guinea pigs ($n=8$) and of guinea pigs with acute renal failure ($n=10$). Bars represent means ± S.E.; $^{a}P<0.05$, $^{b}P<0.01$, vs. values in control hearts at 30th min, $^{e}P<0.05$, $^{g}P<0.001$ vs. values in ARF hearts at 30th min.

the 100th min, 6.25 ± 1.37 vs. 3.88 ± 0.61 μkat/g min, respectively; $P<0.05$), but at the end of the experiments (in the 150th min), it halved (1.50 ± 0.30 μkat/g min; $P<0.001$; Fig. 3). However, in the control hearts, the LDH release rate increased fivefold in the 100th min compared to the value at the end of equilibration time (12.73 ± 2.31 vs.

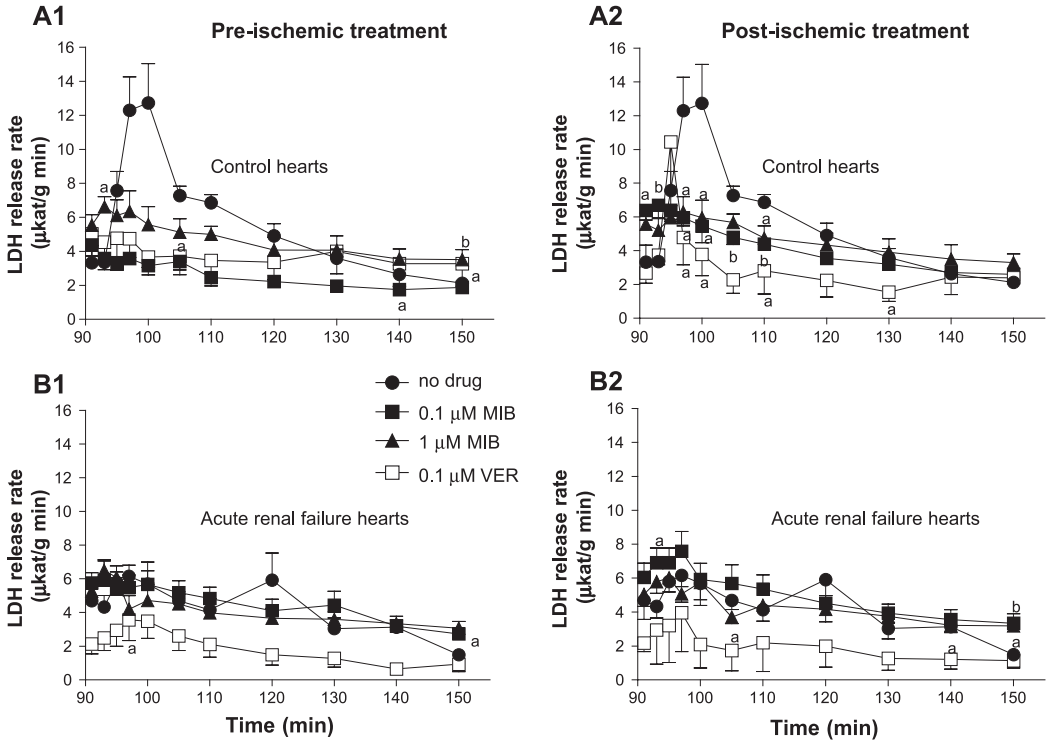

Fig. 4. Lactate dehydrogenase (LDH) release rate during reperfusion in the isolated control hearts of guinea pigs (panel A1 and B1) and in the hearts of animals with acute renal failure (panel A2 and B2). Effects of mibefradil or verapamil added before ischemia till the end of the experiment (pre-ischemic treatment—A1 and A2) or during reperfusion only (post-ischemic treatment—B1 and B2). Values are means ± S.E., $n=5-10$, $^{a}P<0.05$; $^{b}P<0.01$ vs. respective 'no drug' group.

2.54 ± 0.73 μkat/g min, respectively; $P < 0.001$) and remained increased (4.90 ± 1.72 μkat/g min, $P < 0.05 - P < 0.01$) till the 130th min of experiment (Fig. 3).

3.4.2. Effects of mibefradil and verapamil on the LDH release rate during reperfusion

In the hearts of animals with acute renal failure, the pre- and post-ischemic application of verapamil decreased the LDH release rate, whereas the pre-ischemic application of mibefradil (0.1 and 1μM) did not influence it (Fig. 4, B1 and B2). In the hearts of animals with acute renal failure, the post-ischemic application of 1 μM mibefradil even increased the LDH release rate at the beginning and at the end of reperfusion (Fig. 4, B2). However, in the control hearts, the pre- and post-ischemic application of both 1 μM mibefradil or verapamil significantly ($P < 0.05 - P < 0.01$) decreased the maximum LDH release rate in the 100th min of the experiment (from 12.73 ± 2.31 to 5.92 ± 0.95 and 3.48 ± 1.02 μkat/g min, 1 μM mibefradil and verapamil, respectively; Fig. 4, A1 and A2).

4. Discussion

In the present work, we studied the influence of acute renal failure on the extent of ischemic–reperfusion injury. We also examined the possible involvement of T- and L-type Ca^{2+} channels in these processes. For this purpose, we perfused the hearts of guinea pigs having acute renal failure and those of controls with two specific Ca^{2+} channel antagonists, mibefradil (a T- and L-type antagonist) or verapamil (an L-type antagonist), either pre- or post-ischemically. We observed the incidence of reperfusion arrhythmias, the changes in heart contractility and the extent of myocardial cell damage during reperfusion.

We noticed a higher incidence and longer duration of sustained ventricular fibrillation and of contractile failure in the isolated hearts of animals with acute renal failure than in the control hearts during reperfusion. The LDH release rate, which we used to assess the extent of myocardial damage, increased more in the control hearts than in the hearts of animals with acute renal failure. For the experiments with both Ca^{2+} channel antagonists, we used mibefradil in two concentrations: in 0.1 μM concentration, which blocks only T-type Ca^{2+} channels, and in 1 μM concentration, which blocks both T- and L-type Ca^{2+} channels (Abernethy, 1997; Clozel et al., 1997; Hermsmeyer, 1998). These concentrations were near the concentrations used for mibefradil pharmacokinetic and pharmacodynamic population analysis (Welker and Banken, 1998). Verapamil, which blocks only L-type Ca^{2+} channels, was used only in 0.1-μM concentration because in our pilot experiments on guinea pigs, 1 μM verapamil had a strong negative inotropic effect.

In the hearts of animals with acute renal failure, 0.1 and 1 μM mibefradil terminated the sustained ventricular fibrillation, whereas 0.1 μM mibefradil only improved the contractility (Table 2, B2 and C2). Verapamil, however, decreased the LDH release rate in the group of hearts of guinea pigs with acute renal failure during reperfusion (Fig. 4).

4.1. Normoxia

We were injecting gentamicin for 8 days because a longer procedure (12 days, as Yeo et al., 1994 reported for rats) led to death of some guinea pigs. In the guinea pigs that were injected with gentamicin (Table 1), the serum concentrations of urea and of creatinine were respectively three and eight times higher than in the control animals. Robinson et al. (1992) and Raine (1993) reported about four times higher serum concentrations of urea and creatinine in the rats with acute renal failure and with subtotal nephrectomy, respectively. Similarly in rats, serum urea and creatinine increased by 883% and 480%, respectively, as reported by Kumar et al. (2000). As we did not find any laboratory criteria for acute renal failure in guinea pigs in the literature, we accepted the state of acute renal failure at the concentrations of urea and creatinine found in our experiments. Histologically, in the kidneys of gentamicin-treated animals in our experiments, epithelial degeneration and urine cylinders in the proximal tubules, as well as interstitial mononuclear infiltration, were found. The most remarkable finding in all preparations was the proximal tubular epithelial cell degeneration with apoptosis. These findings are consistent with reports of Al-Majed et al. (2002), Cunha and Schor (2000), Kopple et al. (2002) and Ali et al. (2003).

The heart rate and LVP were similar in both the hearts of animals with acute renal failure and the control hearts (Table 1). We expected a lower LVP in the hearts of animals with acute renal failure than in the control ones, as Raine et al. (1993) had reported a diminished inotropic responsiveness of rat hearts to some positive inotropic agents in the glycerol model of acute renal failure. However, more ventricular ectopic beats were observed in some hearts of animals with acute renal failure than in the control hearts during early equilibration (till 20th min, data not yet published). Coronary flow was higher by about 30% in the hearts of animals with acute renal failure than in the control hearts (Grašič Kuhar and Budihna, 2000).

4.2. Ischemia and reperfusion

In the pilot experiments on the isolated hearts of non-treated guinea pigs, we demonstrated a high incidence of reversible ventricular arrhythmias, especially ventricular fibrillation, and an increased LDH release rate if 50-min zero-flow ischemia was performed. We thus chose this experimental model as the basis of our study.

4.3. Arrhythmias

In our experiments, the ventricular fibrillation was on average six times longer in the hearts of animals with acute

renal failure than in the corresponding control hearts (47.6 vs. 7.7 min, $P < 0.05$; Table 2, A1 and A2)). The contractile elements in the hearts of animals with acute renal failure may not have been able to cope with intracellular ionic disturbances at the beginning of reperfusion, which caused sustained ventricular fibrillation. The incidence and duration of ventricular tachycardia were similar in the hearts of animals with acute renal failure and in control hearts.

In the hearts of animals with acute renal failure, both concentrations of mibefradil applied either before or after ischemia terminated the ventricular fibrillation arising during reperfusion (Table 2, B2 and C2). The termination of ventricular fibrillation might have been achieved by blocking T-type Ca^{2+} channels only. T-type Ca^{2+} channels participate in pacemaking function of the heart and are involved in spontaneous depolarisation (Hermsmeyer, 1998). Involvement of L-type channels might have been necessary also, but it would not be enough to terminate ventricular fibrillation during reperfusion. Farkas et al. (1999) demonstrated that, in dogs, 0.6 μM verapamil and mibefradil prevented ventricular fibrillation during ischemia but not during reperfusion. They explained the higher efficacy of both drugs by a prolonged higher extracellular potassium concentration during ischemia, which increased only transitionally during early reperfusion. However, in the hearts of animals with acute renal failure, 0.1 and 1 μM mibefradil decreased the incidence of ventricular tachycardia and terminated the arising ventricular fibrillation. Mibefradil in the concentration of 1 μM, when applied pre-ischemically, decreased ventricular fibrillation. These actions could be specific for the changes associated with acute renal failure, e.g. higher extracellular potassium concentration. In the hearts of animals with acute renal failure, various ions could produce disturbance of the membrane potential or the membrane depolarisation. In contrast to mibefradil, verapamil affected neither the incidence nor the duration of ventricular tachycardia and of ventricular fibrillation.

In the control hearts, neither mibefradil nor verapamil in the concentrations mentioned affected the above parameters during reperfusion.

4.4. Heart rate

Most of the hearts of guinea pigs with acute renal failure fibrillated during reperfusion, and the difference between nonfibrillating hearts of animals with acute renal failure and the control groups was not significant. However, tachycardia was absent in the nonfibrillating hearts of animals with acute renal failure but was observed in the control hearts. In the 'ischemic no drug' hearts of animals with acute renal failure, the heart rate during reperfusion was similar to that before ischemia. The addition of 0.1 μM mibefradil or verapamil to hearts of animals with acute renal failure did not change the heart rate. However, the addition of 1 μM mibefradil decreased the heart rate during late reperfusion period. The timing (pre- or post-ischemic application) did not influence these effects (Table 3).

However, in the control hearts, mibefradil in both concentrations and verapamil decreased heart rate; 1 μM mibefradil was more potent (Table 3, B1). The mechanism of the reduction of heart rate by verapamil might have depended partly on various degrees of atrioventricular block, as Billman and Hamlin (1996) reported for dogs, and partly on the inhibition of sinus node. Billman and Hamlin (1996) also demonstrated that mibefradil shortened the refractory period in dog heart. L-type Ca^{2+} channels participated in action potential-phase 2 (Kerins et al., 2001). These mechanisms were probably also involved in the heart rate-reducing effect of verapamil and mibefradil. Mibefradil (1 μM) was probably more potent because it affected both the L- and T-type Ca^{2+} channels. A sympatholytic effect of mibefradil through N-type Ca^{2+} channels could also be involved (Pfaffendorf et al., 2000). We cannot explain the lack of a heart rate-reducing effect of 0.1 μM mibefradil and of verapamil during reperfusion in the hearts of animals with acute renal failure (Table 3, C2).

4.5. Left ventricular pressure

In our experiments, most of the hearts of animals with acute renal failure fibrillated. In 2 of 10 of the nonfibrillating hearts of animals with acute renal failure, however, LVP during reperfusion decreased as it had in the control hearts (Table 4A). Under normoxic conditions, LVP was similar in the control hearts and in the hearts of animals with acute renal failure (Table 4, A), and LVP in both groups decreased to the same extent after pre-ischemic application of 1 μM mibefradil and 0.1 μM verapamil (Table 4, B1 and B2). Thus, in the hearts of guinea pigs with acute renal failure, the contractile apparatus, when able to cope with intracellular ionic disturbances at the beginning of reperfusion, seemed to be normally sensitive to Ca^{2+} ions. Raine et al. (1993), however, found a decreased cardiac output due to the decreased stroke volume in the rats with chronic renal failure.

In the myocardium of guinea pigs, both T- and L-type Ca^{2+} channels are present (Hoischen et al., 1998). In our experiments, post-ischemically added 0.1 μM mibefradil improved contractility in the hearts of animals with acute renal failure during reperfusion (Table 4, C2). However, during normoxia, 0.1 μM mibefradil did not affect LVP in the hearts of animals with acute renal failure. Mibefradil in the higher concentration (1 μM), given pre- and post-ischemically, decreased LVP in the hearts of animals with acute renal failure in early reperfusion (Table 4, B2 and C2). Farkas et al. (1999) showed that 0.6 μM mibefradil and 0.1 μM verapamil decreased LVP in rat hearts due to an action through the L-type Ca^{2+} channels when the extracellular potassium concentration was increased. In our experiments, mibefradil, given post-ischemically at both concentrations, improved LVP in control hearts during reperfusion, i.e. it decreased stunning, probably by preventing Ca^{2+} influx.

Verapamil in the concentration used did not terminate ventricular fibrillation and, consequently, could not influence LVP in the hearts of animals with acute renal failure. In our experiments, verapamil in the concentration used did not affect LVP in the control hearts either. Our group (Budihna et al., 1995) reported that verapamil at the same concentration as used in the present experiments improved contractility during reperfusion more if given pre- than post-ischemically.

4.6. Lactate dehydrogenase release rate

In the hearts of animals with acute renal failure, the LDH release rate increased during reperfusion. The increase was about fourfold smaller than in the control hearts (Fig. 3). This finding could indicate a lower extent of myocardial cell necrosis in the hearts of animals with acute renal failure. However, during ventricular fibrillation, the LDH release rate was low and increased when ventricular fibrillation stopped. Effective stroke volume enables an effective perfusion of small coronary vessels and a consecutive washout of the enzyme LDH. Since most hearts of animals with acute renal failure fibrillated during all the reperfusion period, an effective LDH washout was probably not possible. In the control hearts, we really found the maximal LDH release rate during a 15-min interval after ventricular fibrillation stopped (Fig. 4). Another reason for the low LDH release rate in the isolated hearts of animals with acute renal failure during reperfusion could be the release of most of the LDH pool from the hearts before the isolated hearts were rendered ischemic. Namely, in the hearts of animals with acute renal failure, the LDH release rate during equilibration time was high: at the 20th min, 6.68 ± 1.09 μkat/g min compared to 1.55 ± 0.25 μkat/g min in control hearts ($P < 0.05$). However, at the end of equilibration time, the LDH release rates in both groups were similar (Table 1).

In the hearts of animals with acute renal failure, verapamil decreased the LDH release rate during reperfusion. On the contrary, mibefradil even increased it at the beginning as well as at the end of reperfusion (Fig. 4B). This action of mibefradil was not expected as both mibefradil and verapamil had decreased the LDH release rate in the control hearts. However, mibefradil terminated ventricular fibrillation; during ventricular fibrillation, LDH could accumulate because of cell damage and, after discontinuation of ventricular fibrillation by verapamil (from 5th to 20th min of reperfusion), a large increase of LDH release rate could be expected. Because of the supposed protective effect of mibefradil, possibly through T-channels, the cell damage was smaller, fibrillation stopped and accumulated LDH was released. Verapamil did not terminate ventricular fibrillation, so the accumulated LDH could not be released. The LDH release rate was therefore lower if compared with that of the 'no-drug' group of the hearts of animals with acute renal failure.

In summary, we found greater ischemic–reperfusion injury (more sustained ventricular fibrillation, decreased LVP and eventually also more extensive cell necrosis) in the hearts of animals with acute renal failure than in the hearts of control guinea pigs. Mibefradil (0.1 and 1 μM) was more effective than verapamil (0.1 μM) to terminate, in the 1-μM concentration even to prevent, ventricular fibrillation, and, in the 0.1-μM concentration, to improve LVP in the hearts of guinea pigs with acute renal failure. Verapamil, however, decreased the post-ischemic LDH release rate in the hearts of guinea pigs with acute renal failure. Our experiments suggest a more important role of T-type than of L-type Ca^{2+} channels in the ischemic–reperfusion injury in the isolated hearts of guinea pigs with acute renal failure.

The calcium channel antagonist, mibefradil, is effective to restore the contractile and heart-beating function of guinea pig hearts suffering from acute renal failure during reperfusion. As the effects were already seen at a concentration of 0.1 μM, we suppose that the action could be mediated at least partly through T-type Ca^{2+} channels. Other actions of mibefradil could also contribute to the results of our experiments.

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