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Ischemia/reperfusion-induced arrhythmias in anaesthetized rats: a role of Na^+ and Ca^{2+} influx

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Abstract

We hypothesized that by limiting the Na^+ and Ca^{2+} loading by a blocker/inhibitor of the Na^+ channel (lidocaine), Na^+ overload (R56865: *N*-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-*N*-methyl-2-benzothiazolamine), Ca^{2+} channel (verapamil), Na^+–H^+ exchange (ethylisobutyl amiloride) or of Na^+–Ca^{2+} exchange (No. 7943: 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulfonate), it should be possible to reduce ischemia/reperfusion-induced arrhythmias. To test this hypothesis, we used anaesthetized rats subjected to 5 min of coronary artery occlusion followed by 10 min of reperfusion to study antiarrhythmic effects of above compounds on reperfusion-induced ventricular premature beats, ventricular tachycardia, and reversible and irreversible ventricular fibrillation. Compound or saline was administered as an intravenous bolus injection at 5 min before ischemia. Pretreatment with lidocaine (5 mg/kg), verapamil (0.63 mg/kg), R56865 (0.63 mg/kg) or ethylisobutyl amiloride (1.25 mg/kg) significantly reduced or abolished all types of ventricular arrhythmias. However, pretreatment with verapamil was associated with second or third degree heart block in 3 out of 12 animals. Pretreatment with No. 7943 did not significantly influence the ischemia/reperfusion-induced ventricular arrhythmias. The present results suggest that both intracellular Na^+- and Ca^{2+}-loading play important roles in reperfusion-induced ventricular arrhythmias and the inhibition of Na^+–Ca^{2+} exchange to limit Ca^{2+} loading probably does not play any important role in ischemia/reperfusion-induced arrhythmias in anaesthetized rats.

Keywords: Reperfusion; Ventricular arrhythmia; Ionic channel; (Rat)

1. Introduction

Reperfusion of myocardium subjected to a transient ischemia rapidly induces ventricular arrhythmias including ventricular premature beats, ventricular tachycardia and ventricular fibrillation in both experimental animals and man (Pogwizd and Corr, 1987; Baxter and Yellon, 1993; Lu et al., 1993b, 1995). Although the exact mechanisms of ischemia/reperfusion-induced arrhythmias are not fully understood, intracellular Na^+- and Ca^{2+}-loading have been implicated to reperfusion arrhythmogenesis (Sweies et al., 1990; Park et al., 1995; Tani et al., 1996). In fact, reperfusion of the ischemic myocardia results in an increase in intracellular Na^+ and Ca^{2+} (Park et al., 1995; Van Emous et al., 1997). Subsequently, the Na^+ and Ca^{2+} overload occurring in myocardial cells appear to be very important etiologic factors in myocardial injuries including arrhythmias during reperfusion sequence (Tani et al., 1996).

Protons (H^+) accumulate in the extracellular space during ischemia, and the rapid washout of extracellular H^+ during reperfusion may create an intracellular to extracellular H^+ gradient, resulting in an influx of Na^+ via the Na^+–H^+ exchanger. Inhibition of the Na^+–H^+ exchange would, therefore, be expected to limit Na^+ overload during reperfusion. Indeed, inhibition of the Na^+–H^+ exchange during ischemia by more or less selective inhibitors—amiloride, and its derivatives such as 5-(*N*-ethyl-*N*-isopropyl) amiloride or cariporide (HOE694)—results in attenuation of the increase in Na^+ and Ca^{2+} in the cardiac tissues (Murphy et al., 1991; Pike et al., 1993; Lai et al., 1994) and provides cardiac protection against myocardial dysfunction, infarct size and reperfusion-induced arrhythmias (Karmazyn, 1988). One of the other pathways for Na^+ influx to induce Na^+ loading during ischemia and reperfusion may be involved in the Na^+ channels. Using a fast Na^+ channel blocker such as lidocaine or a non-inactive Na^+ channel inhibitor, R56865, has been shown to reduce Na^+ influx and protect hearts against myocardial dysfunction (Bergey et al., 1982; Van Emous et al., 1997) or

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arrhythmias (Garner et al., 1990; Lu and De Clerck, 1993; Lu et al., 1993a).

Intracellular Ca^{2+} overloading is another well-recognized feature in myocardial ischemia and reperfusion damage and arrhythmias (Bentfeld et al., 1977; Szekeres et al., 1987). The exact mechanism for Ca^{2+} influx into the myocardial cells remains unsolved; it is, however, generally thought that both Ca^{2+} slow channels and Na^+–Ca^{2+} exchangers play important roles in the Ca^{2+} overload during the myocardial ischemia and reperfusion (Murphy et al., 1991; Lai et al., 1994). Actually, blockade of Ca^{2+} slow channels (l-type) by verapamil shows to reduce reperfusion-induced arrhythmias (Bergey et al., 1984; Sweies et al., 1990). In the pathological conditions such as ischemia/reperfusion, the intracellular Na^+ concentration may rise via the Na^+–Ca^{2+} exchange system, which in turn increases the intracellular Ca^{2+} concentration through the Na^+–Ca^{2+} exchange (Allen et al., 1993; Wantano et al., 1996). This Ca^{2+} increase leads to Ca^{2+} overload which may induce various pathological conditions including arrhythmias and cell death. By using an effective inhibitor of Na^+–Ca^{2+} exchanger, it may, therefore, provide the new therapy for Ca^{2+} overload induced by ischemia/reperfusion. In fact, a heavy metal La^{3+} at certain concentrations has been shown to reduce Na^+- and Ca^{2+}-overloading induced by ischemia and reperfusion and this effect was associated with a reduction of reperfusion-induced ventricular arrhythmias in isolated rat hearts (Maulik et al., 1996). The authors speculated that the protective effect of La^{3+} is most probably by the modulating Na^+–Ca^{2+} exchanger in isolated rat hearts (Maulik et al., 1996), although La^{3+} is also known to block Ca^{2+} channels. Most recently, Wantano et al. (1996) reported that No. 7943, a synthetic compound, is a potent inhibitor for the Na^+–Ca^{2+} exchanger in isolated cardiomyocytes. Using a potent inhibitor of the exchanger makes it possible to study a role of Na^+–Ca^{2+} exchange in reperfusion-induced arrhythmias in vivo.

Although the ventricular arrhythmias resulting from ischemia/reperfusion, have been studied extensively in experimental animal preparations, few comparable studies investigated the possible role of both Na^+- and Ca^{2+}-loading in reperfusion-induced ventricular arrhythmias in vivo. We, therefore, decided to expand the above observations, using anaesthetized rat model, to examine the possible role of Na^+- and Ca^{2+}-channels, H^+–Na^+ exchange and Na^+–Ca^{2+} exchange in ischemia/reperfusion-induced ventricular arrhythmias using their specific inhibitors.

2. Materials and methods

2.1. Animal preparation

This investigation conformed to The Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication no. 85-23, revised 1985). In all experiments, male Wistar rats, weighing approximately 340 g, were used. Animals were anaesthetized with Na^+ pentobarbitone (60 mg/kg, intraperitoneally); after tracheotomy, they were ventilated with room air (stroke volume of 1 ml/100 g, at 55 strokes/min). Body temperature was maintained at 37 ± 0.5°C with an electrical heating pad. The right femoral artery and vein were cannulated for arterial blood pressure monitoring and intravenous (i.v.) access, respectively. Electrodes were attached to the four limbs for surface electrocardiographic (ECG) recordings. Heart rate was then calculated from the R–R interval.

2.2. Experimental protocol

When preparation was completed, 10 min was allowed for equilibration. Rats ($n = 12$ for each group) intravenously received saline or one of following compounds: lidocaine (5 mg/kg; Sigma-Aldrich, Belgium), verapamil (0.63 mg/kg; Sigma-Aldrich), R56865: *N*-[1-[4-(4-fluorophenoxy)butyl]- 4-piperidinyl]- *N*-methyl-2-benzothiazolamine (0.63 mg/kg; Janssen Pharmaceutica, Belgium) (Lu and De Clerck, 1993), 5-(*N*-ethyl-*N*-isopropyl) amiloride (1.25 mg/kg; Hoechst Japan, Tokyo, Japan) (Tani et al., 1996), or No. 7943: 2-[2-[4-(4-nitrobenzyloxy)phenyl]-ethyl]isothiourea methanesulfonate (1.25 mg/kg; Kanebo, Osaka, Japan) (Wantano et al., 1996). At 5 min after the treatment, rats were subjected to 5 min coronary artery occlusion followed by 10 min reperfusion.

Successful coronary artery occlusion was indicated by a decrease in arterial blood pressure and by ECG changes showing ischemic signs (ST-segment elevation). Reperfusion was confirmed by an increase in arterial blood pressure and by ECG changes, i.e., reversal of ST-segment elevation immediately upon release of the ligation. At the end of the experiment, the heart was excised and flushed via the aorta with 3 ml saline; the ligature was retightened, and 3 ml 0.25% Evans blue dye solution was slowly injected for 30 s via the aorta. The dye-free area was taken to reflect the area of the ventricle irrigated by the occluded coronary artery, according to the method by Johnston et al. (1993). The dye-stained area was then carefully cut from the unstained heart; both sections were weighed, in order to calculate the size of the occluded area (expressed as a percentage of the weight of the whole heart).

2.3. Arrhythmia analysis

The ECG and arterial blood pressure were recorded throughout the experiments and at a fast speed throughout ischemia and reperfusion. Ventricular arrhythmias induced during reperfusion were defined and quantified in accordance with the guideline of the Lambeth Conventions for the analysis of experimental arrhythmias (Walker et al., 1988). 25 mm/s ECG recordings were analyzed for the incidence of and the mean time to onset of ventricular

Table 1
Summary of values from anaesthetized rats subjected to 5 min of coronary occlusion followed by 10 min of reperfusion

Group (mg/kg, i.v.)	5 min after compound	End of ischemia	10 min after reperfusion	Occluded size (%)
(a) Heart rate (beats/min)				
Saline control	−2 ± 1	−6 ± 1	−55 ± 14	31 ± 1
Lidocaine (5)	−11 ± 1[a]	−3 ± 1	2 ± 1[a]	32 ± 2
Verapamil (0.63)	−23 ± 3[a]	−28 ± 9[a]	−27 ± 10	34 ± 5
R56865 (0.63)	−24 ± 1[a]	−23 ± 4[a]	−15 ± 2	33 ± 1
Ethylisobutyl amiloride (1.25)	−27 ± 3[a]	−30 ± 4[a]	−25 ± 3	29 ± 2
No. 7943 (1.25)	3 ± 1	4 ± 1	−17 ± 11	32 ± 1
(b) Mean arterial blood pressure (mmHg)				
Saline control	6 ± 2	−37 ± 7	−49 ± 16	–
Lidocaine (5)	−16 ± 3[a]	−26 ± 7	−5 ± 4	–
Verapamil (0.63)	−55 ± 6[a]	−55 ± 7[a]	−38 ± 11	
R56865 (0.63)	−31 ± 3[a]	−39 ± 5	−12 ± 4	–
Ethylisobutyl amiloride (1.25)	−4 ± 3	−17 ± 8[a]	−2 ± 5	–
No. 7943 (1.25)	15 ± 4	−11 ± 6	−10 ± 13	–

[a] $P < 0.05$ vs. the saline control group.
The results are mean ± S.E.M. of 12 experiments.
The values are expressed as a percentage change from its baseline value.

premature beats, ventricular tachycardia (defined as four or more consecutive ventricular premature beats), ventricular fibrillation and number of ventricular premature beats (n/10 min during ischemia and reperfusion). Irreversible ventricular fibrillation, defined as ventricular fibrillation for three continuous minutes, was also determined in each group. Additionally, the mean times to onset (s) of ventricular tachycardia and ventricular fibrillation during ischemia and reperfusion were quantified in each experimental group.

2.4. Statistical analysis

All values are expressed as mean ± standard error of the mean (mean ± S.E.M.) except for the number of ventricular premature beats (mean and range). For evaluation of the differences between the incidence of ventricular premature beats, ventricular tachycardia, ventricular fibrillation and irreversible ventricular fibrillation, Fisher's exact test was used. For analysis of the onset time of ventricular tachycardia and ventricular fibrillation, the number of ventricular premature beats, occluded size, heart rate, mean arterial blood pressure and ECG parameters, Mann–Whitney U test was applied to determine the statistical significance between the groups. A p-value less than 0.05 was used as criterion for statistical significance.

3. Results

3.1. Heart rate and mean arterial blood pressure

The mean baseline values of heart rate and mean arterial blood pressure in anaesthetized rats were not significantly different between the experimental groups (data not

Table 2
Effect of pharmacological interventions on the severity of reperfusion-induced cardiac arrhythmias in anaesthetized rats, subjected to 5 min of coronary artery occlusion

Groups (mg/kg i.v.)	Ventricular premature beats (VPBs)		Ventricular tachycardia (VT)		Ventricular fibrillation (VF)		Survival (n)
	Number	(n/10 min)	Incidence (n)	Onset time (s)	Incidence (n)	Onset time (s)	
Saline control	12/12	545 (31–1327)	12/12	20 ± 5	11/12	31 ± 4	6/12
Lidocaine (5)	5/12[a]	3 (1–4)[a]	1/12[a]	35	0/12[a]	–	12/12[a]
Verapamil (0.63)[b]	0/12[a]	0	0/12[a]	–	0/12	–	12/12[a]
R56865 (0.63)	4/12[a]	1 (0–5)[a]	0/12[a]	–	0/12[a]	–	12/12[a]
Ethylisobutyl amiloride (1.25)	5/12[a]	164 (0–1832)	2/12[a]	31 ± 11	0/12[a]	–	12/12[a]
No. 7943 (1.25)	11/12	369 (0–785)	9/12	15 ± 5	8/12	40 ± 8	10/12

[a] $P < 0.05$ vs. the control group.
[b] Verapamil induced type II/III a–v block in 3 out of 12 animals, and two of these three animals died from cardiac arrest due to the a–v block.
Values are taken as mean ± standard error except for the number of ventricular premature beats (mean and range). If the duration of ventricular fibrillation was over 180 s, it was taken as 180 min to calculate the mean value.

shown). Heart rate varied from 350 to 480 beats/min and mean arterial blood pressure from 70 to 100 mmHg.

At 5 min after the administration of lidocaine (5 mg/kg, i.v.), verapamil (0.63 mg/kg, i.v.), R56865 (0.63 mg/kg, i.v.), or ethylisobutyl amiloride (1.25 mg/kg, i.v.), heart rate and mean arterial blood pressure except for ethylisobutyl amiloride (mean arterial blood pressure: −4% vs. 6% with solvent; $p > 0.05$) were significantly lower than those in the saline control group. In contrast, the administration of No. 7943, a Na^+–Ca^{2+} exchange inhibitor, did not significantly change heart rate and mean arterial blood pressure (Table 1a and b).

At the end of 5 min coronary occlusion, lidocaine did not significantly change heart rate and mean arterial blood pressure when compared to the saline control group, whereas verapamil still significantly lowered heart rate and mean arterial blood pressure. R56865 significantly reduced heart rate, but not mean arterial blood pressure when compared to the saline group. Ethylisobutyl amiloride also significantly lowered heart rate, but it significantly prevented fall in mean arterial blood pressure when compared to the saline group. Furthermore, No. 7943 did not significantly influenced heart rate and mean arterial blood pressure when compared the effect of saline (Table 1a and b).

At the end of 10 min reperfusion, lidocaine significantly prevented fall in heart rate (2% from its baseline vs. −55% with saline; $p < 0.05$); and it also prevented fall in mean arterial blood pressure (−5% from its baseline value vs. −49% with solvent; $p > 0.05$). Verapamil, R56865, ethylisobutyl amiloride and No. 7943 partly prevented fall in heart rate and mean arterial blood pressure, but these effects were not statistically different from those with saline (Table 1a and b).

3.2. Effects of pharmacological interventions ischemia/reperfusion-induced ventricular arrhythmias

During reperfusion after 5 min coronary artery occlusion, almost all rats pretreated with saline developed ventricular beats, ventricular tachycardia, reversible ventricular fibrillation and 50% rats developed irreversible ventricular fibrillation (Table 2). Pretreatment with lidocaine (5 mg/kg, i.v.), a Na^+ channel blocker, dramatically reduced the incidence of ventricular premature beats from 100% in the saline group to 42% ($p < 0.05$), decreased the number of ventricular premature beats (3 vs. 545 in the saline group; $p < 0.05$) and the incidence of ventricular tachycardia from 100% to 8% ($p < 0.01$), and abolished the reversible ventricular fibrillation and irreversible ventricular fibrillation ($p < 0.001$ vs. saline control group; Table 2). Pretreatment with verapamil (0.63), a L-type Ca^{2+} channel blocker, abolished all ischemia/reperfusion-induced ventricular arrhythmias. However, verapamil at a dose of 0.63 mg/kg, i.v., induced type II and III atria–ventricular (a–v) block in 3 out of 12 animals and two out of these three animals died during occlusion because of the cardiac arrest. R56865 (0.63 mg/kg, i.v.), a Na^+ overload inhibitor, also significantly reduced the incidence of ventricular premature beats to 33% and abolished the incidence of ventricular tachycardia and ventricular fibrillation (Table 2). Furthermore, ethylisobutyl amiloride (1.25 mg/kg, i.v.) also significantly reduced the incidence of ventricular premature beats to 42%, ventricular tachycardia to 17% and abolished the incidence of both reversible ventricular fibrillation and irreversible ventricular fibrillation. However, pretreatment with No. 7943 (1.25 mg/kg, i.v.), a Na^+–Ca^{2+} exchange inhibitor, did not significantly reduce all types of ischemia/reperfusion-induced ventricular arrhythmias (Table 2 and Fig. 1). None of the animals pretreated with saline or compound except for verapamil developed a–v block.

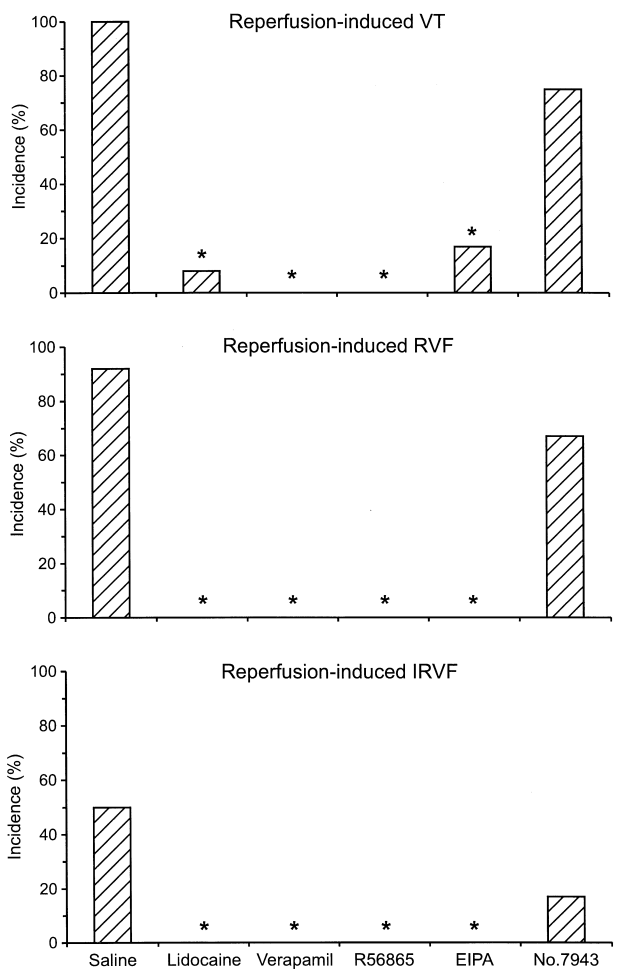

Fig. 1. Effects of pharmacological interventions on reperfusion-induced ventricular tachycardia, and reversible and irreversible ventricular fibrillation after 5 min coronary artery occlusion in anaesthetized rats. Saline, lidocaine (5 mg/kg), verapamil (0.63 mg/kg), R56865 (0.63 mg/kg), ethylisobutyl amiloride (EIPA: 1.25 mg/kg) or No. 7943 (1.25 mg/kg) was administered 5 min before ischemia. $* P < 0.05$ as compared with the saline group.

3.3. Size of occluded zone

The size of the ischemic zone was not significantly different within groups; it varied between 24% and 38% of the whole heart weight (Table 1a and b).

4. Discussion

Our results of this study demonstrate that blockade of Na^+ channels, Ca^{2+} channel or inhibiting H^+–Na^+ exchange reduces ischemia/reperfusion-induced ventricular arrhythmias. These effects indicate that both Na^+- and Ca^{2+}-loading play important roles in ischemia/reperfusion-induced ventricular arrhythmias in anaesthetized rats. Our results further indicate that inhibition of Na^+–Ca^{2+} exchange with No. 7943, a selective inhibitor for the exchange (Wantano et al., 1996), does not play a central role in ischemia/reperfusion-induced ventricular arrhythmias in rats.

Although the exact mechanisms of ischemia/reperfusion-induced arrhythmias are not fully understood, intracellular Na^+- and Ca^{2+}-loading have been implicated to reperfusion arrhythmogenesis (Sweies et al., 1990; Park et al., 1995; Tani et al., 1996). The Ca^{2+} overload is also known to be caused by a preceding intracellular Na^+ accumulation during ischemia and reperfusion (Park et al., 1995; Tani et al., 1996). It may be the final common pathways leading to reperfusion arrhythmias that are related to delayed afterdepolarization (Ferrier et al., 1985). Several pathways for Na^+ and Ca^{2+} influx have been proposed: (1) the tetrodotoxin-sensitive fast Na^+ channel, (2) the slow Ca^{2+} channel, (3) the non-inactivating Na^+ current which is evoked by veratridine, (4) the H^+–Na^+ exchange, and (5) the Na^+ and Ca^{2+} exchange. Therefore, blockade of one of these pathways would be expected to protect against ischemia/reperfusion-induced cardiac arrhythmias.

The role of the fast Na^+ channel in the accumulation of Na^+ loading during ischemia and reperfusion and in myocardial ischemic/reperfusion damage is well-evidenced in several studies (Manning and Hearse, 1984; Kojima and Miura, 1991; Butwell et al., 1993; Van Emous et al., 1997). In particular, Van Emous et al. (1997) recently reported that blocking fast Na^+ channel with lidocaine limits Na^+ influx during ischemia and reduces Na^+ content during reperfusion and improves post-ischemic functional and metabolic recovery in isolated rat hearts. As shown in the present study, lidocaine is very effective against reperfusion-induced ventricular arrhythmias in anaesthetized rats. The protective effect may be achieved by directly preventing the cellular Na^+ overload during ischemia/reperfusion via blocking the fast Na^+ channel. Clinical data and experimental data have also shown that blockade of the fast Na^+ channel is beneficial for the treatment of digitalis toxication (Lu et al., 1993a). This condition of Na^+ overload induced by ouabain is thought to be similar to that by ischemia/reperfusion (Bentfeld et al., 1977; Szekeres et al., 1987; Garner et al., 1990).

The use of non-inactivating Na^+ current blocker R56865 is reported to be effective against Na^+- and Ca^{2+}-overload under various conditions (Garner et al., 1990; Ver Donck and Borgers, 1991; Lu et al., 1993a). Myocardial ischmemia and reperfusion is thought to cause long-lasting bursts of opening and prolong the inactivation process of this Na^+ channel, which results in Na^+- and Ca^{2+}-overload. Garner et al. (1990) reported that R56865 has very potent effect against reperfusion-induced ventricular arrhythmias in isolated rat hearts. The present results with R56865 confirm the protective effects against ischemia/reperfusion-induced arrhythmias in rats in vivo.

The slow Ca^{2+} channel may represent an important pathway of Ca^{2+} loading during myocardial ischemia and reperfusion, although the results of using its antagonists to prevent reperfusion-induced arrhythmias are contradictory (see review, Manning and Hearse, 1984). Anyway, blockade of the slow Ca^{2+} channel has been shown to be effective against reperfusion-induced ventricular arrhythmias in rats (Bergey et al., 1984; Sweies et al., 1990; Baxter and Yellon, 1993) and ouabain-induced arrhythmias in guinea pigs (Lu et al., 1993a). This is in agreement with our present data obtained with verapamil in rats. However, the use of verapamil (0.63 mg/kg, i.v.) is complicated by incidence of a–v block, that has been also observed in other studies (Goldberger and Aronson, 1992; Lu and De Clerck, 1993). Nevertheless, the effect obtained with verapamil suggests that Ca^{2+} overload via the slow Ca^{2+} channel could play an important role in the ischemia/reperfusion-induced arrhythmias in rats.

The intracellular acidosis produced by the accumulation of H^+ during ischemia is thought to be restored during reperfusion by washout of metabolites, influx of bicarbonate, and H^+–Na^+ exchange (Vandenberg et al., 1993). This may create an intracellular to extracellular H^+ gradient, resulting in an influx of Na^+ via the Na^+–H^+ exchange. Such an influx of Na^+ could result in an increase in intracellular Na^+, which in turn would favor an increase in intracellular Ca^{2+} via Na^+–Ca^{2+} exchange (Wantano et al., 1996). The hypothesis about the role of Na^+–H^+ exchange in ischemia/reperfusion injury including arrhythmias is well-supported by the observations that inhibitors of Na^+–H^+ exchange such as ethylisobutyl amiloride or cariporide can limit the accumulation of Ca^{2+} and reduce reperfusion-induced arrhythmias (Duff et al., 1989; Scholz et al., 1995; Xue et al., 1996; Aye et al., 1997). In the present study, ethylisobutyl amiloride (1.25 mg/kg, i.v.) potently reduced reperfusion-induced incidence of ventricular premature beats and tachycardia, and prevented ventricular fibrillation in anaesthetized rats with ligation of the left coronary artery. The results are comparable to other results with HOE494 and with amiloride or ethylisobutyl amiloride in isolated rat hearts and in anaesthetized rats (Scholz et al., 1995; Xue et al., 1996).

Surprisingly, we did not find that the Na^+–Ca^{2+} exchange plays an important role in ischemia/reperfusion-induced arrhythmias in the present study by using its potent antagonist: No. 7943. No. 7943 has been shown to block the Na^+–Ca^{2+} exchange with IC_{50} value of 0.32 μM in isolated guinea-pig ventricular cells (Wantano et al., 1996). However, to our knowledge, the data of its activity in vivo is lacking. The reason for the negative finding of the compound at 1.25 mg/kg, i.v., is unknown in the present study; even when the dose of the compound was up to 5 mg/kg, i.v., it failed to reduce ischemia/reperfusion-induced ventricular arrhythmias in rats (our preliminary date; not shown here). A heavy metal La^{3+} is known to block the Na^+–Ca^{2+} exchange (Kimura et al., 1987) and reduce reperfusion-induced arrhythmias (Maulik et al., 1996). However, La^{3+} is not specific because it also blocks Ca^{2+} channel. Furthermore, the protective effects of La^{3+} against reperfusion-induced arrhythmias in isolated rat hearts are not very potent: it moderately reduces reperfusion-induced ventricular tachycardia and fibrillation only at concentrations of 0.05 and 0.1 mg/l, but not at 1 and 5 mg/l (Maulik et al., 1996). It has also been reported that the roles of Na^+–Ca^{2+} exchange vary in a species-dependent way (Bers et al., 1996). Moreover, enhanced Na^+–Ca^{2+} exchange has only been found in chronic heart diseases (Menick et al., 1996; Litwin and Bridge, 1997), but not in acute myocardial ischemia (Bersohn et al., 1982). Anyway, our findings could suggest that blockade of Na^+–Ca^{2+} exchange may not be beneficial for ischemia/reperfusion-induced arrhythmias at least in anaesthetized rats.

It is well-known that digitalis toxicity mimics ischemia/reperfusion condition both in vitro and in vivo. The rise of Na^+ (i) by the inhibiting Na^+–K^+ pump with digitalis, especially in a condition of digitalis toxicity, causes cardiac arrhythmias as seen during ischemia/reperfusion (Levi et al., 1997). In the present study, we have not investigated a possible role of the Na^+–K^+ pump in ischemia/reperfusion-induced arrhythmias in rats. Additional experiments are needed, if we wish to determine further that ischemia/reperfusion may inhibit the Na^+–K^+ pump, thereby resulting in Na^+ overload in myocardium during ischemia/reperfusion, and inducing ischemia/reperfusion arrhythmias in rats.

In the present study, we found that protective effects afforded by lidocaine, verapamil, R56865 or ethylisobutyl amiloride appear to be associated with significant reduction in heart rate or arterial blood pressure. Furthermore, we pretreated rats before ischemia not prior to reperfusion. The results, therefore, do not make it possible to elucidate the role of heart rate and blood pressure in reperfusion-induced arrhythmias nor rule out the possibility of compounds that they may directly modify the 'severity' of ischemia. Additional experiments are needed, if it is essential to prove the important role of these compounds in changing heart rate and arterial blood pressure or in their effects during ischemia in reperfusion-induced arrhythmias in rats.

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