Inhibitory Effects of Pre-ischemic and Post-ischemic Treatment With FR 168888, a Na\(^+\)/H\(^+\) Exchange Inhibitor, on Reperfusion-Induced Ventricular Arrhythmias in Anesthetized Rat

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ABSTRACT—Effects of pre-ischemic and post-ischemic treatment with FR 168888 (5-hydroxymethyl-3-(pyrrol-l-yl) benzoylguanidine methanesulfonate), a Na\(^+\)/H\(^+\) exchange inhibitor, on reperfusion-induced ventricular arrhythmias were examined in an ischemia/reperfusion model of anesthetized rat. FR 168888 (0.3 mg/kg) significantly reduced the incidence of ventricular fibrillation (VF) and mortality induced by reperfusion following 5-min coronary occlusion, when it was intravenously administered 5 min before coronary artery occlusion. Post-ischemic treatment with FR 168888 (0.3 – 10 mg/kg), i.e. given 3 min after the start of occlusion, reduced the incidence of VF and mortality. In order to examine the optimal time of administration, FR 168888 (3 mg/kg) was administered 1 or 3 min after the start of occlusion or immediately before reperfusion. There was no significant difference in the reduction of VF and mortality among the three post-ischemic treatment groups. FR 168888 (3 and 10 mg/kg) significantly increased the blood pressure during ischemia without affecting the heart rate. These results indicate that FR 168888 has antiarrhythmic effects on reperfusion-induced arrhythmias even administered after coronary occlusion.

Keywords: Na\(^+\)/H\(^+\) exchange, Arrhythmia, Ischemia, Reperfusion, FR 168888

Recent studies have suggested that Na\(^+\)/H\(^+\) exchange (NHE) is involved in myocardial ischemia/reperfusion injury (1 – 5). Intracellular Ca\(^{2+}\) overload resulting from intracellular Na\(^+\) overload, which is induced by activated NHE during ischemia and especially soon after reperfusion, leads to myocardial cell death, arrhythmias and stunning.

Although there are no clinically successful drugs for treatment against myocardial ischemia/reperfusion injury, many experimental studies have shown that inhibition of the NHE can protect against injuries induced in in vitro and in vivo hearts (6 – 10). FR 168888 (FR, 5-hydroxymethyl-3-(pyrrol-l-yl) benzoylguanidine methanesulfonate) is a new NHE inhibitor that significantly inhibits cell swelling induced by activated NHE in rat thymic lymphocytes (11). Ohara et al. reported that FR was more potent than amiloride in inhibiting NHE in rat lymphocytes, and FR had a weak inhibitory effect on Na\(^+\)/Ca\(^{2+}\) exchange of rat cardiac sarcolemmal vesicles, but the Na\(^+\)/Ca\(^{2+}\) exchange inhibition may not contribute to the cardio-protective mechanism for ischemia/reperfusion injury (12). They also showed that when FR was administered before coronary occlusion, it displayed a protective effect on arrhythmias induced by ischemia/reperfusion in rats. In most clinical situations, cardioprotective agents can only be administered immediately before or shortly after reperfusion, rather than before the occurrence of coronary occlusion. The present study was designed to examine the antiarrhythmic effects of FR when it was administered before or after occlusion, simultaneously to show the dose-related effects and the optimal time of administration in post-ischemic application.

MATERIALS AND METHODS

Induction of coronary ischemia/reperfusion injury in rats

Male Sprague-Dawley rats (body weight 250 to 400 g) were purchased from the Animal Laboratory for Research of Yamanashi Medical University. All experiments were carried out under the Guideline for Animal Experiments of Yamanashi Medical University. As reported earlier (13), under anesthesia with pentobarbital sodium (60 mg/kg,
intraperitoneally), the femoral vein was cannulated to allow drug administration, and the trachea was cannulated for artificial ventilation. The systemic blood pressure was monitored via a catheter inserted into the carotid artery and a standard limb lead I electrocardiogram (ECG) was continuously monitored on a recorder (RM-62001; Nihon Kohden, Tokyo). The chest was opened by left thoracotomy at approximately 2 mm to the left of the sternum, followed by sectioning the 4th and 5th ribs. Artificial ventilation was immediately started using room air (volume 1.5 ml/100 g, rate 54 strokes/min) to maintain PCO$_2$, PO$_2$ and pH within the normal range. After incising the pericardium, the heart was exteriorized by applying gentle pressure on the rib cage, and a 6/0 braided silk suture (attached to a 10 mm micropoint reverse cutting needle) was placed around the left coronary artery. The heart was placed back into the chest and the animal was allowed to stabilize.

Transient 5 min regional myocardial ischemia was induced by passing the threads through a small plastic tube and pressing the tube against the epicardium, and reperfusion was initiated by releasing the ligature and removing the plastic tube. According to a study on the time-course appearance of ischemia-induced arrhythmias (14) and also to ours (10), there was no severe arrhythmia during the 5 min of coronary occlusion. Thus, the inhibitory effects of FR on ischemia-induced arrhythmias were not evaluated in this study. After reperfusion, the responses were observed for 10 min. Ischemia and reperfusion were confirmed as described previously (15). In short, successful occlusion was confirmed by the increase of the height of the R wave during the first few seconds of each occlusion (16) and a 20–30% reduction in the arterial blood pressure compared to the pre-ischemic values. Successful reperfusion was confirmed by the return of the arterial blood pressure to the pre-ischemic values.

**Definition of arrhythmias and analysis**

Definitions of arrhythmias were based on the description of the Lambeth Conventions (17). Ectopic ventricular activity was categorized as a single premature ventricular contraction (PVC), ventricular tachycardia (VT, 4 or more consecutive PVC) or ventricular fibrillation (VF, inability to distinguish individual QRS complexes and to measure the rate). Complex forms (e.g., bigeminy) were included in the count of PVC and were not analyzed separately. Reference was made to the blood pressure tracings to confirm which type of ectopic activity was occurring, particularly to distinguish between the polymorphic VT and VF. When the former occurred, the blood pressure was usually still pulsatile, whereas with VF the blood pressure fell rapidly towards zero and was no longer pulsatile. VF may be sustained or may revert spontaneously to a normal sinus rhythm in the rat (14). In all experiments, the incidence of VT, VF and mortality (due to terminal VF sustained for 3 min or more) was noted.

**Exclusion criteria**

Experiments were terminated or excluded from the final data analysis, if any of the following occurred (13, 18): arrhythmias prior to coronary artery occlusion; mean arterial pressure less than 60 mmHg prior to drug or vehicle administration and atrioventricular block during the first 5 min of ischemia (probably caused by ligature occluding the septal branch of the left coronary artery). Eighty-nine rats were used in this study. Two of them were excluded for absence of signs of ischemia, one for low blood pressure before occlusion and one for atrioventricular block during the 5 min of ischemia.

**Experimental protocols**

The present study consisted of 3 protocols (Fig. 1). In protocol I (pre-ischemic treatment, n = 10 each), FR 0.3 mg...
/kg or saline was administered 5 min before the coronary artery occlusion followed by 10 min of reperfusion. In protocol II, FR at 0.3, 1, 3 or 10 mg/kg was administered 3 min after the onset of occlusion (n=10 each). In protocol III, FR at 3 mg/kg was infused 1 min or 3 min after occlusion, or immediately before reperfusion, termed, respectively, 1-min post-ischemic treatment group, 3-min post-ischemic treatment group or immediately before reperfusion treatment group (n=10 each). The volume of injection was adjusted to 0.7 ml with saline. The ECG and blood pressure were continuously recorded throughout the experiment.

Drugs
FR was kindly supplied by Fujisawa Pharmaceutical Co., Ltd. (Osaka). Pentobarbital sodium was purchased from Tokyo Kasei Kogyo (Tokyo). FR was dissolved in saline on the day of the experiments.

Statistics
Statistical analysis was based upon the guidelines for statistics (19) with modification for the study of arrhythmias using rat hearts (17, 20). All data are expressed as means ± S.E.M. Student’s t-test and one-way ANOVA (analysis of variance) followed by Dunnett’s multiple comparison test were used to test drug effects on hemodynamic parameters. Differences in the incidence of arrhythmias among groups were analyzed by Fisher’s exact probability test.

RESULTS

Inhibitory effects of FR on reperfusion-induced arrhythmias in the pre-ischemic treatment group
In the saline control group, the incidence of reperfusion-induced VT, VF and mortality was high (VT, 100%; VF, 100%; and mortality, 90%, Fig. 2). FR at 0.3 mg/kg significantly decreased the VF incidence to 10% and mortality to 0% (P<0.01), whereas there was no significant reduction in the VT incidence (80%).

Inhibitory effects of FR on reperfusion-induced arrhythmias in the 3-min post-ischemic treatment group
Administration of FR at 0.3 – 10 mg/kg at 3 min after occlusion caused reduction in the incidence of VF and mortality (Fig. 3). The groups of 0.3 and 1 mg/kg had the same degree of reduction in the incidence of VF (both to 70%) and mortality (both to 50%), while the 3 and 10 mg/kg groups showed stronger inhibitory effects on the incidence of VF and mortality than the lower dose groups (to 60% in VF and to 30% in mortality, P<0.05). The incidence of VT was not significantly reduced in all treated rats.

The changes of hemodynamics when FR was administered 5 min before occlusion
When FR at 0.3 mg/kg was administered 5 min before occlusion, the mean blood pressure (mBP) of the rats increased transiently, and 4 min later, it returned to the pre-administration value (Fig. 4A). No change of the heart rate was observed in the pre-ischemic treatment (Fig. 4B).

The changes of hemodynamics when FR was administered after occlusion
FR at 3 or 10 mg/kg increased the mBP when given 3 min after occlusion. After 1 min of administration, the mBP increased by 11 and 10 mmHg, respectively (P<0.01 vs predrug values, Fig. 5A), and after 2 min of administration, the mBP increased by 9 and 12 mmHg (P<0.05 and 0.01 vs predrug values, respectively), whereas in the saline group, mBP decreased during occlusion. The administration of FR at 3 or 10 mg/kg had no significant effects on the heart rate (Fig. 5B).

The time-dependent effect of FR on reperfusion-induced arrhythmias in the post-ischemic treatment groups
The administration of FR at 3 mg/kg at 1 min, 3 min after occlusion or with-reperfusion showed the same degree of inhibition in mortality (to 30%, P<0.05, Fig. 6). VF incidence was reduced to 60% in the 1-min and 3-min post-ischemic treatment groups and to 30% in the with-reperfusion group (P<0.05).
Fig. 3. Prevention of reperfusion-induced ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality in rat hearts by post-ischemic treatment with FR at 0.3, 1, 3, 10 mg/kg (n = 10 each) intravenous administered 3 min after coronary occlusion. Numbers above bars give incidence of arrhythmias or mortality. *P<0.05 vs control group.

Fig. 4. Effect of the pre-ischemic treatment of FR on mean blood pressure (A) and heart rate (B) in the anesthetized rat. Saline (n = 8) or FR (n = 10) was administered by intravenous bolus injection 5 min before occlusion. Values are the means ± S.E.M. 0 min: before administration of drug or saline. *P<0.05, vs predrug value.

Fig. 5. Effect of FR on mean blood pressure (A) and heart rate (B) during ischemia in the anesthetized rat. Saline (n = 5) or FR (3, 10 mg/kg; n = 10 each) was administered by intravenous bolus injection 3 min after occlusion. Values are the means ± S.E.M. 0 min: before occlusion, 3 min: drug or saline was administered, 5 min: the onset of reperfusion. *P<0.05, **P<0.01 vs predrug value (3 min).
DISCUSSION

This study demonstrated that FR, a NHE inhibitor, markedly suppressed reperfusion-induced arrhythmias when it was given not only before but also after occlusion in anesthetized rats. In the pre-ischemic treatment group, FR significantly reduced the incidence of VF and mortality at a dose of 0.3 mg/kg. In the post-ischemic treatment groups and with-reperfusion group, it was shown that post-ischemic application could also reduce the incidence of VF and mortality, although higher doses were necessary for a significant inhibition.

The NHE mechanism has been recognized to contribute to both ischemic and reperfusion injuries. Reperfusion was reported to cause reactivation of NHE exchange when rapid removal of acidic extracellular fluid occurred to extrude H\(^+\) from cells for exchange of Na\(^+\) (21, 22). Accumulated intracellular Na\(^+\) leads to an increase of Ca\(^{2+}\) influx by a Na\(^+\)/Ca\(^{2+}\) exchange mechanism, resulting in intracellular Ca\(^{2+}\) overload which is thought to enhance myocardial damage and cause the reperfusion arrhythmias (6, 23, 24). Extensive studies about NHE inhibitors, including our previous studies, have shown that NHE inhibition can decrease the incidence of VT, VF and mortality, as well as limit the extent of infarction when these compounds were administered before the onset of occlusion in anesthetized dogs and rats (9, 10, 12, 25).

FR has been reported to be a potential NHE inhibitor and have inhibitory effects on reperfusion-induced arrhythmias, when it was administered before occlusion (12). In the present study, we demonstrated that FR has a powerful antiarrhythmic effect in anesthetized rats not only in the pre-ischemic treatment group at a dose of 0.3 mg/kg, but also in the post-ischemic treatment group in which FR was administered during ischemia, although a 10 times higher dose was necessary for significant decrease in the incidence of VF and mortality compared with the pre-ischemic treatment group. In the groups of 1 min, 3 min post-ischemic treatment or with-reperfusion treatment, FR 3 mg/kg had the same inhibitory effects on the mortality (to 30%, P<0.05), although a significant reduction of VF incidence was observed only in the with-reperfusion group (to 30%, P<0.05) (Fig. 6). It has been known that there are no functional coronary collaterals in rats (26), and thus the inhibitor that was administered after coronary artery occlusion might not reach the ischemic myocardium until the onset of reperfusion. Therefore, higher doses might be necessary in the post-ischemic application, compared with the administration before occlusion. The tissue concentrations of the inhibitor that is administered immediately before reperfusion might be higher in the ischemic myocardium than the concentration attained by the drug administered during early ischemia because of the decrease of the plasma concentration by time and the lack of functional coronary collaterals in rats. This may be reflected in a stronger inhibitory effect of the with-reperfusion group on the incidence of VF than of the post-ischemic treatment groups. A clinical study (27) also showed that cariporide, a NHE inhibitor, attenuated reperfusion injury in patients with acute anterior myocardial infarction undergoing direct percutaneous trans-
luminal angioplasty (PTCA), in which a high dose of cariporide (40 mg) was infused 10 min before reperfusion. Previous animal studies also demonstrated that the post-ischemic application of NHE inhibitors reduced myocardial cell death after ischemia and reperfusion and limited infarct size (10, 28, 29).

It is assumed that activation of NHE occurs during both ischemia and reperfusion. During the ischemic period, intracellular acidosis must activate NHE and leads to intracellular Na\(^+\) and Ca\(^{2+}\) overload (2, 30). Then at reperfusion, NHE is further activated by rapid removal of acidic extracellular fluid, resulting in an abrupt increase in transmembrane H\(^+\) gradient (21). In this rat model of 5-min ischemia followed by 10-min reperfusion, FR applied before ischemia might have reached the ischemic area and thus inhibit NHE more effectively than FR applied only at reperfusion. Thus the application of FR before the onset of occlusion might strongly protect against the reperfusion-induced arrhythmias.

It has been suggested that pre-ischemic application of some NHE inhibitors, including FR, has no effect on the mBP and HR (7, 9, 10, 12). Gumina et al. (31) also suggested that BIIB 513, a NHE inhibitor, when it was administered (3 mg/kg) during ischemia, did not change the hemodynamics of dogs during ischemia. The present study showed that both pre- and post-ischemic treatment with FR increased the mBP in the first several minutes after administration without changing the heart rate. Especially in the post-ischemic treatment, the decrease in the mBP of rats during ischemia was attenuated after administration of 3 or 10 mg/kg of FR in the 3-min post-ischemic treatment group. A similar effect was not observed in the saline group excluding the influence of injection volume. The explanation for this pressor effect is yet to be determined. However, it is a unique effect of FR that might be useful for improving the blood perfusion of the ischemic myocardium and has not been reported for other NHE inhibitors (7, 9, 10, 12 and 31).

The effects of FR on ion channels have not been reported, but since we did not observe any change of the parameters of the electrocardiogram, even when the highest dose of 10 mg/kg was administered (data not shown), there may be no significant ion channel effect. In addition, Ohara et al. reported that FR showed a weak inhibitory effect on Na\(^+\)/Ca\(^{2+}\) exchange in rat cardiac sarcolemmal vesicles (12), but they speculated that this effect may be of no importance in protective effects. Thus, as many other selective NHE inhibitors, the antiarrhythmic effects of FR might be due to its selective NHE exchange inhibitory effect.

In conclusion, the NHE inhibitor FR significantly reduced the incidence of VF and mortality that were induced by reperfusion in anesthetized rats, even given after coronary artery occlusion. FR also showed a transient pressor effect. These results suggest that FR can be used to prevent lethal ventricular arrhythmias under some clinical conditions such as thrombolytic therapy, PTCA, or bypass surgery, all of which are effective management of acute coronary syndromes.

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