Effects of Aprindine on Ischemia/Reperfusion-Induced Cardiac Contractile Dysfunction of Perfused Rat Heart

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ABSTRACT—The present study was undertaken to determine whether aprindine, a class Ib antiarrhythmic agent, exerts beneficial effects on ischemia/reperfusion-induced cardiac contractile dysfunction and metabolic derangement. Isolated rat hearts were subjected to 35-min global ischemia, followed by 60-min reperfusion, and functional and metabolic alterations of the heart were determined with or without aprindine-treatment. Ischemia induced a cessation of left ventricular developed pressure (LVDP), a rise in left ventricular end-diastolic pressure (LVEDP), and an increase in myocardial sodium content and a decrease in myocardial potassium content. When the hearts were reperfused, little recovery of LVDP and sustained rise in LVEDP and perfusion pressure were observed. Ischemia/reperfusion resulted in a release of ATP metabolites and creatine kinase from perfused hearts, an increase in myocardial sodium and calcium contents, and a decrease in myocardial potassium and magnesium contents. Treatment of the perfused heart with either 10 or 30 μM aprindine for the last 3 min of pre-ischemia improved contractile recovery during reperfusion and suppressed changes in myocardial ion content during ischemia and reperfusion. Treatment with the agent also attenuated the release of ATP metabolites and creatine kinase from the heart. However, treatment with high concentrations of aprindine (70 and 100 μM) improved neither cardiac contractile dysfunction, myocardial ionic disturbance nor the release of ATP metabolites and creatine kinase during reperfusion. Two possible mechanisms for the cardioprotection by the agent have been suggested: suppression of transmembrane flux of substrates and enzymes, and prevention of accumulation of myocardial sodium during ischemia.

Keywords: Aprindine, Calcium overload, Antiarrhythmic agent (class Ib), Ischemia/reperfusion, Sodium overload

Aprindine is classified as a class Ib antiarrhythmic agent, a sodium-channel blocker, according to classification of Vaughan Williams (1). Aprindine blocks the sodium current in guinea pig ventricular myocytes and rabbit atrioventricular node (2, 3). The agent can suppress ouabain-induced ventricular fibrillation (4) and lower the mortality of high-risk patients within a month after myocardial infarction (5). The agent is also effective in the therapy of chronic ventricular dysrhythmias such as supraventricular arrhythmias (6). Since the plasma level of this agent can be maintained at a sufficient level due to its long half life in humans, this drug is considered to be appropriate for long-term therapy of arrhythmias (7). Iwasaki et al. (8) showed that aprindine inhibited ischemia/reperfusion-induced arrhythmia in perfused rat hearts, which may obviously be beneficial for recovery of cardiac contractile function in ischemic/reperfused hearts. Besides arrhythmogenesis, ischemia/reperfusion is recognized to induce contractile dysfunction depending upon the ischemic duration. There is little information available concerning the effects of aprindine on ischemia/reperfusion-induced contractile dysfunction and metabolic impairment. The present study was undertaken to determine whether aprindine improves post-ischemic contractile function and metabolism of ischemic/reperfused hearts.

MATERIALS AND METHODS

Animals
Male Wistar rats, weighing 210–250g (Japan SLC Inc., Hamamatsu), were used in the present study. The
animals were conditioned to $23 \pm 1^\circ\text{C}$ with a constant humidity of $55 \pm 5\%$, a cycle of 12-hr light and 12-hr dark, and allowed free access to food and tap water, according to the Guidelines of Experimental Animal Care issued by the Prime Minister's Office of Japan.

**Perfusion of the hearts**

The perfusion of isolated rat hearts was carried out as described previously (9). The rats were stunned by a blow to the head. After thoracotomy, the hearts were rapidly isolated and immersed in ice-cold and oxygenated Krebs-Henseleit bicarbonate buffer with the following composition: 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 1.25 mM CaCl$_2$, 25 mM NaHCO$_3$, and 11 mM glucose. Isolated hearts were quickly transferred to glass organ bath of the non-working, non-recirculating Langendorff apparatus and perfused at $37^\circ\text{C}$ with the Krebs-Henseleit buffer as described above at a constant flow rate of 9 ml/min using a micropump (MP3B; Tokyo Rikakiki, Tokyo). The perfusion buffer was equilibrated with a gas mixture of 95% oxygen and 5% carbon dioxide. A latex balloon with an uninflated diameter of 3.7 mm, connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo), was inserted into the left ventricular cavity through the mitral opening, and secured with a ligature that included the left atrial remnants. The heart was loaded with an initial left ventricular end-diastolic pressure (LVEDP) of 5 mmHg. After 15 min of equilibration, the heart was paced at 300 beats/min and further equilibrated for 15 min. The perfusion pressure was monitored through a branch of the aortic cannula by means of a pressure transducer (TP-200T, Nihon Kohden, Tokyo) connected to a carrier amplifier (AP-621G, Nihon Kohden). Left ventricular developed pressure (LVDP) was monitored by another pressure transducer and recorded on a thermal pen recorder (WT-645G, Nihon Kohden) throughout the experiment.

After 30-min equilibration, the perfusion was stopped, and the hearts were submerged in an organ bath filled with Krebs-Henseleit buffer in which the 11 mM glucose was replaced with 11 mM Tris/HCl. This buffer was previously equilibrated with a gas mixture of 95% nitrogen and 5% carbon dioxide, pH 7.4, and maintained at $37^\circ\text{C}$ to avoid hypothermia-induced cardioprotection. After 35 min of ischemia, the buffer in the organ bath was drained, and the hearts were reperfused for 60 min at $37^\circ\text{C}$ with the normal Krebs-Henseleit buffer equilibrated with a gas mixture of 95% oxygen and 5% carbon dioxide. The perfused hearts were paced throughout the experiment except for the first 15 min of reperfusion, to prevent contractile irregularities that might frequently occur during this period. For the purpose of comparison, hearts were perfused for 35 min under normoxic conditions, followed by 60 min of normoxic perfusion (normoxic group).

Treatment of the perfused hearts with different concentrations of aprindine ranging from 10 to 100 $\mu$M as a final concentration was carried out by infusing the appropriate concentrations of aprindine into the Krebs-Henseleit buffer only for the last 3-min pre-ischemia. Aprindine dissolved in a perfusion buffer was infused through an injection port located just prior to the aortic cannula at a flow rate of 200 $\mu$l/min by means of an infusion-pump (STC-523; Terumo Co., Tokyo).

**Determination of myocardial ion content**

At an appropriate time in the experimental sequence (0, 35 or 95 min), the perfusion of hearts was stopped and the vascular space of hearts was washed with 8 ml of a ice-cold washing buffer composed of 320 mM sucrose and 20 mM Tris/HCl, pH 7.4. The myocardial ion content was measured according to the method described previously (10). The myocardium was washed with the sucrose buffer. After determining its wet weight, the myocardium was dried at $120^\circ\text{C}$ for 48 hr. Then the dried myocardium, after determining its dry weight, was digested at 180°C with concentrated HNO$_3$ until the solution evaporated. The resulting residue was reconstituted with 2.5 ml of 0.75 N HNO$_3$ and used for determination of myocardial ion content by means of an atomic absorption spectrometer (AA-680; Shimadzu Seisakusyo, Kyoto).

**Determination of creatine kinase activity and ATP metabolites in the perfusate eluted from heart**

The perfusate was collected during reperfusion or normoxic perfusion. Creatine kinase activity and ATP metabolites in the perfusate eluted from perfused heart were determined. The total amount of creatine kinase released in the buffer from perfused hearts during reperfusion was determined by measuring its activity according to the method of Bergmeyer et al. (11). ATP metabolites were determined by using HPLC as described previously (12). Briefly, ATP metabolites were separated through a column of C$_{18}$ cellulose acetate (Cosmosil 5C18; Nacalai Tesque, Kyoto) with a diameter of 4.6 mm and length of 15 cm, by elution with 0.25 M NH$_4$H$_2$PO$_4$ containing 3.5% CH$_3$CN (pH 6.0), at a flow rate of 1.0 ml/min (L-6000; Hitachi, Tokyo). The absorbance of these metabolites in the perfusate was monitored at 254 nm (L-4000, Hitachi) and recorded using a data-chromatoprocessor (D-2000, Hitachi).

**Statistics**

Results are expressed as means±S.E.M. Statistical significance was evaluated by analysis of variance followed by Dunnett's multiple comparison when the effects of different concentrations of aprindine on several
parameters of perfused hearts were examined, while Student's t-test was employed when two groups were compared. A confidence level of more than 95% was considered significant (P < 0.05).

RESULTS

Changes in functional parameters of perfused rat hearts

Functional alterations of the perfused rat heart subjected to 35 min of ischemia and subsequent 60 min of reperfusion were determined in the presence and absence of various concentrations of aprindine. Changes in LVDP, LVEDP and perfusion pressure of ischemic/reperfused hearts treated with and without aprindine are shown in Fig. 1. The LVDP of the pre-ischemic heart (control value) generated with an initial LVEDP of 5 mmHg was 78.9 ± 4.6 mmHg, and perfusion pressure was 75.5 ± 5.2 mmHg (n = 30). Ischemia rapidly induced a decline in LVDP and the LVDP decreased to zero within 2 min of ischemia (Fig. 1, upper panel). When the hearts were reperfused, no recovery of LVDP was observed. LVEDP was increased approximately 15 min after the onset of ischemia and reached peak levels at approximately 25 min of ischemia (Fig. 1, middle panel). The LVEDP was further increased during the reperfusion period, and the increased level of LVEDP was sustained throughout reperfusion. Ischemia induced an immediate decline in the perfusion pressure of all hearts examined. The perfusion pressure of the untreated heart increased upon reperfusion by approximately 140% of the pre-ischemic value (Fig. 1, lower panel).

LVDPs at the end of the pre-ischemic perfusion of the heart treated with either 10 or 30 µM aprindine were 39.6 ± 15.0% or 3.8 ± 1.7% of the initial value, respectively, whereas those of the heart treated with either 70 or 100 µM aprindine completely ceased (Fig. 1, upper panel). That is, aprindine suppressed LVDP generated during pre-ischemic perfusion in a concentration-dependent manner. This negative inotropic effect of aprindine was reversible under normoxic conditions. When the normoxic heart was treated with 100 µM aprindine, its LVDP completely recovered within 20 min after stopping aprindine-administration. LVDPs of the hearts treated with 10 and 30 µM aprindine recovered to approximately 20% and 60% of the pre-ischemic value, respectively (Fig. 1, upper panel). The increase in LVEDP during ischemia and reperfusion was significantly suppressed in the hearts treated with 30 µM aprindine and tended to be suppressed in those treated with 10 µM aprindine (Fig. 1, upper panel). The increase in coronary perfusion pressure during reperfusion was also prevented by treatment with 30 µM aprindine, but not 10 µM aprindine (Fig. 1, lower panel). In contrast, treatment with 70 and 100 µM aprindine failed to recover the LVDPs and to suppress the rise in LVEDP during both the ischemia and reperfusion periods (Fig. 1, upper and middle panel). Treatment with 100 µM aprindine, but not 70 µM, induced a significant increase in perfusion pressure during reperfusion (Fig. 1, lower panel).
Examination of perfusate eluted from hearts

Creatine kinase activity: The creatine kinase activity in the perfusate was measured. During the pre-perfusion period, the release of creatine kinase activity in the perfusate was negligible (less than 3 nmol NADPH/min/g wet tissue). Creatine kinase was markedly released from the myocardium during the reperfusion period (Fig. 2). Treatment with different concentrations of aprindine...
caused biphasic effects; treatments with 10 and 30 μM aprindine suppressed the release of the enzyme by approximately 50% and 40% of the values, respectively, whereas those with 70 and 100 μM aprindine failed to suppress the release of the enzyme (Fig. 2).

Release of ATP metabolites: Release of ATP metabolites during reperfusion was determined (Fig. 3). The ATP metabolites released from the perfused hearts during reperfusion were hypoxanthine, inosine and adenosine, nucleosides or bases metabolized from ATP (12–14). A minimal release of ATP metabolites was detected during normoxic perfusion (less than 0.1 μmol/g wet tissue). The ATP metabolites were markedly released from reperfused hearts. Treatment with 10 and 30 μM aprindine suppressed the release of ATP metabolites during reperfusion by approximately 20° and 40%, respectively, whereas that with 70 and 100 μM aprindine failed to suppress the release of ATP metabolites (Fig. 3).

Myocardial ion content

Myocardial sodium, potassium calcium and magnesium contents of the perfused hearts at the end of reperfusion (95 min) with and without various concentrations of aprindine were determined (Fig. 4). Pre-ischemic values of these ion contents were 56.92±2.88, 361.50±4.13, 2.09±0.07 and 36.32±0.98 μmol/g dry tissue, respectively (n=5). Myocardial sodium and calcium contents significantly increased upon reperfusion (approximately 2.7- and 5-fold higher than the pre-ischemic values, respectively), whereas myocardial potassium and magnesium in the hearts significantly decreased (approximately 40% and 60% of the pre-ischemic values, respectively). Treatment with either 10 or 30 μM aprindine during pre-ischemic perfusion significantly suppressed these changes in ion content in reperfused hearts in a dose-dependent manner. Treatment with either 70 or 100 μM aprindine failed to suppress ischemia/reperfusion-induced changes in these ion contents.

In another set of experiments, the rat hearts pre-treated with and without 30 μM aprindine were subjected to 35 min ischemia (Fig. 5). Sodium, potassium, calcium and magnesium contents of the heart treated with 30 μM aprindine at the end of pre-ischemia were 55.51±3.52, 354.77±5.57, 2.18±0.18 and 36.20±0.88 μmol/g dry tissue, respectively (n=4). Ischemia caused an increase in myocardial sodium content (2.3-fold higher than the pre-ischemic value) and a decrease in myocardial potassium content (50% of the pre-ischemic value). There were no

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![Fig. 5](image_url)  
Myocardial sodium, potassium, calcium and magnesium contents of the hearts subjected to 35-min normoxia (Nor: striped column) and ischemia without (open column) and with 30 μM aprindine treatment (dotted column). Each value represents the mean ± S.E.M. of 4 to 6 experiments. *Significantly different from the untreated group and #significantly different from the value of the normoxic group (P < 0.05).
changes in myocardial calcium and magnesium contents during the ischemic period. Treatment with 30 \( \mu \)M aprindine significantly suppressed the changes in myocardial sodium and potassium contents at the end of ischemia. Myocardial calcium and magnesium contents of the ischemic hearts were not altered regardless of treatment with or without the agent. To examine the myocardial ion profile of the ischemic heart treated with a high concentration of aprindine, the heart was exposed to 100 \( \mu \)M aprindine during pre-ischemia, and myocardial sodium, potassium, calcium and magnesium contents at the end of ischemia were determined. Treatment with 100 \( \mu \)M aprindine significantly suppressed an increase in myocardial sodium content (69.17 \( \pm \) 5.12 \( \mu \)mol/g dry tissue) and a decrease in myocardial potassium content (242.83 \( \pm \) 10.61 \( \mu \)mol/g dry tissue). There were no significant changes in myocardial calcium and magnesium content of the heart at the end of ischemia (2.19 \( \pm \) 0.12 and 36.13 \( \pm \) 1.67 \( \mu \)mol/g dry tissue, respectively) \( (n=4) \).

**DISCUSSION**

In the present study, posts ischemic recovery of cardiac contractile function was enhanced by treatment with either 10 or 30 \( \mu \)M aprindine for the last 3 min pre-ischemia. This treatment decreased the reperfusion-induced disturbance in the contents of myocardial ions such as sodium, potassium, calcium and magnesium: at the end of ischemia, ischemia-induced accumulation of myocardial sodium and reduction of myocardial potassium were suppressed by treatment with 30 \( \mu \)M aprindine. In contrast, ischemia-induced changes in myocardial calcium and magnesium were not affected by this treatment. Recently, ischemia and hypoxia have been shown to induce an increase in myocardial sodium content, that is, sodium overload \( (15-18) \). Furthermore, a decrease in high energy phosphates due to ischemia may enhance sodium overload and decrease in myocardial potassium, since reduction of ATP content causes a suppression of sodium/potassium ATPase activity in the sarcolemma \( (19, 20) \). Tani and Neely \( (15, 21-23) \) suggested that sodium-overload induced during ischemia caused abnormal calcium influx, the so-called "calcium overload", during subsequent reperfusion. They also suggested an involvement of sodium/calcium exchanger as the mechanism for calcium overload. Calcium-overload is considered to be a cause of cardiac dysfunction: it induced a decrease in mitochondrial activity and activation of protease and phospholipase \( (24-27) \). Thus, the agent that is capable of preventing or suppressing sodium overload during ischemia may prevent calcium accumulation and thereby is a possible cardioprotective agent against ischemia/reperfusion-induced damage to the myocardium.

A possible mechanism for sodium overload in the ischemic myocardium is attributable to sodium flux into cardiac myocytes via sodium channels in the sarcolemma. Several investigators suggested that a sodium channel blockade during ischemia involved an improvement of post ischemic contractile recovery \( (9, 10, 28-30) \). For example, Ver Donck and Borgers \( (31) \) have shown that R 56865 which is a new type of sodium channel blocker protected isolated cardiac myocytes from hypercontracture induced by pathological stimuli such as singlet oxygen, lysophosphaticidylcholine and ouabain in vitro. This agent has also been shown to recover cardiac function, prevent cardiac contracture, normalize calcium distribution and suppress arrhythmias during ischemia/reperfusion possibly through the blockade of sodium channels \( (32-34) \). Our present results agree with the hypothesis of the cardioprotective mechanism of sodium channel blockers.

The recovery of contractile function during reperfusion was associated with suppression of the release of creatine kinase and ATP metabolites from the heart. The release of creatine kinase indicates a loss of cytosolic macromolecular components and is considered to be an indicator of cardiac cell membrane disruption and/or an increase in cell membrane permeability \( (35-37) \). Our above-described observations are considered to represent a non-specific change in cardiac cell membrane permeability across the sarcolemma. Treatment of perfused hearts with aprindine for the last 3 min pre-ischemia resulted in a suppression of the release of enzyme and metabolites from the hearts. These results suggest that aprindine is capable of preventing cardiac cell necrosis and/or detrimental changes in the sarcolemma during ischemia/reperfusion.

We observed that treatment with high concentrations of aprindine failed to improve cardiac contractile function and metabolism after reperfusion, although treatment with 100 \( \mu \)M aprindine suppressed the ischemia-induced increase in myocardial sodium content. The mechanism underlying this failure has not been addressed in the present study. Levine and Hollier \( (38, 39) \) showed that aprindine at high concentrations competitively inhibited calmodulin-stimulated phosphodiesterase and calcium ATPase \( (Ca^{2+}-ATPase) \) due to a decrease in calcium-binding affinity with calmodulin. Calmodulin is a heat-stable protein and known to affect the activities of many enzymes, including cyclic nucleotide phosphodiesterase, \( Ca^{2+}-ATPase \), myosin light chain kinase and phospholipase A2 phosphorylase kinase. In addition, calcium uptake into the sarcoplasmic reticulum is stimulated by calmodulin \( (40) \). It is also suggested that aprindine inhibits calcium transport, maintenance of cyclic
nucleotide level or availability of glucose for energy production (38). Furthermore, Levine and Hollier showed that the ID$_{50}$ value of aprindine for Ca$^{2+}$-ATPase was 84 nM (39). This concentration is comparable to that eliciting the failure to recover postischemic contractile function in the reperfused heart in the present study.

In summary, aprindine, a sodium-channel blocker, is capable of exerting beneficial effects on the contractile function of ischemic/reperfused hearts. Since the inhibition of transmembrane flux of ions, macromolecules and metabolites was detected, it could be concluded that protection of membrane integrity by treatment with the agent, particularly suppression of ischemia-induced sodium overload and reperfusion-induced calcium overload, plays a significant role in the improvement of postischemic contractile recovery and metabolism of the perfused hearts.

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