Effects of Bunazosin, a Selective α_1 -Adrenergic Blocking Agent, on Myocardial Energy Metabolism in Ischemic Dog Heart

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Abstract—Effects of a selective α_1 -adrenergic blocking agent, bunazosin, on myocardial energy metabolism in the ischemic heart were studied. Ischemia was induced by ligating the left anterior descending coronary artery of the dog heart. Bunazosin was injected intravenously either 5 or 20 min before coronary artery ligation. Hearts were removed 3 min after coronary ligation and used for determination of the levels of cardiac tissue metabolites. Ischemia decreased the levels of ATP, creatine phosphate, glycogen and glucose, and increased the levels of ADP, AMP, hexose monophosphates and lactate. The energy charge potential (ECP) calculated was decreased by ischemia. Pretreatment with bunazosin inhibited the decrease in ATP and the increase in AMP caused by ischemia, resulting in the high value of ECP in the ischemic myocardium. Bunazosin also prevented the changes in carbohydrate metabolism caused by ischemia. It is concluded that bunazosin may reduce the influence of ischemia on the myocardium.

According to the receptor binding assay, not only β -adrenergic receptors but also α_1 -adrenergic receptors exist on the myocardial cell membrane (1, 2). During ischemia, released catecholamines activate β -adrenergic receptors and make the myocardial damage worse (3). There are reports indicating that β -adrenergic blocking agents, such as propranolol, can reduce the myocardial injury caused by ischemia (4–6). Either norepinephrine or epinephrine released during ischemia affects both β - and α_1 -adrenergic receptors. In the recent studies, stimulation of α_1 -adrenergic receptors of the myocardium activates phosphoinositide metabolism, leading to an increase in the intracellular calcium ion concentration (7). The increased calcium ions can produce a positive inotropism (8), cardiac arrhythmia (9). In addition, norepinephrine induces cardiac hypertrophy through α_1 -adrenergic receptors (10). In the non-ischemic normal myocardium, α_1 -adrenergic stimulation plays an important role in many physiological processes. In the ischemic

myocardium, in which an energy deficiency has occurred, however, the stimulated α_1 -adrenergic receptors may extend the influence of ischemia on the myocardium, because of activation of energy consuming processes. A selective α_1 -adrenergic blocking agent can inhibit the activation of phosphoinositide metabolism including the intracellular calcium ion movement (11). Nayler et al. (12) have been reported that prazosin exerts a protective effect on the ischemic myocardium under some in vitro conditions. However, it is still unclear whether an α_1 -adrenergic blocking agent directly protects the myocardium from ischemic damage in vivo. The present study, therefore, was undertaken to examine whether an α_1 -adrenergic blocking agent, bunazosin, possesses a protective effect on the ischemic myocardium.

Materials and Methods

Forty healthy mongrel dogs of either sex weighing 7–18 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and endotracheally intubated and ventilated with a respirator. A left thoracotomy was performed

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between the fourth and fifth ribs to expose the left ventricular wall. A main trunk of the left anterior descending coronary artery (LAD) was dissected free from the adjacent tissues at a distal portion to the first diagonal branch, and it was loosely encircled with a 2-0 silk thread ligature. Ischemia was initiated by ligating the LAD. An ischemic region of the myocardium was assessed by visible cyanosis and the elevation of the ST segment of the ECG, which was introduced by a wire electrode attached on the surface of the left ventricular wall. Heart rate was counted from the ECG taken in the standard limb lead II. Arterial blood pressures were measured via cannula introduced into the left femoral artery. Coronary blood flow was measured by an electromagnetic flow probe positioned just proximal to the ligature.

After a 60-min stabilization period, either saline or bunazosin (0.3 mg/kg) was injected i.v. over a period of 30 sec into the femoral vein. After either 5 or 20 min of injection, the ligature around the LAD was tied in half of the total number of animals receiving bunazosin and also in half of the total number of animals receiving saline. When the ischemia had been produced for 3 min, a full thickness sample of the myocardium was taken from the center of the ischemic area. An equivalent sample was taken from the control animals, in which the LAD was not tied. The dose of bunazosin (0.3 mg/kg) used in the present study was chosen because it was sufficient to produce a maximal hypotensive effect in an intact dog (13). The samples were immediately pressed and frozen with clamps previously chilled in liquid nitrogen in such a way that the endocardial portion of the myocardium could be taken for analysis. The endocardial tissue sample was pulverized in a mortar with a pestle precooled with liquid nitrogen and extracted with 6% perchloric acid. The levels of glycogen, glucose, glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-diphosphate (FDP), pyruvate, lactate, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and creatine phosphate (CrP) in the neutralized perchloric acid extract were determined according to standard enzymatic procedures. Energy charge potential (ECP) was

calculated from the concentration of ATP, ADP, and AMP to estimate how many high-energy phosphates the adenine nucleotides had, according to the following equation (14):

$$\frac{([\text{ATP}] + 1/2[\text{ADP}])}{([\text{ATP}] + [\text{ADP}] + [\text{AMP}])}$$

The ratio of $([\text{G6P}] + [\text{F6P}])/[\text{FDP}]$ was calculated from the concentration of hexose phosphates in order to estimate the rate of glycolytic flux through the phosphofructokinase (PFK) reaction (15). The ratio of lactate to pyruvate (L/P) was also calculated as an index of the cytoplasmic redox state in the myocardial cell.

Data were evaluated using analysis of variance, and a *P* value of 0.05 or less was considered significant.

Results

Hemodynamics: Hemodynamic data of saline- and bunazosin-treated animals whose coronary artery was ligated for 3 min are shown in Fig. 1. Bunazosin was injected either 5 or 20 min before coronary ligation. Hemodynamic changes caused by the drug injection in the non-ischemic groups, in which the coronary artery was not ligated, were the same as those in the ischemic groups (data are not shown). Treatment with saline did not affect blood pressures, heart rate and coronary flow appreciably. Bolus injection of bunazosin decreased arterial blood pressure within a minute. The decreased blood pressure was sustained for at least 20 min. Heart rate slightly increased when bunazosin was administered, probably because of the compensatory mechanism for the hypotension induced by the drug injection. Coronary blood flow was not changed by bunazosin. After coronary artery ligation, by which coronary blood flow became zero, arterial blood pressures slightly decreased, but heart rate did not change in both saline- and bunazosin-treated animals.

Energy metabolism: The levels of adenine nucleotides and calculated ECP are illustrated in Fig. 2. Whether bunazosin was present or not, ischemia significantly ($P < 0.01$) decreased the level of ATP, while it significantly ($P < 0.01$) increased the levels of ADP and

Fig. 1. Hemodynamic changes of animals in which the coronary arteries were ligated. Saline (N=6) or bunazosin (0.3 mg/kg) was injected i.v. either 5 (N=8) or 20 min (N=6) before coronary ligation. The curves for the blood pressure represent the systolic and diastolic pressure, respectively. The values under the arrows, indicating the drug application (-5 min), represent control values obtained just before saline or bunazosin injection. Plots and bars represent means and S.E.M., respectively.

AMP. However, bunazosin administered either 5 or 20 min before coronary ligation kept the ATP level high in the ischemic myocardium compared with that in the saline-treated ischemic myocardium. The level of AMP in the nonischemic myocardium, when bunazosin was injected 5 min before coronary ligation, was significantly ($P < 0.05$) lower than that in the saline-treated nonischemic myocardium. In this case, the increased level of AMP due to ischemia significantly ($P < 0.01$) reduced with bunazosin treatment. The level of AMP in the nonischemic heart, when bunazosin was injected 20 min before ischemia, was significantly higher than that in the saline-treated nonischemic myocardium. Bunazosin injected 20 min before ischemia did not inhibit the increase in AMP level due to ischemia significantly. ECP in the saline-treated heart significantly ($P < 0.01$) decreased from 0.901 ± 0.003 to 0.816 ± 0.006 , when the heart was made ischemic for 3 min. Bunazosin that was injected 5 min and 20 min before ischemia lessened the decrease in ECP due to ischemia significantly ($P < 0.01$ and $P < 0.05$, respectively).

The level of CrP was also determined in this study (Fig. 3). Bunazosin did not modify the level of CrP in the nonischemic myocardium significantly. Ischemia significantly ($P < 0.01$) decreased the CrP level in the saline- and bunazosin-treated myocardium. The decreased level of CrP was lessened by bunazosin injected 5 min before ischemia, but not by bunazosin injected 20 min before ischemia.

Carbohydrate metabolism: In the saline-treated myocardium, ischemia decreased myocardial glycogen and glucose level significantly ($P < 0.01$) (Fig. 4). In the bunazosin-treated myocardium, ischemia decreased the glycogen and glucose levels, but the differences between nonischemic and ischemic myocardium were not significant. Ischemia increased the level of G6P and F6P, whereas it did not change the level of EDP in the saline-treated heart (Fig. 5). Therefore, the ratio of $([G6P] + [F6P]) / [FDP]$ was significantly ($P < 0.01$) increased by ischemia indicating an inhibition of glycolytic flux at the level of PFK. Bunazosin injected 5 min before ischemia significantly ($P < 0.01$) inhibited the increases in G6P and F6P caused

Fig. 2. Effect of bunazosin on changes in the levels of adenine nucleotides and ECP during ischemia. Saline or bunazosin (0.3 mg/kg) was injected i.v. either 5 or 20 min before coronary ligation. Myocardial tissue samples were taken just before (nonischemic myocardium, $\square$; saline, N=6; bunazosin 5 min before ligation, N=8; bunazosin 20 min before ligation, N=6) or 3 min after coronary artery ligation (ischemic myocardium, $\square$; saline, N=6; bunazosin 5 min before ligation, N=8; bunazosin 20 min before ligation, N=6). Data are means $\pm$ S.E.M. (μ mol/g wet tissue). ** P < 0.01, compared with nonischemic control in each group. * P < 0.05; ## P < 0.01, compared with saline group.

by ischemia. However, the increase in $([G6P] + [F6P])/[FDP]$ ratio due to ischemia was not reduced by bunazosin, because the FDP level also decreased during ischemia. When bunazosin was injected 20 min before coronary ligation, the increase in $([G6P] + [F6P])/[FDP]$ appeared to be reduced by the drug, because the levels of G6P and F6P decreased, while the FDP level did not change after coronary ligation.

In the saline-treated heart, the level of lactate significantly (P < 0.01) increased and the level of pyruvate slightly decreased after

Fig. 3. Effect of bunazosin on the creatine phosphate level in the ischemic myocardium. Symbols are the same as those in Fig. 2. Data are means $\pm$ S.E.M. (μ mol/g wet tissue). ** P < 0.01, compared with nonischemic control in each group. ## P < 0.01, compared with saline group.

Fig. 4. Effect of bunazosin on the glycogen and glucose levels in the ischemic myocardium. Symbols are the same as those in Fig. 2. Data are means $\pm$ S.E.M. (μ mol glucose equivalents/g wet tissue for glycogen and μ mol/g wet tissue for glucose). ** P < 0.01, compared with nonischemic control in each group.

coronary ligation, resulting in an increased L/P ratio (Fig. 6). After bunazosin was in-

Fig. 5. Effect of bunazosin on the levels of hexose phosphates and the ratio of $[G6P]+[F6P]/[FDP]$ in the ischemic myocardium. Symbols are the same as those in Fig. 2. Data are means $\pm$ S.E.M. ($\mu\text{mol/g}$ wet tissue). * $P < 0.05$; ** $P < 0.01$, compared with nonischemic control in each group. # $P < 0.05$; ## $P < 0.01$, compared with saline group.

jected either 5 or 20 min before coronary ligation, the increase in the lactate level slightly reduced, and the pyruvate level was slightly increased in the ischemic myocardium, leading to the reduction of the increased level of L/P ratio.

Discussion

β -Adrenergic blocking agents are extensively used for the therapy of angina pectoris. During myocardial ischemia, catecholamine (norepinephrine) is released from the sympathetic nerve endings and stimulates β -adrenergic receptors (3). Stimulation of β -adrenergic receptors induced by ischemia facilitates anaerobic carbohydrate metabolism, resulting in glycogen breakdown, accumula-

Fig. 6. Effect of bunazosin on the pyruvate and lactate levels and the ratio of $[Lactate]/[Pyruvate]$ in the ischemic myocardium. Symbols are the same as those in Fig. 2. Data are means $\pm$ S.E.M. ($\mu\text{mol/g}$ wet tissue). ** $P < 0.01$, compared with nonischemic control in each group. # $P < 0.05$, compared with saline group.

tion of glycolytic intermediates, and myocardial acidosis (16, 17). These metabolic changes are inhibited by propranolol (16, 17). Either norepinephrine or epinephrine can bind to both β - and α_1 -adrenergic receptors and exhibits intrinsic pharmacological activities. Stimulation of α_1 -adrenergic receptors increases inositol trisphosphate, which likely enhances the concentration of intracellular calcium ions (18). Ischemia increases the number of α_1 -adrenergic receptors of the ischemic region of the myocardium as well as that of β -adrenergic receptors (19, 20). These facts indicate that catecholamines released by ischemia may make the myocardial injury worse via β - and α_1 -adrenergic receptors. In the present study, bunazosin, a selective α_1 -adrenergic blocking drug, reduced the decrease in myocardial high energy phosphate levels, and it partially inhibited

the changes in carbohydrate metabolism due to ischemia (Figs. 2, 3 and 4). Although the ratio of $([G6P] + [F6P])/[FDP]$, indicating glycolytic flux, was not influenced by bunazosin in the ischemic heart, the levels of G6P and F6P were lower than those of saline-treated ischemic heart (Fig. 5). Stimulation of α_1 -adrenergic receptors activates the adenosine cyclic 3',5' monophosphate (cyclic AMP) system, including cyclic AMP-dependent protein kinase, glycogen phosphorylase and glucose transport (21). This may be one of the reasons why the levels of G6P, F6P and FDP decreased after bunazosin injection. Inhibition by bunazosin of G6P and F6P accumulation in the ischemic myocardium indicates that myocardial anaerobic metabolism may return to an aerobic one in the bunazosin-treated heart. In addition, reduction of the L/P ratio by bunazosin also suggests that the myocardial metabolism is under aerobic conditions in the bunazosin-treated ischemic myocardium (Fig. 6). Bunazosin did not inhibit anaerobic glycolysis as potently as the β -adrenergic blocking drug did (16). Although both α - and β -adrenergic receptors can affect a number of cellular metabolic processes via cyclic AMP formation (21, 22), the effect of α -adrenergic stimulation on the metabolic processes may not be potent as compared with that of β -adrenergic stimulation.

Stimulation of α_1 -adrenergic receptors of the myocardium and those of the arterial smooth muscle increases intracellular free calcium ions via activation of phosphoinositide metabolism (7, 18). Elevated intracellular calcium ions increase cardiac contraction (8), development of arrhythmias (9), and vasoconstriction (1). Improvement of the cardiac energy state by bunazosin observed in the present study (Figs. 2 and 3) may be due to a reduction of myocardial energy demand that results from decreasing cardiac contraction and arterial blood pressure.

Because bunazosin could sustain its hypotensive effect for more than 5 min, bunazosin was injected either 5 or 20 min before coronary ligation. When bunazosin was treated 5 min before coronary ligation, the ischemic myocardial level of CrP was higher, and the levels of G6P, F6P and FDP were

lower than the respective values in the myocardium treated with bunazosin 20 min prior to coronary ligation. However, the effect of bunazosin injected 20 min before ischemia on the myocardial energy metabolism was not appreciably different from that of the drug injected 5 min before ischemia.

In the present study, an ischemic myocardial sample was obtained from the endocardial portion of the myocardium 3 min after initiation of ischemia. The reason for this is that the endocardial portion of the myocardium is more vulnerable to ischemia (23), and that the ischemic changes occurring within a few minutes after the onset of ischemia are of importance for the patient with angina pectoris. Anginal attack must occur immediately after the coronary blood flow has been interrupted. In fact, the most potent influence of acute ischemia on the myocardial metabolism is observed in the endocardial portion of the myocardium 3 min after coronary artery ligation in the dog (24).

In conclusion, myocardial tissue damage during ischemia may be due partly to stimulation of α_1 -adrenergic receptors in the myocardium, and bunazosin may protect the myocardium from the ischemic damage, probably because of preserving myocardial energy.

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