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Effects of Minoxidil on Ischemia-Induced Mechanical and Metabolic Dysfunction in Dog Myocardium

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ABSTRACT—Effects of minoxidil on ischemia-induced myocardial mechanical and metabolic dysfunction were examined in anesthetized open-chest dogs. A regional portion of the left ventricle was made ischemic for 20 min by ligating the left anterior descending coronary artery, and then reperfused for 120 min. Dimethylsulfoxide or minoxidil (0.3, or 1.0 mg/kg) was injected intravenously 10 min before ligation. Ischemia decreased regional myocardial contraction, and reperfusion recovered it but incompletely. Myocardial metabolic derangement was observed during ischemia, such as decreases in the myocardial levels of ATP and creatine phosphate. These metabolic changes caused by ischemia were restored by reperfusion. Minoxidil injection at 0.3 and 1.0 mg/kg significantly decreased blood pressures but increased coronary flow. Pretreatment with minoxidil significantly enhanced the recovery of myocardial contraction during reperfusion after ischemia. The levels of ATP and creatine phosphate in the ischemic myocardium were significantly preserved by minoxidil at 0.3 mg/kg. No significant effect of minoxidil on the metabolism was observed in the 120 min reperfused myocardium. In conclusion, minoxidil improved the mechanical dysfunction in the reperfused heart and the drug at low dose preserved high-energy phosphates during ischemia.

Keywords: Minoxidil, Ischemia, Reperfusion, K_{ATP} channel, ATP

Minoxidil, 2,4-diamino-6-piperidinylpyrimidine 3-oxide, has the hypotensive action and has proven to be effective in patients with severe hypertension that responds poorly to other hypotensive drugs (1). At present, this drug is used topically for treatment of baldness (2). Minoxidil may induce proliferation of epithelial cells of the hair follicle and vasodilation of scalp blood vessels (3). However, even in topical use of minoxidil, the cardiovascular adverse effects such as tachycardia and acute myocardial infarction have been reported (4, 5). On the other hand, Spindler (6) has demonstrated that deaths occurring during clinical studies of topical minoxidil are the result of causes other than use of minoxidil.

Minoxidil is a prodrug and should be metabolized to its active metabolite, minoxidil sulfate, to relax vascular smooth muscle (7). Minoxidil sulfate activates and opens ATP sensitive potassium (K_{ATP}) channels in the vascular smooth muscle cell and ventricular cell (8 – 10). Because the activation or opening of K_{ATP} channels causes hyperpolarization and/or shortening of action potential duration of the cell and then reduces Ca^{2+} influx through the voltage-dependent Ca^{2+} channels, it dilates the coronary artery and decreases myocardial contractility (11). The coronary dilation increases oxygen supply, and the decrease in myocardial contractility decreases myocardial oxygen demand. Therefore, opening of K_{ATP} channels should exert a beneficial effect on ischemic myocardium. Recently, a clinical study has reported that nicorandil, another K_{ATP} channel opener, improves outcome in patients with stable angina (12). However, nicorandil having a nitro moiety in its structure is also a nitrovasodilator (13), but minoxidil is not. On the other hand, the hypotension caused by systemic vasodilation induces the baroreflex activation of the sympathetic nervous system, leading to an increase in myocardial oxygen consumption. This may be responsible for adverse effects of minoxidil on ischemic heart disease (14). In the present study, we examined the direct effect of minoxidil on ischemic myocardium in terms of changes in myocardial mechanical contraction and myocardial energy metabolism during ischemia and reperfusion.

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MATERIALS AND METHODS

This investigation conforms to the Guiding Principles for the Care and Use of Experimental Animals in Hokkaido College of Pharmacy (published in 2001).

Experiments for mechanical function

Healthy mongrel dogs of either sex, weighing 8 – 34 kg (12.5 ± 0.7 kg, $n = 40$), were anesthetized intravenously with 30 mg/kg sodium pentobarbital and ventilated with room air. A left thoracotomy was performed between the fourth and fifth ribs, and the left ventricle was exposed. After the heart was suspended in a pericardial cradle, the main trunk of the left anterior descending coronary artery was dissected free from the distal end to the first diagonal branch and loosely encircled with a silk thread ligature. The left anterior descending coronary flow was measured using a magnetic flow probe positioned in the left anterior descending coronary artery proximal to the ligature. The left femoral vein was cannulated for administration of drugs, a vehicle, or subsequent anesthetics as needed. To measure the left ventricular end diastolic pressure (LVEDP) and first derivative of left ventricular pressure (LVdP/dT), a polyethylene tube connected to a pressure transducer was inserted into the left ventricular chamber through the cardiac apex. Myocardial contraction was measured as segment shortening in the regions perfused by the left anterior descending coronary artery by a pair of ultrasonic crystals. The two crystals of each pair were implanted at an interval of approximately 1 cm. The leads of the crystals were connected to an ultrasonic amplifier (4105; Nihondenki San-Ei, Tokyo) that transforms the crystal-transmitted sound pulse into an electrical signal proportional to the distance between them. The tracings were monitored with an oscilloscope (Synchroscope SS-7604; Iwatsu, Tokyo). Diastolic segment length (DL) was determined at the beginning of the rising phase of positive LVdP/dT (onset of isovolumic contraction), and systolic segment length (SL) was determined at the peak negative LVdP/dT (15, 16). The segment shortening was calculated using the equation of (DL – SL) / DL. Arterial blood pressures were measured via a cannula introduced from the left femoral artery to near the aortic arch. Heart rate was monitored using limb lead II from the electrocardiogram and a tachograph. All hemodynamics were monitored on a polygraph (360 system, Nihondenki San-Ei).

After the control observations, 100% dimethylsulfoxide (DMSO) or minoxidil at the dose of 0.3 or 1.0 mg/kg dissolved in 100% DMSO was injected intravenously over a period of 30 s into the left femoral vein, in a volume of 0.1 ml/kg. In our preliminary experiment, we determined the dose at which intravenous administration of the dog with minoxidil decreased the diastolic blood pressure by about 20%. This dose (1.0 mg/kg) and the lesser dose (0.3 mg/kg) were used in the present study. The ligature around the coronary artery was tied 10 min after the injection and then released 20 min after coronary ligation. Ischemia was confirmed by visible cyanosis and segment shortening being negative (bulging). Measurements of hemodynamic parameters were continued for a further 120 min after releasing the ligature. A full-thickness transmural sample of the myocardium was taken from the previously ischemic region, at 120 min after reperfusion for determination of tissue metabolites as described below.

Experiments for metabolic function

An additional 37 dogs (body weight; 13.2 ± 1.2 kg) were used for the experiment to determine the tissue levels of ATP and the related intermediary metabolites. The left anterior descending coronary artery was dissected free and loosely encircled with a silk thread ligature in all animals, but the other instruments were not attached to avoid myocardial damage. After the control observations, DMSO or minoxidil at the dose of 0.3 and 1.0 mg/kg was injected intravenously. A myocardial sample that would be ischemic by coronary ligation was transmurally taken from one half of the animals 10 min after each injection (just before the ligation). In another half of the animals, the coronary artery was ligated 10 min after the drug injection, and the myocardial sample was taken from the ischemic region 20 min after the ligation. The myocardial tissue samples obtained either before or after ischemia and after reperfusion obtained in the above experiments were immediately pressed and frozen with freezing clamps chilled in liquid nitrogen, and the subendocardial portion of the frozen sample was used for biochemical assay (17).

Biochemical analyses

The frozen subendocardial samples were pulverized in a mortar with a pestle precooled with liquid nitrogen and extracted with 6% perchloric acid. After neutralization with KOH, the supernatants were used to determine the levels of glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-diphosphate (FDP), pyruvate, lactate, ATP, ADP, AMP and creatine phosphate (CrP), according to standard enzymatic procedures (18) using a spectrometer (DU 640 Spectrophotometer; Beckman Instruments, Inc., Fullerton, CA, USA). The total adenine nucleotide (TAN) was calculated as the sum of adenine nucleotide levels and the energy charge potential (ECP) was calculated by the equation $([ATP] + 0.5[ADP]) / ([ATP] + [ADP] + [AMP])$ to estimate the myocardial energy state (19).

Statistical analyses

All values are expressed as means ± S.E.M. Changes in hemodynamics were estimated by an analysis of variance

(ANOVA) for repeated measurements for overall effect of the treatments followed by Dunnett's post-hoc procedures. Differences in hemodynamics within groups were compared using an ANOVA followed by Dunnett's post-hoc procedures. The significance of differences in the metabolites between DMSO-treated and minoxidil-treated groups or between before ischemia, after ischemia, and after reperfusion groups was evaluated using an unpaired Student's *t*-test. Differences were considered statistically significant when $P<0.05$.

RESULTS

Mortality and exclusion

Initially, 40 dogs were used in the experiment for mechanical function. However, 13 dogs were excluded because of ventricular fibrillation that occurred during ischemia or just after reperfusion: 3 out of 12 DMSO-treated dogs; 5 out of 15 minoxidil 0.3 mg/kg-treated dogs; 5 out of 13 minoxidil 1.0 mg/kg-treated dogs. Because neither visible cyanosis nor bulging was observed in 4 dogs during ischemia in spite of coronary ligation, they were excluded: 2 dogs of DMSO-treated and 2 dogs of minoxidil 0.3 mg/kg-treated groups. Thus, a total of 23 dogs were included in the data analysis in this series of experiments: DMSO-treated (n = 7), 0.3 mg/kg minoxidil-treated (n = 8) and 1.0 mg/kg minoxidil-treated (n = 8) groups. There were no significant differences in mortality between the groups.

In the experiments for metabolic function, no dog out of 37 died in this series of experiments. However, 2 dogs in the ischemic group were excluded because some metabolic values were rejectable by statistical analysis. Therefore, 35 dogs were used for data analysis; samples were taken before ischemia from DMSO-treated (n = 5), 0.3 mg/kg minoxidil-treated (n = 6) and 1.0 mg/kg minoxidil-treated (n = 5) groups, and those after ischemia from DMSO-treated (n = 6), 0.3 mg/kg minoxidil-treated (n = 6) and 1.0 mg /kg minoxidil-treated (n = 7) groups.

Mechanical function

Table 1 shows the baseline values of systolic and diastolic blood pressure, heart rate, coronary flow, LVEDP and LVdP/dT. There was a variation in LVdP/dT between groups. Percent changes in the hemodynamic parameters to the respective pre-injection values are shown in Fig. 1, except for LVEDP. Because the pre-injection values of LVEDP were 0 mmHg in some dogs, we could not calculate the percentage. In the DMSO-treated dogs, systolic and diastolic blood pressures slightly but significantly increased after DMSO injection and decreased transiently after the onset of ischemia. After that, the blood pressures did not change until the end of experiment. In the minoxidil-treated dogs, the arterial blood pressures, particularly diastolic pressure, significantly decreased after the drug injection. The decrease in diastolic pressure was dose-dependent. Heart rate was significantly decreased by DMSO injection and sustained during ischemia. After reperfusion, the heart rate that had decreased gradually recovered to its pre-injection level. The heart rate in the 0.3 mg/kg minoxidil-treated group decreased after the injection and during ischemia, and the decreased level was sustained during reperfusion. On the other hand, in the 1.0 mg/kg minoxidil-treated group, heart rate was not changed significantly by the injection and gradually increased after reperfusion. Coronary flow was increased by minoxidil in a dose-dependent manner and decreased to 0 mL/min in either group when the coronary artery was ligated. The reactive hyperemia was observed shortly after the onset of reperfusion. The coronary flow increased beyond the pre-ischemic level returned to its baseline level within 15 min after reperfusion in the DMSO-treated group. However, the increased coronary flow was sustained in the minoxidil-treated groups. There was a significant difference between the DMSO- and minoxidil-treated groups. LVEDP appeared to increase during ischemia and returned during reperfusion. The LVdP/dT in the DMSO-treated group decreased during ischemia, returned during reperfusion, and

Table 1. The baseline values of hemodynamics obtained before DMSO or minoxidil injection

Parameters	DMSO-treated	Minoxidil-treated	
		0.3 mg/kg	1.0 mg/kg
SBP (mmHg)	158 ± 10	139 ± 7	147 ± 4
DBP (mmHg)	116 ± 9	108 ± 7	113 ± 2
HR (beats/min)	178 ± 9	161 ± 8	167 ± 3
CF (mL/min)	14 ± 3	24 ± 14	10 ± 1
LVEDP (mmHg)	1.7 ± 0.9	1.5 ± 0.6	2.1 ± 0.4
LVdP/dT (mmHg/s)	7209 ± 344	5424 ± 474*	4766 ± 487*

Data are means ± S.E.M. of 7 or 8 observations in each treated group. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; CF, coronary flow; LVEDP, left ventricular end diastolic pressure; LVdP/dT, first derivative of left ventricular pressure. *$P<0.05$, compared with corresponding values in the "DMSO-treated" group.

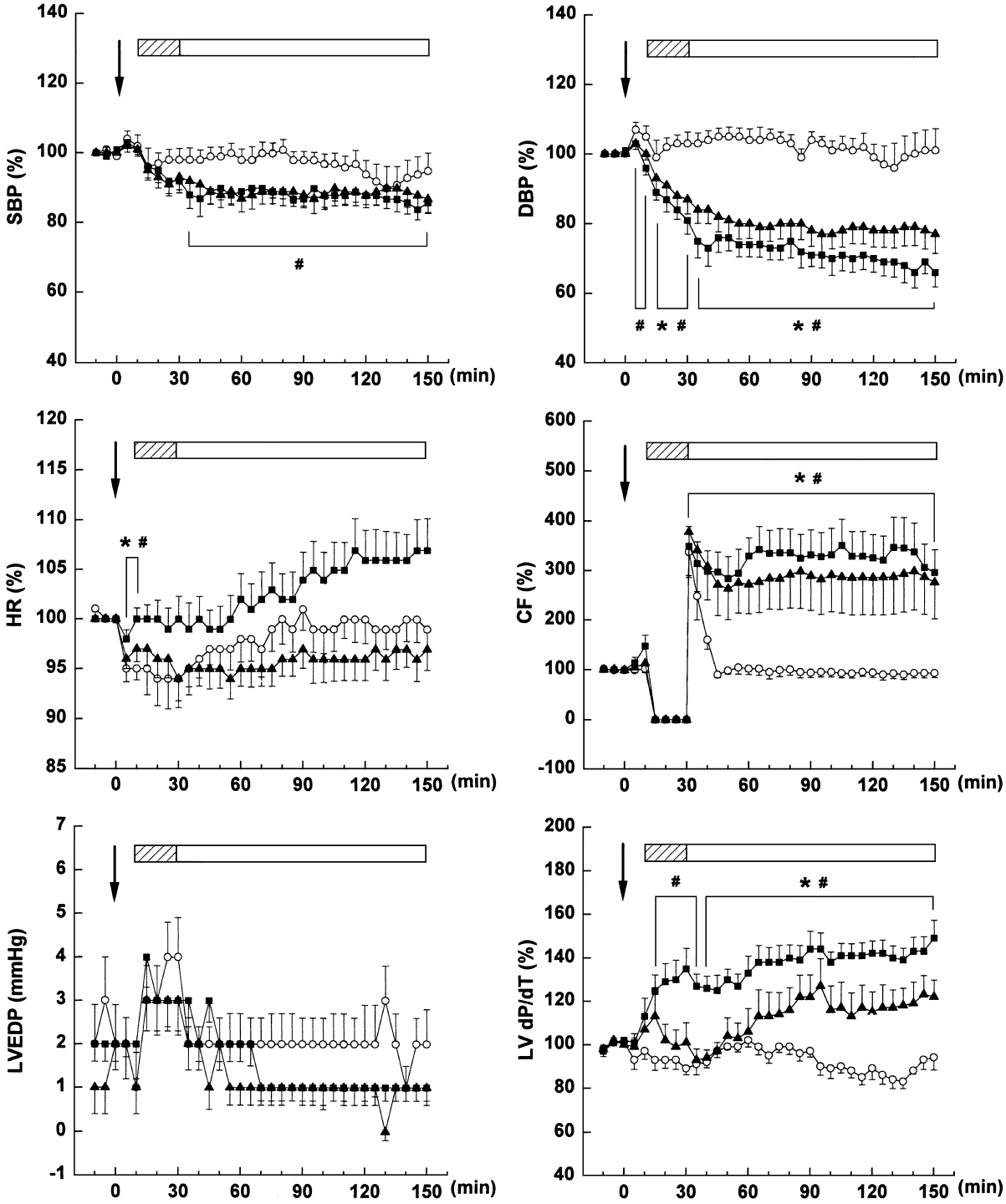

Fig. 1. The effect of minoxidil on systolic and diastolic blood pressures (SBP and DBP), heart rate (HR), coronary flow (CF), LVEDP and LVdP/dT during ischemia and reperfusion. The values of hemodynamic parameters were normalized to the respective pre-injection values and expressed as % changes, except for LVEDP (see Results for detail). After 10 min of control measurement, either dimethylsulfoxide (DMSO) (circle) or minoxidil (0.3 mg/kg, triangle or 1.0 mg/kg, square) was injected intravenously over a period of 30 s at the injection volume of 0.1 ml/kg (arrows). The left anterior descending coronary artery was ligated 20 min after the injection (ischemia shown by hatched column). After 20 min of coronary ligation, the ligated coronary artery was released, so that the ischemic myocardium was reperfused (reperfusion shown by empty column). Symbols indicating significance within groups are not shown to avoid complexity. $^{*}P<0.05$, difference in overall changes after injection, during ischemia, or during reperfusion between the DMSO-treated and 0.3 mg/kg minoxidil-treated groups. $^{\#}P<0.05$, difference in overall changes after injection, during ischemia, or during reperfusion between the DMSO-treated and 1.0 mg/kg minoxidil-treated groups.

again decreased in the latter phase of reperfusion. The LVdP/dT in the 0.3 mg/kg minoxidil-treated group increased, although it transiently decreased during ischemia. Minoxidil increased the LVdP/dT; the values in the 1.0 mg/kg minoxidil-treated group during ischemia and those in the 0.3 and 1.0 mg/kg minoxidil-treated groups during reperfusion were significantly higher than those in the DMSO-treated group, respectively.

Percent changes of rate-pressure products calculated by heart rate × systolic blood pressure are shown in Fig. 2. Basal values in the DMSO-treated, 0.3 mg/kg minoxidil-treated and 1.0 mg/kg minoxidil-treated groups were 28 ± 3, 23 ± 2 and $25 \pm 1 \cdot 10^3$ mmHg/min, respectively. There was no significant difference among the values. DMSO and minoxidil did not alter the rate-pressure product. In the DMSO-treated group, the rate-pressure product decreased by about 7% during ischemia. Reperfusion recovered the rate-pressure product that had decreased during ischemia. In the minoxidil-treated groups, the rate-pressure product also decreased during ischemia by about 12% and decreased further even after reperfusion. The rate-pressure products during reperfusion in both minoxidil-treated groups were lower than that in the DMSO-treated group, and that in the 1.0 mg/kg minoxidil-treated group appeared to be higher than that in the 0.3 mg/kg minoxidil-treated group.

There was no significant differences in basal myocardial contraction calculated as segment shortening between the groups: 0.198 ± 0.024 in the DMSO-treated, 0.195 ± 0.007 in the 0.3 mg/kg minoxidil-treated, and 0.218 ± 0.030 in the 1.0 mg/kg minoxidil-treated groups. The values of segment shortening were normalized to the respective pre-injection values and expressed as % changes. Changes in myocardial contraction before, during, and after ischemia are shown as % segment shortening in Fig. 3. In the DMSO-treated group, myocardial contraction decreased by ischemia and returned toward its pre-ischemic level by reperfusion. However, the recovery of myocardial contraction during reperfusion was incomplete, indicating the stunning phenomenon (20). Minoxidil at either dose did not modify myocardial contraction before and during ischemia. However, the recovery of myocardial contraction during reperfusion was significantly enhanced by minoxidil as compared to that in the DMSO-treated group. There was no significant difference in the recovery between 0.3 and 1.0 mg/kg minoxidil-treated groups.

Metabolic changes

The levels of the energy and carbohydrate metabolites before and 20 min after ischemia and 120 min after reperfusion are summarized in Table 2. In the DMSO-treated group, ischemia significantly decreased the levels of ATP, TAN, ECP, and CrP, and increased those of G6P, F6P and lactate. The altered levels of these metabolites were returned by reperfusion towards their pre-ischemic levels, but the levels of ATP and TAN were still low and that of CrP became high as compared to the respective pre-ischemic levels. In the minoxidil-treated groups, similar responses to ischemia/reperfusion were observed. However, the changes in the levels of ATP, TAN, ECP, CrP, G6P, F6P and lactate were significantly attenuated by pretreatment with minoxidil at 0.3 mg/kg. There was no significant difference in the metabolite levels between the DMSO- and

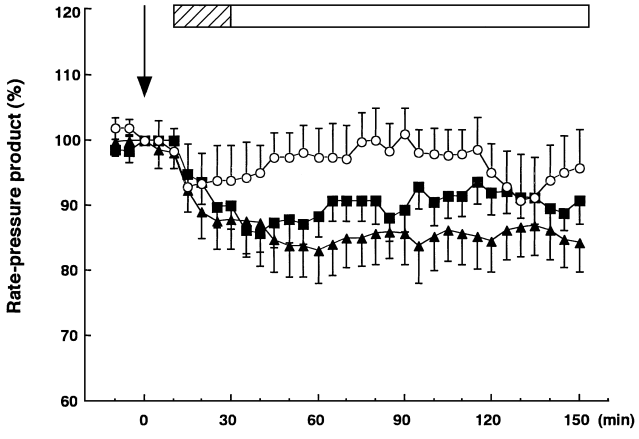

Fig. 2. Calculated rate-pressure products. Rate-pressure products were calculated from the values of heart rate and systolic blood pressure shown in Fig. 1. Symbols and experimental protocols are the same as those in Fig. 1.

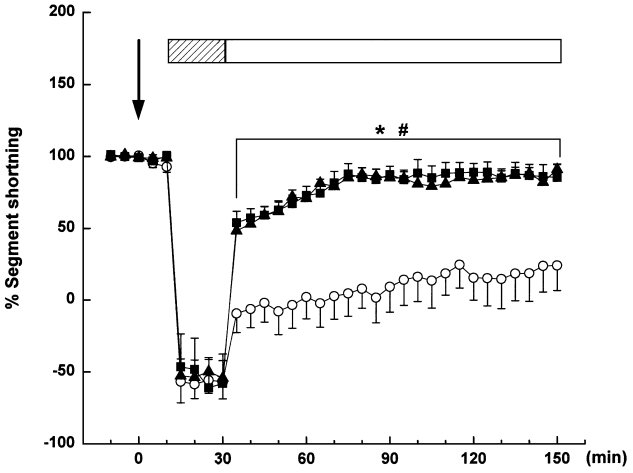

Fig. 3. The effect of minoxidil on myocardial contraction during ischemia and reperfusion. Myocardial contraction is expressed as % segment shortening, which is a percentage of the value obtained just before the injection. Symbols and experimental protocol are the same as those in Fig. 1. *P<0.05, difference in overall changes during reperfusion between the DMSO-treated and 0.3 mg/kg minoxidil-treated groups. $^{\#}P$<0.05, difference in overall changes during reperfusion between the DMSO-treated and 1.0 mg/kg minoxidil-treated groups.

Table 2. Metabolic data of the myocardium

Metabolites		DMSO-treated	Minoxidil-treated	
			0.3 mg/kg	1.0 mg/kg
ATP	before	4.95 ± 0.14	5.22 ± 0.13	5.25 ± 0.13
	ischemia	2.23 ± 0.23*	3.60 ± 0.39*,#	2.67 ± 18*
	reperfusion	3.43 ± 0.21*	3.74 ± 0.22*	3.90 ± 0.15*
ADP	before	0.90 ± 0.07	1.06 ± 0.11	1.00 ± 0.06
	ischemia	0.87 ± 0.06	0.96 ± 0.05	0.97 ± 0.05
	reperfusion	0.74 ± 0.07	0.77 ± 0.07*	0.78 ± 0.07*
AMP	before	0.27 ± 0.03	0.28 ± 0.01	0.25 ± 0.03
	ischemia	0.30 ± 0.03	0.28 ± 0.03	0.29 ± 0.02
	reperfusion	0.10 ± 0.02*	0.11 ± 0.02*	0.14 ± 0.04*
TAN	before	6.13 ± 0.17	6.57 ± 0.22	6.51 ± 0.15
	ischemia	3.40 ± 0.22*	4.84 ± 0.40*,#	3.93 ± 0.18*
	reperfusion	4.27 ± 0.25*	4.62 ± 0.27*	4.81 ± 0.18*
ECP	before	0.88 ± 0.01	0.88 ± 0.01	0.88 ± 0.01
	ischemia	0.78 ± 0.02*	0.84 ± 0.01*,#	0.80 ± 0.01*
	reperfusion	0.89 ± 0.01	0.89 ± 0.01	0.89 ± 0.01
CrP	before	5.88 ± 0.77	4.89 ± 0.85	5.13 ± 0.75
	ischemia	1.90 ± 0.46*	3.30 ± 0.39#	1.36 ± 0.18*
	reperfusion	9.41 ± 0.46*	8.99 ± 0.72*	8.51 ± 0.59*
G6P	before	0.29 ± 0.13	0.36 ± 0.13	0.24 ± 0.08
	ischemia	0.87 ± 0.12*	0.27 ± 0.05#	0.84 ± 0.15*
	reperfusion	0.12 ± 0.02	0.12 ± 0.03	0.14 ± 0.03
F6P	before	0.05 ± 0.02	0.07 ± 0.02	0.04 ± 0.02
	ischemia	0.23 ± 0.03*	0.06 ± 0.02#	0.22 ± 0.04*
	reperfusion	0.02 ± 0.01	0.02 ± 0.01*	0.03 ± 0.01
FDP	before	0.06 ± 0.02	0.08 ± 0.03	0.08 ± 0.04
	ischemia	0.07 ± 0.04	0.02 ± 0.01	0.03 ± 0.01
	reperfusion	0.01 ± 0.01*	0.01 ± 0.01*	0.03 ± 0.02
Pyr	before	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.02
	ischemia	0.14 ± 0.08	0.06 ± 0.01	0.05 ± 0.01
	reperfusion	0.06 ± 0.03	0.10 ± 0.08	0.07 ± 0.02
Lac	before	2.72 ± 0.83	2.54 ± 0.76	2.34 ± 0.46
	ischemia	20.95 ± 1.84*	10.10 ± 2.68*,#	19.10 ± 1.61*
	reperfusion	1.02 ± 0.46	0.75 ± 0.42*	0.50 ± 0.16*

Myocardial samples were obtained before ischemia (before), 20 min after ischemia (ischemia) and 120 min after reperfusion (reperfusion) in the DMSO- and minoxidil-treated groups. Data are means ± S.E.M. (μmol/g wet tissue weight except for ECP) of 5 – 8 observations in each treated group. TAN, total adenine nucleotide; ECP, energy charge potential; CrP, creatine phosphate; G6P, glucose 6 phosphate; F6P, fructose 6 phosphate; FDP, fructose 1,6 diphosphate; Pyr, pyruvate; Lac, lactate. *P<0.05, compared with "before" in each group. #P<0.05, compared with corresponding values in the "DMSO-treated" group.

minoxidil (1.0 mg/kg)-treated groups.

DISCUSSION

In the present study, pretreatment with minoxidil significantly improved myocardial contractile dysfunction due to ischemia/reperfusion termed stunning (Fig. 3). Braunwald and Kloner (20) have demonstrated that a period of coronary occlusion shorter than 20 min is not associated with the development of myocardial necrosis. Twenty minutes of ischemia used in the present study fits their definition. It has been shown that myocardial stunning is associated with a decrease in the tissue concentration of ATP (21), a loss of Ca^{2+} homeostasis (22), and formation of oxygen-derived free radicals (23). Minoxidil at 0.3 mg/kg significantly preserved the level of ATP in the 20-min ischemic heart, but was not effective on that in the 120-min reperfused heart (Table 2). The levels of TAN and CrP and ECP value in the 0.3 mg/kg minoxidil-treated ischemic myocardium were also higher than those in the DMSO-treated

ischemic myocardium. Overshoot phenomenon of CrP is often observed after reperfusion following brief ischemia (24). In the present study, the higher level of CrP was clearly observed during reperfusion as compared to that before ischemia (Table 2). Minoxidil at either dose did not affect the level of CrP obtained after reperfusion. The level of high-energy phosphates just before the onset of reperfusion, but not after reperfusion, could be responsible for the improvement of myocardial contractile dysfunction (25). However, at the higher dose of minoxidil (1.0 mg/kg), no significant preservation of high-energy phosphate was observed, although contractile function recovery after reperfusion was similar in extent to that of the 0.3 mg/kg minoxidil-treated group. The effect of minoxidil on myocardial contraction was not simply explained by preservation of high-energy phosphate during ischemia.

Minoxidil may affect the myocardial contractile function through its hemodynamic actions. Systolic and diastolic blood pressures were significantly decreased, and coronary flow during reperfusion was significantly increased by minoxidil (Fig. 1). Reduction of blood pressures decreases the cardiac work, resulting in a decrease in myocardial oxygen demand, whereas increased coronary flow increases myocardial oxygen supply. These findings may contribute to improve myocardial stunning. On the other hand, reduction of perfusion pressure of the coronary arteries due to the decrease in diastolic pressure could decrease blood supply for the ischemia/reperfusion area despite the increasing of coronary flow (coronary steal phenomenon) (26). Furthermore, minoxidil at 1.0 mg/kg gradually but significantly increased LVdP/dT during reperfusion, and it increased heart rate. These positive inotropic and chronotropic actions that are reflex responses to decreased blood pressure increase myocardial oxygen consumption (27). In fact, the rate-pressure product, calculated as an index of myocardial oxygen consumption, in the 1.0 mg/kg minoxidil-treated group was higher than that in the 0.3 mg/kg minoxidil-treated group, although there was no significant difference between them. (Fig. 2). These unfavorable effects of minoxidil, particularly at 1.0 mg/kg, may diminish its favorable effects on myocardial stunning and metabolism. This may be one of reasons why the effect of minoxidil at 1.0 mg/kg on myocardial stunning did not exceed that at the lower dose, 0.3 mg/kg, in the present study.

The levels of some glycolytic intermediates were measured, because oxygen deficiency could activate the glycolysis to produce ATP anaerobically. In fact, ischemia increased the myocardial levels of G6P, F6P, and lactate in the present study. If the accumulation of G6P, F6P and lactate due to ischemia reflects the anaerobic status of the myocardium, attenuation of the metabolite accumulation may mean the conversion of energy status of the myocardium from an anaerobic to aerobic one. Because minoxidil at 0.3 mg/kg significantly preserved ATP and attenuated the accumulation of G6P, F6P and lactate caused by ischemia, the myocardium appeared to be under aerobic conditions (Table 2).

In conclusion, intravenous injection of minoxidil at lower dose (0.3 mg/kg) clearly improved myocardial contractile dysfunction during reperfusion after brief ischemia in association with preservation of high-energy phosphates in the ischemic myocardium. Minoxidil at higher dose (1.0 mg/kg) also improved the contractile dysfunction during reperfusion, although it did not show any effects on the ischemia-induced metabolic derangement. Augmentation of hypotension caused by minoxidil at high dose may cause some reflex responses that attenuate its beneficial effects on myocardial metabolism. We have to pay attention to patients with ischemic heart disease when using minoxidil at high dose or frequently. However, the possible serum concentration of minoxidil is extremely low in topical use. From our rough calculation, the serum concentration of 1% topical minoxidil may be equivalent to that of 0.0005 mg/kg intravenous minoxidil.

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