COMPARISON OF THE EFFECTS OF DOBUTAMINE AND ISOPROTERENOL IN ISCHEMIC HEARTS BY PHOSPHORUS-31 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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We compared the effects of 2 inotropic agents, dobutamine and isoproterenol, on regional coronary blood flow, contractile function, hemodynamics and levels of phosphate compounds in the acutely ischemic canine heart. Dogs were instrumented to determine regional coronary blood flow (non-radioactive microsphere method), contractile function (sonomicrometry), and hemodynamics. Myocardial phosphate compounds were measured simultaneously by the phosphate-31 (^{31}P) magnetic resonance spectroscopic technique. Both drugs augmented the global performance of the heart, but produced no significant improvement in regional contractile function in the ischemic region. Isoproterenol significantly increased the ratio of inorganic phosphate to phosphocreatine in the ischemic region, as compared to dobutamine. However, no significant differences were seen in myocardial lactate consumption with inotropic stimulation between the 2 groups. ^{31}P magnetic resonance spectroscopy could be used to differentiate between the effects of dobutamine and isoproterenol on energy metabolism in ischemic myocardium despite the lack of significant differences in regional function and myocardial lactate consumption. The significant tachycardia without an augmentation of systemic blood pressure induced by isoproterenol may account for this unfavorable effect on myocardial energy metabolism.

(*Jpn Circ J* 1994; **58**: 338–350)

MAGNETIC resonance spectroscopy (MRS) is an attractive probe for studying cardiac physiology^{1–7}. Some clinical studies have evaluated the usefulness of 31 phosphorus (^{31}P) MRS for identifying the presence of myocardial disease^{2,3} while other experimental trials have investigated its abil-

ity to differentiate between reversible and irreversible ischemic myocardial injury⁴ and to evaluate myocardial salvage after ischemia and reperfusion by measuring concentrations of high-energy phosphate compounds⁵.

We designed the present study to determine whether ^{31}P MRS could differentiate between the effects of 2 synthetic cardioactive sympathomimetic amines, dobutamine and isoproterenol, on the metabolic status of flow-limited myocardium.

Key words:

Magnetic resonance spectroscopy
Inotropic agents
Ischemia
Myocardial phosphate compounds

(Received May 11, 1993; accepted October 20, 1993)

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Fig. 1. Schema of animal preparation

The coronary artery was narrowed by a 1-0 silk ligature once or twice around a 16 or 18-gauge needle and the LAD, respectively. The needle was withdrawn immediately after the knot was tied, so that the artery was not totally occluded.

Abbreviations; Ao; aorta, LAD; left anterior descending coronary artery, LCX; left circumflex coronary artery, PA; pulmonary artery

METHODS

Animal Preparation

A total of 39 adult mongrel dogs of either sex weighing between 9 and 15 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and intubated. The animals were then ventilated at a rate of 20/min and anesthetized with 0.75 to 1.50% halothane. Tidal volume and FIO_2 were adjusted to maintain arterial pH between 7.35 and 7.45 and PO_2 above 100 mmHg. Muscle relaxation was obtained by intravenous administration of pancuronium bromide (0.2 mg/kg).

A 6F polyethylene catheter was inserted into the femoral artery to monitor blood pressure and for sampling of arterial blood. Drugs were administered through another polyethylene catheter inserted into the femoral vein. A 7F high-fidelity micromanometer was passed retrogradely through the

carotid artery into the left ventricle to measure left ventricular pressure and its first derivative. A 5F Swan-Ganz catheter was introduced into the pulmonary artery via a left common cervical vein to measure cardiac output.

The chest was opened through the left fifth intercostal space and the heart was exposed by excising the pericardium parallel to the phrenic nerve. The left anterior descending coronary artery was dissected free, proximal to the first major epicardial left ventricular branch and encircled by a 1-0 silk ligature. A 6F polyethylene catheter was placed in the left atria for the injection of non-radioactive colored microspheres. Another polyethylene catheter was inserted into the great cardiac vein via the right atrial appendage for blood sampling. A surface coil, 20 mm in diameter, was then attached to the epicardial surface of the left ventricu-

lar wall over the region that became cyanotic (as determined by visual inspection) when the left anterior descending coronary artery was occluded. A pair of ultrasonic crystals was implanted in the myocardium below the coil to measure systolic function (Fig. 1). The animals were wrapped in a water-circulating blanket to maintain a body temperature and were placed in a spectroscopy unit.

Experimental Protocol

The study was divided into 2 parts. In Part 1, we administered dobutamine or isoproterenol to non-ischemic dogs to compare their effects on cardiac performance and myocardial concentrations of phosphate compounds and to determine the appropriate doses of these inotropic agents for use in the ischemic condition. In Part 2, we compared the effects of dobutamine and isoproterenol on cardiac performance and myocardial concentrations of phosphate compounds in the ischemic condition and evaluated the ability of ^{31}P -MRS to differentiate between the effects of these drugs on the metabolic status of flow-limited myocardium.

Baseline hemodynamic measurements and ^{31}P nuclear magnetic resonance (NMR) spectra were initially obtained during the control state. Spectroscopic data were acquired over a 30-min period in 3 consecutive 10-min acquisitions. Data from the last 10-min acquisition was used as the pre-ischemic control. After NMR data were obtained, we measured regional contractile function and cardiac output. Microspheres were then injected to determine regional coronary blood flow.

Part 1: After control measurements were obtained, dobutamine was administered via the femoral vein at doses of 5 and subsequently $10\text{ }\mu\text{g/kg/min}$ for 30 min (group D). Isoproterenol was administered at doses of 0.1 and subsequently $0.2\text{ }\mu\text{g/kg/min}$ in the same fashion (group I). After ^{31}P NMR spectra and hemodynamic measurements were obtained simultaneously during each period, regional contractile function and cardiac output were measured again. Microspheres were injected to determine regional coronary blood flow.

Part 2: After control measurements were obtained, the coronary artery was narrowed

by tying a 1-0 silk ligature once or twice around a 16- or 18-gauge needle, respectively, and the left anterior descending coronary artery. The needle was withdrawn immediately after the knot was tied to avoid totally occluding the artery. We selected the needle most suited to the size of each coronary artery. After a period of stabilization, spectral data were acquired for 30 min, and hemodynamic measurements were obtained simultaneously. After NMR data acquisition, regional contractile function and cardiac output were measured, and microspheres were again injected to determine regional coronary blood flow. After obtaining measurements during the ischemic baseline period, dobutamine ($10\text{ }\mu\text{g/kg/min}$) or isoproterenol ($0.1\text{ }\mu\text{g/kg/min}$) was administered and the measurements were repeated.

Following the experiment, each animal was killed with a lethal dose of potassium chloride. The location of the coil was marked and the heart was excised. All surgical procedures and experimentation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Measurement of Myocardial Blood Flow

Myocardial blood flow was measured with non-radioactive colored microspheres⁸. Microspheres, $15\text{ }\mu\text{m}$ in diameter, were agitated in an ultrasonic mixer before being injected. Approximately 1.5×10^6 microspheres were injected into the left atrial catheter and flushed with 10 ml of saline for each measurement. A reference arterial blood specimen was withdrawn through the femoral catheter, at a rate of 4 ml/min, starting 5 sec before the injection of microspheres and continuing for 180 sec. After the experiment, duplicate myocardial specimens were obtained from the anterior wall directly under the surface coil (ischemic region), as well as from the inferior wall (control region) of the left ventricle.

Measurement of Regional Contractile Function

Pairs of ultrasonic crystals were implanted in the myocardium to determine wall thickness to measure regional contractile function⁹. One crystal (2 mm in diameter) was inserted tangentially through the myocardium

Fig. 2. Representative spectra and hemodynamic tracings obtained in non-ischemic (control) myocardium. Group D (Fig. 2a) and group I (Fig. 2b). Both inotropic agents augmented regional contractile function and increased regional coronary blood flow. No significant changes were detected in Pi, Pcr or ATP peaks. Abbreviations; Ant; anterior wall, ATP; adenosine triphosphate, CBF; regional coronary blood flow, dP/dt; the first derivative of LVP, ED; end-diastole, ES; end-systole, Inf; inferior wall, LVP; left ventricular pressure, Pcr; phosphocreatine, Pi; inorganic phosphate, WT; wall thickness

to the subendocardium. The second crystal (4 mm in diameter), which was attached to a Dacron patch, was sewn to the epicardial surface over the subendocardial crystal. The pairs of crystals were placed below the coil to measure wall thickness in the ischemic region. Due to radiofrequency interference from the sonomicrometer cables, spectral data were obtained with the cables disconnected. End-diastolic dimensions were determined at the beginning of left ventricular contraction (at the onset of a positive first derivative of the left ventricular pressure signal). End-systolic wall thickness was determined approximately 20 ms before the peak negative first derivative of left ventricular pressure. The position of the crystals was confirmed at necropsy.

Spectrometer Preparation

All measurements were performed with cardiac and respiratory gating in a 25-cm bore 2.0-T spectroscopy unit (BEM 250/80; Otsuka Electronics). The ^{31}P and ^1H frequencies were 32.34 and 79.88 MHz, respectively. Radiofrequency transmission and signal detection were performed using a movable surface coil, 20 mm in diameter. The H_2O signal detected at 79.88 MHz through this surface coil was used to homogenize the magnetic field and to adjust the position of the animal in the magnet. We chose the most suitable pulse duration between 22 to 25 μs to obtain the maximal signal-to-noise ratio. The pulse interval was 3 sec and each measurement represented the sum of 200 induction decays, resulting in a total acquisition time of 10 min for each spectrum.

TABLE I HEMODYNAMIC AND METABOLIC DATA IN GROUP D NON-ISCHEMIC DOGS

	Control	Dobutamine	
		5 μ g/kg/min	10 μ g/kg/min
SBP (mmHg)	109 $\pm$ 8	124 $\pm$ 12	125 $\pm$ 9*
DBP (mmHg)	75 $\pm$ 7	85 $\pm$ 11	86 $\pm$ 9
HR (/min)	154 $\pm$ 8	166 $\pm$ 7*	184 $\pm$ 6†‡
RPP (KmmHg/min)	15.5 $\pm$ 1.9	18.7 $\pm$ 2.1*	20.9 $\pm$ 2.3*‡
dP/dt (KmmHg/sec)	1.35 $\pm$ 0.14	2.03 $\pm$ 0.24†	2.45 $\pm$ 0.26†§
%WT (%)	24.9 $\pm$ 3.0	30.4 $\pm$ 3.6†	31.4 $\pm$ 9.8†
CO (l/min)	1.51 $\pm$ 0.15	1.74 $\pm$ 0.11*	1.87 $\pm$ 0.13†
CBF (Ant) (ml/min/g)	0.92 $\pm$ 0.10	1.26 $\pm$ 0.13†	1.64 $\pm$ 0.13†
CBF (Inf) (ml/min/g)	0.95 $\pm$ 0.09	1.18 $\pm$ 0.12	1.53 $\pm$ 0.19*
pH	7.20 $\pm$ 0.05	7.25 $\pm$ 0.04	7.18 $\pm$ 0.06
Pi/Pcr	0.22 $\pm$ 0.05	0.22 $\pm$ 0.03	0.21 $\pm$ 0.05
Pcr/ATP	1.68 $\pm$ 0.11	1.69 $\pm$ 0.08	1.71 $\pm$ 0.08
MVO ₂ (ml/min/100 g)	8.9 $\pm$ 1.5	10.7 $\pm$ 1.5	13.3 $\pm$ 1.1*
Lactate consumption (mg/min/100 g)	7.1 $\pm$ 1.4	7.4 $\pm$ 1.5	7.1 $\pm$ 1.3

All measurements are mean $\pm$ S.E. (n=6)

Abbreviations; Ant; anterior wall, ATP; adenosine triphosphate, CBF; regional coronary blood flow, CO; cardiac output, DBP; diastolic blood pressure, dP/dt; the first derivative of left ventricular pressure, HR; heart rate, Inf inferior wall, MVO₂; myocardial oxygen consumption, Pcr; phosphocreatine, Pi; inorganic phosphate, RPP; rate pressure products, SBP; systolic blood pressure, %WT; % wall thickening

*p<0.05, †p<0.01 versus control. ‡p<0.05, §p<0.01 versus low-dose dobutamine.

Spectral Analysis

Tissue levels of myocardial phosphate compounds were estimated from the areas under individual peaks, which were measured manually with a planimeter. We evaluated the myocardial energy metabolism from the ratios of phosphate compounds (Pi/Pcr and Pcr/ATP) to avoid the possibility of tissue injury which may have been induced by the implantation of an internal reference to measure the absolute values of these compounds and to minimize the variations in data acquisition between the different animal preparations. In Part 2 of the experiment, the ratio of Pcr/ATP was not estimated. Since myocardial ATP and Pcr content both decreased (changed in the same direction) during ischemia, the ratio of Pcr/ATP may not be a reliable marker for ischemia in this condition. The chemical shift difference (S) between the Pi and Pcr resonances was measured to calculate the change in intracellular pH with ischemia and inotropic stimulation using the following equation¹⁰:

$$\text{pH} = 6.90 - \log ((S - 5.85)/(3.29 - S))$$

Calculations

The rate pressure product (K mmHg/min)

=systolic blood pressure (mmHg) $\times$ heart rate (/min)

Myocardial oxygen consumption (ml/min/100 g)

=the difference in oxygen content between arterial and coronary sinus blood (ml/ml) $\times$ regional coronary blood flow (ml/min/g) $\times$ 100.

Myocardial lactate consumption (mg/min/100 g)

=the difference in lactate concentrations between arterial and coronary sinus blood (mg/ml) $\times$ regional coronary blood flow (ml/min/g) $\times$ 100.

Statistical Analysis

Data are expressed as mean $\pm$ SE. Differences between values obtained in the same animal during non-ischemic and ischemic conditions were analyzed using Bonferroni's multiple comparisons test. Differences between values in groups D and I were analyzed using Student's unpaired t-test. A P value of less than 0.05 was considered statistically significant. We used the data in group D from our previous report.¹¹

RESULTS

Part 1: Effects of inotropic agents in the non-ischemic heart

Japanese Circulation Journal Vol.58, May 1994

TABLE II HEMODYNAMIC AND METABOLIC DATA IN GROUP I NON-ISCHEMIC DOGS

	Control	Isoproterenol	
		0.1 $\mu\text{g/kg/min}$	0.2 $\mu\text{g/kg/min}$
SBP (mmHg)	112 $\pm$ 5	114 $\pm$ 4	114 $\pm$ 5
DBP (mmHg)	73 $\pm$ 6	65 $\pm$ 4	63 $\pm$ 4
HR (/min)	143 $\pm$ 6	216 $\pm$ 8†	232 $\pm$ 7†§
RPP (KmmHg/min)	16.2 $\pm$ 1.1	24.7 $\pm$ 1.7†	26.5 $\pm$ 1.7†§
dP/dt (KmmHg/sec)	1.42 $\pm$ 0.07	2.80 $\pm$ 0.25†	3.28 $\pm$ 0.26†§
%WT (%)	22.7 $\pm$ 3.2	35.7 $\pm$ 2.6†	36.9 $\pm$ 3.1†
CO (l/min)	1.55 $\pm$ 0.07	2.32 $\pm$ 0.18†	2.61 $\pm$ 0.22†
CBF (Ant) (ml/min/g)	0.88 $\pm$ 0.06	1.41 $\pm$ 0.15*	1.60 $\pm$ 0.20*
CBF (Inf) (ml/min/g)	0.92 $\pm$ 0.07	1.26 $\pm$ 0.11*	1.55 $\pm$ 0.15*
pH	7.11 $\pm$ 0.02	7.06 $\pm$ 0.03	7.03 $\pm$ 0.02
Pi/Pcr	0.22 $\pm$ 0.02	0.18 $\pm$ 0.01	0.19 $\pm$ 0.01
Pcr/ATP	1.53 $\pm$ 0.07	1.64 $\pm$ 0.08	1.61 $\pm$ 0.05
MVO ₂ (ml/min/100 g)	7.6 $\pm$ 0.4	13.7 $\pm$ 1.5*	15.2 $\pm$ 1.7†§
Lactate consumption (mg/min/100 g)	5.0 $\pm$ 0.8	10.6 $\pm$ 1.9	10.4 $\pm$ 2.5

All measurements are mean $\pm$ S.E. (n=7)

Abbreviations; Ant; anterior wall, ATP; adenosine triphosphate, CBF; regional coronary blood flow, CO; cardiac output, DBP; diastolic blood pressure, dP/dt; the first derivative of left ventricular pressure, HR; heart rate, Inf inferior wall, MVO₂; myocardial oxygen consumption, Pcr; phosphocreatine, Pi; inorganic phosphate, RPP; rate pressure products, SBP; systolic blood pressure, %WT; % wall thickening

*p<0.05, †p<0.01 versus control. ‡p<0.05, §p<0.01 versus low-dose isoproterenol.

Representative spectra and hemodynamic tracings from a non-ischemic dog are shown in Fig. 2. Dobutamine (Fig. 2a) and isoproterenol (Fig. 2b) augmented regional contractile function and increased regional coronary blood flow. No significant changes in Pi, Pcr or ATP peaks were detected (Signal/noise ratio 15.9 in Fig. 2a, 32.5 in Fig. 2b).

Hemodynamic and Metabolic Effects of Inotropic Agents

Hemodynamic and metabolic data of non-ischemic hearts in groups D and I are summarized in Tables I and II, respectively. Dobutamine produced significant dose-dependent inotropic effects and an increase in regional coronary blood flow (Table I). Despite an increase in myocardial oxygen consumption, no significant changes were observed in Pi/Pcr, Pcr/ATP or intracellular pH with dobutamine. No significant changes in myocardial lactate consumption were seen in these non-ischemic animals.

Isoproterenol also produced significant dose-dependent inotropic effects and an increase in regional coronary blood flow (Table II). However, there was no marked

change in either systolic or diastolic blood pressure. Despite an increase in myocardial oxygen consumption, no consistent changes were observed in Pi/Pcr, Pcr/ATP or intracellular pH with isoproterenol. No significant changes in myocardial lactate consumption were seen in these non-ischemic animals.

The hemodynamic and metabolic changes associated with dobutamine at a dose of 10 $\mu\text{g/kg/min}$ and isoproterenol at a dose of 0.1 $\mu\text{g/kg/min}$ in non-ischemic myocardium are compared in Fig. 3. Pre-treatment control values are shown below the bars, and significant changes from pre-treatment control values are indicated by asterisks. No significant differences were observed between the 2 groups regarding the changes of regional coronary flow, regional contractile function ($P<0.10$), Pi/Pcr, intracellular pH and myocardial lactate consumption from control values.

Since the change in regional contractile function with dobutamine at a dose of 10 $\mu\text{g/kg/min}$ was not significantly different from that with isoproterenol at a dose of 0.1 $\mu\text{g/kg/min}$, we considered these concentrations of the drugs as equivalent doses in

Fig.3. Comparison of changes from pretreatment values induced by dobutamine and isoproterenol in non-ischemic (control) hearts with regard to regional coronary blood flow, regional contractile function, Pi/Pcr, intracellular pH and myocardial lactate consumption. Group D (open bars); group I (shaded bars).

*p<0.05, **p<0.01; significant changes from pretreatment values.

Abbreviations; CBF; regional coronary blood flow, Lac; myocardial lactate consumption, Pcr; phosphocreatine, Pi; inorganic phosphate, %WT; % wall thickening

Part 2 of the experiment.

Part 2: Effects of inotropic agents in the ischemic heart

Representative spectra and hemodynamic tracings from ischemic hearts are shown in Fig. 4. Regional coronary blood flow and regional contractile function were markedly decreased in the anterior wall after induction of coronary arterial stenosis. The increase in the Pi spectrum was accompanied by a decrease in the Pcr spectrum. In group D (Fig. 4a), dobutamine increased regional coronary blood flow and improved regional contractile function in the ischemic region. There were no marked changes in levels of myocardial phosphate compounds in the ischemic region. In group I (Fig. 4b), isoproterenol paradoxically reduced regional coronary blood flow and caused a deterioration in regional contractile function. Pi/Pcr increased prominently in the ischemic region (Signal/noise ratio 17.2 in Fig. 4a, 26.0 in Fig. 4b).

Effects of Ischemia on Hemodynamics and Myocardial Phosphate Compounds

Hemodynamic and metabolic data from ischemic hearts in both groups are summarized in Table III. After the induction of coronary arterial stenosis in both groups, there were no significant changes in systolic or diastolic blood pressure, heart rate, rate pressure products, the first derivative of left ventricular pressure or cardiac output. A significant decrease was observed in regional coronary blood flow in the anterior wall. No significant change was seen in regional coronary blood flow in the inferior wall. Regional contractile function was also reduced in both groups and a mild paradoxical motion occurred. Pi/Pcr increased markedly and intracellular acidosis was also observed. Myocardial consumption of oxygen and lactate decreased significantly in both groups. There were no significant differences in the control and pre-treatment ischemic baseline

Fig. 4. Representative spectra and hemodynamic tracings obtained in ischemic myocardium. After coronary arterial stenosis was induced, regional coronary blood flow and regional contractile function showed marked decreases in the anterior wall. The increase in the Pi spectrum was accompanied by a decrease in the Pcr spectrum. In group D (Fig. 4a), dobutamine increased regional coronary blood flow and improved regional contractile function. No significant changes were seen in the levels of myocardial phosphate compounds in the ischemic region. In group I (Fig. 4b), isoproterenol paradoxically reduced regional coronary blood flow and led to a deterioration in regional contractile function. Pi/Pcr increased prominently in this region.

Abbreviations; Pre; pre-treatment (ischemic baseline) value. Other abbreviations are the same as in Fig. 2.

values between groups D and I.

Hemodynamic and Metabolic Effects of Inotropic Agents

Hemodynamic and metabolic effects of inotropic stimulation in the ischemic state are presented in Table III. Dobutamine increased both systolic and diastolic blood pressure, although the change in systolic blood pressure was not statistically significant ($P < 0.10$). On the other hand, isoproterenol changed neither systolic nor diastolic blood pressure. Thus, there were significant differences in both systolic and diastolic blood pressure during inotropic stimulation between the 2 groups. Dobutamine and iso-

proterenol significantly increased heart rate, rate pressure products, the first derivatives of left ventricular pressure and cardiac output. These systemic hemodynamic parameters with inotropic agents were similar between the 2 groups.

Dobutamine induced a modest, but not statistically significant, increase in regional coronary blood flow in the ischemic regions. On the other hand, there was no significant change in regional coronary blood flow in the anterior wall in group I. Neither group showed a significant change in regional contractile function. Dobutamine produced no significant changes in intracellular pH or Pi/Pcr in the ischemic region. In contrast,

TABLE III COMPARISON OF HEMODYNAMIC AND METABOLIC DATA IN ISCHEMIC DOGS

	Ischemia					
	Control		Pretreatment		Treatment	
	group D	group I	group D	group I	group D	group I
SBP (mmHg)	123±5	122±6	125±4	121±5	155±7	126±9
DBP (mmHg)	82±4	78±5	86±3	80±5	103±5‡	69±4 ¶
HR (/min)	144±4	146±5	145±4	145±5	198±5§	216±9§
RPP (KmmHg/min)	17.6±0.7	17.8±0.9	18.0±0.6	17.4±0.6	30.3±1.3§	27.2±2.0§
dP/dt (KmmHg/sec)	1.55±0.05	1.55±0.11	1.42±0.04	1.36±0.07	3.21±0.14§	2.83±0.22§
%WT (%)☆	21.8±2.2	25.3±3.2	-3.5±0.8†	-0.6±3.3†	-1.9±1.8	-3.2±3.1
CO (l/min)	1.71±0.07	1.59±0.17	1.52±0.08	1.38±0.12	2.24±0.13§	2.61±0.29§
CBF (Ant) (ml/min/g)	0.98±0.06	1.04±0.14	0.44±0.04†	0.43±0.08†	0.65±0.10	0.37±0.06
CBF (Inf) (ml/min/g)	1.01±0.04	1.00±0.13	1.07±0.08	0.97±0.11	1.80±0.13§	1.67±0.19‡
pH	7.13±0.02	7.18±0.05	6.88±0.05†	6.99±0.06*	6.93±0.02	6.88±0.04
Pi/Pcr	0.24±0.01	0.26±0.04	0.95±0.11†	1.04±0.12*	1.23±0.15	2.17±0.35§ ¶
Pcr/ATP	1.56±0.04	1.60±0.07	N.M.	N.M.	N.M.	N.M.
MVO ₂ (ml/min/100 g)☆	9.3±0.6	9.4±1.0	4.1±0.4†	4.0±0.6†	6.6±1.0‡	3.9±0.6
Lactate consumption (mg/min/100 g)☆	11.1±1.1	9.2±2.2	3.7±0.7†	3.5±1.3*	4.8±1.0	2.8±0.8

All measurements are mean±S.E. (group D N=18 ☆N=16, group I N=8)

Abbreviations; Ant; anterior wall, ATP; adenosine triphosphate, CBF; regional coronary blood flow, CO; cardiac output, DBP; diastolic blood pressure, dP/dt; the first derivative of left ventricular pressure, HR; heart rate, Inf inferior wall, MVO₂; myocardial oxygen consumption, N.M.; not measured, Pcr; phosphocreatine, Pi; inorganic phosphate, RPP; rate pressure products, SBP; systolic blood pressure, %WT; % wall thickening

*p<0.05, †p<0.01 versus control. ‡p<0.05, §p<0.01 versus pretreatment (ischemic) value. ||p<0.05,

¶p<0.01 group D versus I.

isoproterenol produced a significant increase in Pi/Pcr. Thus, significant differences in regional coronary blood flow of the anterior wall and in Pi/Pcr were seen between the 2 groups following inotropic stimulation.

Dobutamine also increased myocardial oxygen consumption, without any significant effects on myocardial lactate consumption. Isoproterenol produced no significant changes in either myocardial oxygen or lactate consumption. Myocardial oxygen consumption during inotropic stimulation was significantly greater in group D than in group I.

The hemodynamic and metabolic changes associated with dobutamine at a dose of 10 µg/kg/min and isoproterenol at a dose of 0.1 µg/kg/min in ischemic myocardium are compared in Fig. 5. Baseline pre-treatment ischemic values are shown below the bars, and significant changes from baseline ischemic values are indicated by asterisks. Baseline ischemic hemodynamic and metabolic data in group I were similar to those in group D. The changes induced by isopro-

terenol in regional coronary blood flow, Pi/Pcr, and intracellular pH in the ischemic region were significantly different than those with dobutamine.

DISCUSSION

Inotropic agents have been used to treat "low output" status in patients with ischemic heart disease and dilated cardiomyopathy. However, sustained inotropic stimulation of the failing heart may produce deleterious effects that could reduce, or even negate, the clinical benefits of these agents¹²⁻¹⁸. Since the deleterious effects of inotropic stimulation have been reported to be related in part to a deterioration of the metabolic status of the ischemic myocardium^{12,13}, useful methods for estimating myocardial metabolic status have been sought. ³¹P MRS has been used to identify the presence of myocardial disease¹, to differentiate between reversible and irreversible ischemic myocardial injury⁴, to evaluate the response of myocardium to various drug therapies⁵, and to investigate the

Fig. 5. Comparison of changes from pretreatment ischemic values induced by dobutamine and isoproterenol in ischemic hearts with regard to regional coronary blood flow, regional contractile function, Pi/Pcr, intracellular pH and myocardial lactate consumption. Group D (open bars); group I (shaded bars).

*p < 0.05, **p < 0.01; significant changes from pretreatment values. Abbreviations are the same as in Fig. 3.

correlation between myocardial contractile function and high-energy phosphate compounds⁶ in various experimental situations. Alterations in phosphate metabolism in patients with myocardial infarction² and dilated cardiomyopathy³ have been recently reported. In this study, dobutamine and isoproterenol each augmented the global hemodynamical performance of the ischemic heart, but failed to improve regional contractile function in the ischemic region. Thus, with regard to functional aspects, we found no significant differences in the response of regional function in ischemic myocardium to dobutamine or isoproterenol at the doses used in our experiments. However, ³¹P-MRS distinguished between the effects of these drugs on myocardial phosphate compounds. In particular, isoproterenol significantly increased Pi/Pcr and slightly reduced intracellular pH, while dobutamine produced no significant changes in these parameters.

It has been well established that concen-

trations of phosphate compounds, especially Pcr and Pi, are sensitive to an imbalance in the supply and demand of oxygen.¹⁴ A reduction in the supply of oxygen to the heart induces a decrease in Pcr, an increase in Pi, and a decrease in intracellular pH. If the ischemic episode is sufficiently severe or prolonged, myocardial ATP levels progressively decline, which eventually leads to irreversible injury of myocardial cells. Our results demonstrated that isoproterenol significantly advanced ischemia in the flow-limited region, as compared with dobutamine, which agrees with previous reports.¹⁵⁻¹⁷ The deterioration of myocardial energy metabolism may be responsible for the previously reported adverse effects associated with isoproterenol.¹⁵⁻¹⁷

The adverse effects of isoproterenol on myocardial energy metabolism are responsible in part for its inability to increase regional coronary blood flow in the ischemic region despite the increase in cardiac work. With

significant coronary arterial stenosis, the flow distal to the stenosis depends on the duration of diastole and on the coronary perfusion pressure.¹⁸ In this study, isoproterenol failed to augment systolic pressure, which is equivalent here to perfusion pressure, and thus induced significant tachycardia. Since significant tachycardia would be expected to shorten the duration of diastole,¹⁹ prominent tachycardia without augmentation of systemic pressure with isoproterenol seems to be responsible for its inability to increase regional coronary blood flow in the ischemic region.

Two major metabolites in myocardium are regulated by two different adrenoceptors. The release of free fatty acids and glucose are stimulated primarily by beta-1 and beta-2 adrenoceptors, respectively.^{20,21} It has been reported that dobutamine primarily stimulates beta-1 adrenoceptors while isoproterenol stimulates beta-1 and 2 adrenoceptors non-selectively.²² The difference in the selectivity for adrenoceptors between the 2 drugs may be account for their different effects on myocardial energy metabolism.

Lactate metabolism (lactate production and extraction) has also been used to assess myocardial ischemia.²³ We measured myocardial lactate consumption to estimate the amount of anaerobic glycolysis that was induced by inotropic stimulation. We observed no significant changes in myocardial lactate consumption despite a marked increase in Pi/Pcr in group I. The effect of dilution from the non-ischemic region of the left ventricle may account for this dissociation.

In our study, the regional contractile function did not significantly differ between the 2 groups despite significant differences in Pi/Pcr and intracellular pH. Pi/Pcr is well known to be closely correlated with regional contractile function in ischemic myocardium.²⁴ The precise mechanisms of this dissociation of myocardial phosphate compounds from regional contractile function are not known. Inotropic actions of catecholamines may modify this relation through their effects on actin-myosin crossbridge formation, or by influencing the calcium transients required for excitation and contraction coupling.²⁵ We estimated myocardial energy metabolism by measuring the relative content of phosphate compounds with ³¹P-

MRS. ³¹P-MRS does not detect all of the phosphate compounds in tissue. The comparison of the concentration of myocardial phosphate compounds determined by ³¹P-MRS with those obtained by chemical analysis suggests that some compartments of intracellular ATP are not detected by ³¹P-MRS.²⁶ However, the levels of ATP measured by the two methods are reported to change in parallel during ischemia.²⁶ The release of phosphate compounds from ischemic myocardium may have also affected the results in our study, which did not measure the absolute values of myocardial phosphate compounds. Under conditions of moderate ischemia, myocardial ATP levels decline during the first 30 min after induction of ischemia and are nearly stable thereafter for 4 or 5 h.²⁷ Therefore, we believe that the changes in the levels of phosphate compounds induced by inotropic stimulation may reflect myocardial energy metabolism in our experiment.

Another limitation of this study is related to the difficulty in accurately measuring phosphate metabolites and pH using ³¹P MRS. While the peak resonances of Pcr and ATP can be measured with great confidence in these spectra, it is difficult to detect Pi resonance, especially under non-ischemic conditions. Accurate measurement of the intensity and chemical shift of Pi resonance was difficult in some cases in our study. We assumed the Pi resonance to be the most up-field peak that allowed a relatively consistent assignment of the Pi chemical shift. During ischemia, Pi resonance was easily identified and its area and chemical shift were measured accurately. However, the contribution of overlapping signals from phosphomonoesters or 2, 3-diphosphoglycerate in the blood pool and the heterogeneity of pH in the ischemic region cannot be estimated. We considered that the inotropic effects of dobutamine and isoproterenol at the doses in Part 2 of our experiment were equivalent since there were no significant differences in the changes in regional contractile function between the 2 groups. However, the increase in regional contractile function from pretreatment ischemic values induced by isoproterenol was almost twice that with dobutamine. Therefore, isoproterenol may increase the demand for myocardial oxygen beyond

that with dobutamine. This difference in the inotropic action between 2 drugs may have influenced the results of our study.

In summary, dobutamine and isoproterenol augmented the global contractile function of the left ventricle. However, neither dobutamine nor isoproterenol significantly changed regional contractile function in the ischemic region. Although neither of these inotropic agents induced a significant change in myocardial lactate consumption, isoproterenol significantly deteriorated energy metabolism in flow-limited myocardium, which was detected by ^{31}P -MRS, as compared with dobutamine.

Acknowledgments

The authors thank Keiko Koga, Takayuki Sogabe and Mari Fukunaga (Bioenergetics Research Center, Tokushima Research Institute, Otsuka Pharmaceutical Company, Ltd.) for their valuable suggestions and assistance in this investigation.

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