Effect of Preischemic Catecholamine Treatment on Ischemia-Reperfusion Injury of the Myocardium

— Subtype, Dose, and Temperature Dependency —

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Preischemic adrenergic stimulation may affect postischemic cardiac function. Using an isolated working heart model, we investigated the effects of preischemic catecholamine treatment on postischemic recovery. Hearts from Wistar rats were perfused in working mode for 20 min, in Langendorff mode for 15 min, and again in working mode for 20 min (W2). Hearts were treated with isoproterenol (8.0 and 40.0 nmol/L), phenylephrine (0.06, 0.30, and 1.50 μ mol/L), or epinephrine (16 and 80 nmol/L) during the W2 period and then arrested with St Thomas' Hospital cardioplegic solution (STH) and subjected to global ischemia (37 °C or 20 °C), followed by reperfusion. At 37 °C, isoproterenol had a beneficial effect at the lower dose but a harmful effect at the higher dose; phenylephrine and epinephrine had a harmful effect at all doses. At 20 °C, isoproterenol and epinephrine had a harmful effect at a high dose; phenylephrine had no harmful effect at any dose. In a separate study, the influence of calcium modulators (diltiazem and ryanodine, added in the STH) on the catecholamine effect was investigated. The harmful effect of preischemic treatment with isoproterenol (24.0 nmol/L) or phenylephrine (0.9 μ mol/L) was abolished by the calcium modulators. Thus, preischemic β -adrenergic or $\alpha + \beta$ -adrenergic stimulation has a deleterious effect on postischemic recovery of the myocardium. The effect could be altered depending on the subtype and dose of catecholamine and the ischemic temperature. Intracellular calcium movement could be involved in the mechanism responsible for the harmful effect of preischemic catecholamine treatment. (*Jpn Circ J* 1998; 62: 517–526)

Key Words: Ischemia-reperfusion-induced injury; Catecholamine; Rat

Positive inotropic agents such as catecholamines are sometimes required preoperatively for patients with unstable hemodynamics caused by valvular or ischemic heart disease. In addition, most heart transplantation donors undergo medical treatment with positive inotropic agents before their hearts are harvested.^{1,2} Some of those patients occasionally suffer from severe low cardiac output syndrome as a result of ischemia-reperfusion injury of the myocardium even after a satisfactory operation.³ Catecholamines may alter the extent of myocardial damage during ischemia and reperfusion when given before or during myocardial ischemia. Little is known, however, about the effect of preischemic catecholamine treatment upon ischemia-reperfusion injury of the myocardium and, in particular, the appropriate choice of catecholamines and their optimal doses.

We have previously demonstrated, using an isolated rat heart preparation, that a β -adrenergic agent (isoproterenol), a phosphodiesterase inhibitor (milrinone), and an exogenous cyclic AMP agent (dibutyl cyclic AMP) have

deleterious effects on postischemic recovery when given before ischemia^{4,5} suggesting that an increase in cyclic AMP might accelerate myocardial injury during ischemia and reperfusion. Cyclic AMP is known to enhance transsarcolemmal calcium influx through cyclic AMP-dependent protein kinase activation. An α -adrenergic agent is known to have some inotropic effect on the myocardium, although the mechanism of its inotropism is different from that of a β -adrenergic agent, which may alter the extent of myocardial damage during ischemia and reperfusion. Hypothermic cardioplegia with topical cooling of the heart is an important method of myocardial protection in open heart surgery. It is a crucial point in a clinical setting to determine how catecholamines work when given before hypothermic ischemia.

Questions that arise are: (1) Is there dose dependency in the deleterious effect of preischemic β -adrenergic stimulation on the ischemia-reperfusion injury? (2) Is there any effect of preischemic α -adrenergic stimulation on the ischemia-reperfusion injury? (3) Is there dose dependency in the effect of calcium modulators on the catecholamine-induced injury? (4) Is there temperature dependency in the effects of catecholamines or calcium modulators? The objectives of the present study were to determine: (1) the effect of catecholamines (β -adrenergic agent, α -adrenergic agent, and $\alpha + \beta$ -adrenergic agent) at different concentrations on ischemia-reperfusion injury; (2) whether calcium modulators (diltiazem and ryanodine) influence the effect of preischemic catecholamine treatment; and (3) whether there are differences in those effects between normothermic ischemia (37 °C) and hy-

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Fig 1. Experimental protocols. After the first 20-min working perfusion (W1), hearts were Langendorff perfused, followed by the second 20-min working perfusion (W2) with various catecholamines. Hearts were then arrested with a 3-min infusion of St Thomas' Hospital cardioplegic solution (STH) and subjected to normothermic (37°C) or hypothermic (20°C) global ischemia. This was followed by 15 min of Langendorff reperfusion and the third 20-min working perfusion (W3). Protocol A: Time-matched controls; protocol B, C, and D: global ischemia and reperfusion (diltiazem was added to STH in protocol C; ryanodine was added to STH in protocol D).

pothemic ischemia (20°C).

Materials and Methods

Animals

Hearts were obtained from male Wistar strain rats, weighing 250–350 g. The animals were taken care of according to the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No 80–23, revised in 1978).

Perfusion Methods

The isolated perfused working heart model, which has been described in detail by Hearse et al⁶ is a left heart preparation, in which an oxygenated perfusion medium enters the cannulated left atrium at a hydrostatic pressure of 12 cmH₂O and is passed into the left ventricle, from which it is spontaneously ejected via an aortic cannula against a hydrostatic pressure of 100 cmH₂O. Coronary effluent leaving from the right heart can be sampled for the measurement of creatine kinase (CK) leakage or pooled and recirculated with the aortic flow. Langendorff non-working perfusion is introduced by clamping the left atrial cannula and introducing perfusion medium into the aorta from a reservoir located 100 cm above the heart. Ischemic cardiac arrest can be induced in this preparation by clamping the aortic cannula. The cardioplegic solution is infused using a reservoir located 60 cmH₂O above the heart. The perfusion medium was a modified Krebs-Henseleit bicarbonate buffer (KHBB) of the following composition (in mmol/L): NaCl, 118.5; NaHCO₃, 25.0; KCl, 4.8; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 1.4; and glucose, 11.0. The buffer was filtered before use and was gassed with 95% oxygen and 5% carbon dioxide. St Thomas' Hospital cardioplegic solution was used as a cardioplegic solution, and has the following composition (in mmol/L): NaCl, 110.0; NaHCO₃, 10.0; KCl, 16.0; MgCl₂, 16.0; and CaCl₂, 1.2. The pH of the cardioplegic solution was adjusted to 7.8 at any temperature before use.

The rats were anesthetized with diethyl ether, and heparin (200 IU) was injected into the femoral vein. The hearts were placed into a 4°C KHBB solution after rapid extirpation, and cannulation of the aorta was performed within 1 min. Hearts were subjected to 5 min of aerobic Langendorff perfusion, followed by a first working perfusion (20 min), a Langendorff perfusion (15 min), and a second working perfusion (20 min). Hearts were then arrested with a 3-min infusion of St Thomas' Hospital cardioplegic solution and subjected to normothermic or hypothermic global ischemia (30 or 33 min at 37°C and 120 min at 20°C). This was followed by a Langendorff reperfusion (15 min) and a third working perfusion (20 min). The duration of ischemic arrest was selected from the preliminary experiments, which identified the periods resulting in about 70% of postischemic recovery of aortic flow at each temperature (the data are not shown). CK leaked into the coronary effluent during 15-min Langendorff reperfusion was measured by the Oliver UV method⁷.

Experimental Protocols (Fig 1)

Preischemic Treatment with a β -Adrenergic Stimulator

Time-Matched Controls Isolated hearts were subjected to continuous aerobic perfusion without ischemia to serve as time-matched controls. Isoproterenol (40.0 nmol/L) was treated during the second working perfusion (protocol A). This was followed by aerobic perfusion (15-min Langendorff perfusion and the third 20-min working perfusion). The dose of isoproterenol was chosen according to our previous studies^{4,5}.

Global Ischemia and Reperfusion Two different levels of concentration (ie a low dose and a high dose) were employed. Isoproterenol (8.0 and 40.0 nmol/L) was used for preischemic treatment during the second working perfusion (protocol B). Hearts were arrested with St Thomas' Hospital cardioplegic solution and then subjected to global ischemia (37°C or 20°C), followed by reperfusion.

In a separate study, the effect of diltiazem (protocol C) or ryanodine (protocol D) on the preischemic catechol-

Table 1 Changes in Indices of Cardiac Function During and After Isoproterenol or Phenylephrine Treatment (Time-Matched Controls)

Concentration of catecholamines	Aortic flow		Coronary flow		Heart rate		Aortic pressure	
	W2/W1 (%)	W3/W1 (%)	w2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)
Control	107.5±1.5	85.6±1.6	98.9±3.0	98.7±2.2	93.0±2.1	96.6±1.8	101.1±0.9	97.9±0.9
Isoproterenol (40.0 nmol/L)	124.2±3.4*	83.2±1.4	133.7±6.7*	95.7±3.2	114.3±2.5*	97.5±2.9	110.7±1.5*	94.9±1.0
Phenylephrine (1.50 µmol/L)	128.9±2.6*	88.5±1.7	131.7±4.2*	107.2±4.3	102.9±2.9*	104.3±6.9	115.6±2.5*	96.3±2.2

Values are expressed as mean±standard error of the mean. * $p<0.05$ vs the control group. W1, the control value measured during the first working perfusion; W2, the value measured during the second working perfusion; W3, the value measured during the third working perfusion. $n=6$.

amine treatment was examined. Isoproterenol (24.0 nmol/L) was used for preischemic treatment during the second working perfusion. Hearts were then arrested with St Thomas' Hospital cardioplegic solution containing diltiazem (0, 0.5, 1.0 and 5.0 µmol/L) or ryanodine (0, 0.175, 1.750 and 8.750 nmol/L) and subjected to global ischemia (37°C or 20°C), followed by reperfusion. The doses of diltiazem and ryanodine were chosen according to our previous studies⁸

Preischemic Treatment With an α -Adrenergic Stimulator
Time-Matched Controls Isolated hearts were subjected to continuous aerobic perfusion without ischemia to serve as time-matched controls. Phenylephrine (1.50 µmol/L) was used in treatment during the second working perfusion (protocol A). This was followed by aerobic perfusion (15-min Langendorff perfusion and the third 20-min working perfusion). The dose of phenylephrine was chosen according to our previous studies^{4,5}

Global Ischemia and Reperfusion Three different levels of concentration were employed. Phenylephrine (0.06, 0.30, and 1.50 µmol/L) was used for preischemic treatment during the second working perfusion (protocol B). Hearts were arrested with St Thomas' Hospital cardioplegic solution and then subjected to global ischemia (37°C or 20°C), followed by reperfusion.

In a separate study, the effect of diltiazem (protocol C) or ryanodine (protocol D) on the preischemic catecholamine treatment was examined. Phenylephrine (0.90 µmol/L) was used for preischemic treatment during the second working perfusion. Hearts were then arrested with St Thomas' Hospital cardioplegic solution containing diltiazem (0, 0.5, 1.0, and 5.0 µmol/L) or ryanodine (0, 0.175, 1.750, and 8.750 nmol/L) and subjected to global ischemia (37°C), followed by reperfusion.

Preischemic Treatment with $\alpha+\beta$ -Adrenergic Stimulator
Global Ischemia and Reperfusion Two different levels of concentration (ie a low dose and a high dose) were employed in preischemic catecholamine treatment. Adrenaline (16.0 and 80.0 nmol/L) was used for preischemic treatment during the second working perfusion (protocol B). Hearts were arrested with St Thomas' Hospital cardioplegic solution and then subjected to global ischemia (20°C or 37°C), followed by reperfusion. The concentrations in the lower dose group and the higher dose group were equivalent to respectively twice and 10-fold that of the serum concentration in patients administered as an initial dose in a clinical setting^{9,10}

Expression of Results

Change in Cardiac Function The following indices were measured as the cardiac function: aortic flow (AF), coronary flow (CF), heart rate (HR), and aortic pressure (AP). The change in cardiac function during adrenergic stimulation was calculated by the following equation.

$$\text{Change during catecholamine treatment} = \frac{W2}{W1} \times 100 (\%)$$

The postperfusion change (ie, recovery) in cardiac function in groups of time-matched controls was calculated using the following equation:

$$\text{Postperfusion recovery} = \frac{W3}{W1} \times 100 (\%)$$

The postischemic change (ie, recovery) in cardiac function in groups treated with global ischemia and reperfusion was calculated using the following equation:

$$\text{Postischemic recovery} = \frac{W3}{W1} \times 100 (\%)$$

where W1=the value of AF, CF, HR, or AP measured during the first working perfusion period; W2=the value of AF, CF, HR, or AP measured during the second working perfusion period; and W3=the value of AF, CF, HR, or AP measured during the third working perfusion period.

Statistical Analysis

Data are reported as means±standard error of the mean. All levels of statistical significance were calculated with 1-way analysis of variance in each study and multiple comparison (Dunnet's t test) was then performed. Values of $p<0.05$ were considered statistically significant.

Results

Preischemic Treatment With a β -Adrenergic Stimulator
Time-Matched Controls The change in AF during isoproterenol treatment (W2/W1 in Table 1) was significantly greater than for the control group. There was no difference in the postperfusion recovery of AF (W3/W1 in Table 1) between the control group and the treatment group. CK leakage resulted in no difference between the control group and the treatment group (4.6 ± 1.0 and 5.5 ± 0.9 IU/15 min per g dry weight, respectively).

Global Ischemia and Reperfusion At 37°C, the change in AF during the preischemic treatment (W2/W1 in Table 2) was significantly greater than in the control group in

Table 2 Changes in Indices of Cardiac Function During Isoproterenol Treatment and After Ischemia (Global Ischemia and Reperfusion)

Temperature during ischemia (°C)	Isoproterenol concentration (nmol/L)	Aortic flow		Coronary flow		Heart rate		Aortic pressure	
		W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)
37	Control	93.0±2.8	65.0±2.6	99.6±2.9	32.7±2.9	99.4±3.4	97.4±3.6	98.5±2.2	93.6±1.8
	8.0	105.1±1.5*	75.7±2.1*	109.3±2.7*	58.6±2.6	104.0±1.6	91.3±3.4	103.0±0.9	98.3±1.8
	40.0	89.7±5.0	12.6±5.2*	139.1±3.6*	46.2±19.1	126.0±5.6*	50.0±20.5*	110.1±2.5*	38.7±15.9*
20	Control	102.7±1.7	67.3±2.3	100.1±1.6	88.2±3.0	95.2±1.5	97.4±2.0	104.0±2.5	95.3±3.9
	8.0	115.7±4.7*	73.9±2.8	120.9±4.4*	94.3±3.2	99.7±0.7	94.6±3.7	105.8±2.2	92.8±2.0
	40.0	132.3±5.2*	20.5±8.7*	145.7±7.0*	57.9±18.5*	120.0±5.6*	63.3±20.1*	112.4±3.4*	56.8±13.1*

Values are expressed as mean±standard error of the mean. * $p<0.05$ vs the control group. W1, the control value measured during the first working perfusion; W2, the value measured during the second working perfusion; W3, the value measured during the third working perfusion. $n=6$.

Fig 2. Postischemic recovery of aortic flow (AF) and creatine kinase (CK) leakage after normothermic (37°C) or hypothermic (20°C) ischemia in hearts treated with isoproterenol during the second 20-min working perfusion. Values are expressed as mean±standard error of the mean. * $p<0.05$ vs the control group. Open columns: recovery of AF; hatched columns: CK leakage.

the 8.0 nmol/L group but not in the 40.0 nmol/L group. However, the changes in CF, HR, and AP in the 40.0 nmol/L group were significantly greater than in the control group (W2/W1 in Table 2). At 20°C, the change in AF during the preischemic treatment (W2/W1 in Table 2) was significantly greater than in the control group. At 37°C, the postischemic recovery of AF (W3/W1 in Table 2) was significantly greater in the 8.0 nmol/L group but significantly less in the 40.0 nmol/L group when compared with the control group (Fig 2, top). CK leakage tended to be greater in the 40.0 nmol/L group than in the control group (Fig 2, top). At 20°C, the postischemic recovery of AF (W3/W1 in Table 2) was significantly less in the 40.0 nmol/L group than in the control group (Fig 2, bottom). CK leakage was significantly greater in the 40.0 nmol/L group than in the control group (Fig 2, bottom).

In hearts arrested with the cardioplegic solution containing diltiazem or ryanodine, the change in AF during the preischemic treatment before the cardioplegic arrest (W2/W1 in Table 3 or Table 4) was significantly greater than in the control group at both ischemic temperatures.

At 37°C, the postischemic recovery of AF (W3/W1 in Table 3) was significantly less in the 0, 0.5, and 5.0

μmol/L diltiazem (plus 24.0 nmol/L isoproterenol) groups than in the control groups but was similar in the 1.0 μmol/L diltiazem (plus 24.0 nmol/L isoproterenol) group and the control group (Fig 3A, top). There was a significant increase in CK leakage in the 0 μmol/L diltiazem (plus 24.0 nmol/L isoproterenol) group than in the control group, but CK leakage was decreased to the control level in the 0.5, 1.0, and 5.0 μmol/L diltiazem (plus 24.0 nmol/L isoproterenol) groups (Fig 3A, top). The post-ischemic recovery of AF (W3/W1 in Table 4) was significantly less in the 0 nmol/L ryanodine (plus 24.0 nmol/L isoproterenol) group than in the control groups, but AF in the 0.175, 1.750, and 8.750 nmol/L ryanodine (plus 24.0 nmol/L isoproterenol) groups recovered to a level similar to that in the control group (Fig 3B, top). There was a significant increase in CK leakage in the 0 nmol/L ryanodine (plus 24.0 nmol/L isoproterenol) group compared with the control group, but CK leakage was decreased to the control level in the 0.175, 1.750, and 8.750 nmol/L ryanodine (plus 24.0 nmol/L isoproterenol) groups (Fig 3B, top).

At 20°C, the postischemic recovery of AF (W3/W1 in Table 3) was significantly less in the 0, 0.5, 1.0, and 5.0 μmol/L diltiazem (plus 24.0 nmol/L isoproterenol) groups than in the control group (Fig 3A, bottom). There was no difference in CK leakage between the control group and any other group (Fig 3A, bottom). The post-ischemic recovery of AF (W3/W1 in Table 4) was significantly less in the 0, 0.175, and 8.750 nmol/L ryanodine (plus 24.0 nmol/L isoproterenol) group than in the control groups, but AF in the 1.750 nmol/L ryanodine (plus 24.0 nmol/L isoproterenol) group recovered to a level similar to that in the control group (Fig 3B, bottom). There was a significant increase in CK leakage in the 0 nmol/L ryanodine (plus 24.0 nmol/L isoproterenol) group compared with the control group, but CK leakage decreased to the control level in the 0.175, 1.750, and 8.750 nmol/L ryanodine (plus 24.0 nmol/L isoproterenol) groups (Fig 3B, bottom).

Preischemic Treatment With α -Adrenergic Stimulator Time-Matched Controls The change in AF during the phenylephrine treatment (W2/W1 in Table 1) was significantly greater than in the control group. There was no difference in the postperfusion recovery of AF (W3/W1 in Table 1) between the control group and the treatment group. CK leakage resulted in no difference between the control group and the treatment group (4.6 ± 1.0 and 5.7 ± 2.0 IU/15 min per g dry weight, respectively).

Table 3 Changes in Indices of Cardiac Function During Isoproterenol Treatment and After Ischemia in Hearts Arrested With St Thomas' Hospital Cardioplegic Solution Containing Various Concentrations of Diltiazem (Global Ischemia and Reperfusion)

Temperature during ischemia (°C)	Isoproterenol (nmol/L)	Diltiazem (μmol/L)	Aortic flow		Coronary flow		Heart rate		Aortic pressure	
			W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)
37	Control	0	101.5±1.6	68.0±3.2	104.4±3.0	94.3±2.8	94.6±3.5	91.3±5.5	102.0±1.2	93.9±0.8
	24.0	0	127.9±2.2*	42.4±2.8*	139.5±4.2*	86.7±5.4	113.4±3.8*	91.4±2.7	117.4±3.7*	97.9±1.2*
	24.0	0.5	125.4±1.7*	48.2±4.4*	140.8±7.2*	85.2±3.8	108.5±2.9*	94.3±3.0	120.6±2.9*	88.8±1.6*
	24.0	1.0	127.1±3.5*	68.7±4.4	142.9±5.3*	91.3±3.3	110.5±1.0*	95.3±4.1	118.6±2.2*	95.3±1.7*
	24.0	5.0	122.8±3.7*	34.2±6.0*	142.1±7.5*	80.9±4.5	105.1±6.1	89.4±4.0	112.4±2.9*	87.7±1.8*
20	Control	0	103.8±1.9	68.9±2.2	99.0±1.5	88.0±3.0	95.8±1.6	95.9±2.2	103.7±2.5	92.2±1.3
	24.0	0	131.1±5.4*	56.1±2.4*	150.5±5.9*	88.1±3.6	108.3±5.5*	103.1±5.4	115.1±3.2*	88.9±3.4
	24.0	0.5	127.1±6.2*	43.8±4.2*	134.3±2.2*	78.2±3.6	112.1±3.7*	92.0±3.7	115.8±2.6*	85.9±1.5
	24.0	1.0	115.1±6.2*	36.3±2.5*	132.5±3.1*	77.8±5.2	107.3±3.6*	92.3±6.4	116.2±1.6*	88.4±3.5
	24.0	5.0	127.0±3.5*	47.9±4.6*	146.8±5.2*	89.4±6.7	112.9±3.5*	99.5±5.8	115.3±1.8*	86.5±1.1

Values are expressed as mean±standard error of the mean. **p*<0.05 vs the control group. W1, the control value measured during the first working perfusion; W2, the value measured during the second working perfusion; W3, the value measured during the third working perfusion. *n*=6.

Table 4 Changes in Indices of Cardiac Function During Isoproterenol Treatment and After Ischemia in Hearts Arrested With St Thomas' Hospital Cardioplegic Solution Containing Various Concentrations of Ryanodine (Global Ischemia and Reperfusion)

Temperature during ischemia (°C)	Isoproterenol (nmol/L)	Ryanodine (nmol/L)	Aortic flow		Coronary flow		Heart rate		Aortic pressure	
			W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)
37	Control	0	101.5±1.6	68.0±3.2	104.4±3.0	94.3±2.8	94.6±3.5	91.3±5.5	102.0±1.2	93.9±0.8
	24.0	0	127.9±2.2*	42.4±2.8*	139.5±4.2*	86.7±5.4	113.4±3.8*	91.4±2.7	117.4±3.7*	87.9±1.2*
	24.0	0.175	118.3±3.1*	65.5±5.5	131.1±4.7*	98.3±5.3	116.7±6.9*	93.5±2.7	114.5±3.3*	91.5±2.3
	24.0	1.750	120.6±5.1*	72.3±2.1	130.6±3.8*	87.5±4.0	105.3±6.4	94.2±3.9	116.6±2.9*	94.1±5.4
	24.0	8.750	127.0±3.5*	67.8±5.0	146.8±5.2*	89.2±4.7	112.9±3.5*	93.0±1.6	115.3±1.8*	89.7±2.1
20	Control	0	103.8±1.9	68.9±2.2	99.0±1.5	88.0±3.0	95.8±1.6	95.9±2.2	103.7±2.5	92.2±1.3
	24.0	0	131.5±5.4*	56.1±2.4*	150.5±5.9*	88.1±3.6	108.3±5.5*	103.1±5.4	115.1±3.2*	88.9±3.4
	24.0	0.175	120.2±4.4*	57.7±3.9*	125.0±5.3*	79.0±5.1	113.3±3.0*	101.1±2.2	105.2±3.5	87.3±1.6
	24.0	1.750	114.8±3.9*	69.0±3.0	123.0±7.6*	85.0±3.1	105.7±6.2	102.7±6.0	105.2±1.6	88.8±2.3
	24.0	8.750	125.7±4.0*	49.3±6.2*	139.4±1.6*	81.8±4.1	116.0±3.7*	98.9±4.7	114.1±3.7*	85.8±2.4

Values are expressed as mean±standard error of the mean. **p*<0.05 vs the control group. W1, the control value measured during the first working perfusion; W2, the value measured during the second working perfusion; W3, the value measured during the third working perfusion. *n*=6.

Fig 3. Postischemic recovery of aortic flow (AF) and creatine kinase (CK) leakage after normothermic (37°C) or hypothermic (20°C) ischemia in hearts treated with isoproterenol during the second 20-min working perfusion and arrested with St Thomas' Hospital cardioplegic solution containing diltiazem (A) or ryanodine (B). Values are expressed as mean±standard error of the mean. **p*<0.05 vs the control group. Open columns: recovery of AF; hatched columns: CK leakage.

Table 5 Changes in Indices of Cardiac Function During Phenylephrine Treatment and After Ischemia (Global Ischemia and Reperfusion)

Temperature during ischemia (°C)	Phenylephrine concentration (μmol/L)	Aortic flow		Coronary flow		Heart rate		Aortic pressure	
		W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)
37	Control	102.1±1.8	67.3±1.8	97.1±2.0	88.8±2.7	97.9±2.3	96.9±2.6	99.8±1.3	89.7±1.7
	0.06	109.3±2.4*	57.6±3.0*	1103.0±2.4	94.4±2.8	95.9±2.1	95.1±3.5	104.0±2.2	89.3±2.0
	0.30	120.5±3.4*	51.0±4.4*	116.9±3.2*	100.7±3.8*	100.2±1.3*	97.2±1.7	112.3±2.7*	88.9±1.5
	1.50	113.1±3.3*	30.2±3.3*	124.8±3.8*	80.2±12.9	98.5±2.0	74.4±11.7*	119.6±6.4*	77.8±12.1
20	Control	102.7±1.7	67.3±2.3	100.1±1.6	88.2±3.0	95.2±1.5	97.4±2.0	104.0±2.5	95.3±3.9
	0.30	116.6±1.5*	70.6±2.5	110.0±2.7*	88.1±3.9	95.9±1.4	100.7±2.2	110.8±4.6	90.5±1.6
	1.50	129.8±3.5*	71.4±1.9	129.5±3.3*	92.2±3.7	101.7±6.0*	100.3±4.9	112.1±2.6	91.8±2.2

Values are expressed as mean±standard error of the mean. * $p<0.05$ vs the control group. W1, the control value measured during the first working perfusion; W2, the value measured during the second working perfusion; W3, the value measured during the third working perfusion. $n=6$.

Fig 4. Postischemic recovery of aortic flow (AF) and creatine kinase (CK) leakage after normothermic (37°C) or hypothermic (20°C) ischemia in hearts treated with phenylephrine during the second 20-min working perfusion and arrested with St Thomas' Hospital cardioplegic solution. Values are expressed as mean±standard error of the mean. * $p<0.05$ vs the control group. Open columns: recovery of AF; hatched columns: CK leakage.

Global Ischemia and Reperfusion At both ischemic temperatures (37°C and 20°C), the change in AF during the pretreatment (W2/W1 in Table 5) was significantly greater than in the control group. At 37°C, the postischemic recovery of AF (W3/W1 in Table 5) was significantly less in the 0.06, 0.30, and 1.50 μmol/L groups than in the control group (Fig 4, top). CK leakage was significantly greater in the 1.50 μmol/L group than in the control group (Fig 4, top). At 20°C, there was no difference between the control groups and any other group in the postischemic recovery of AF (W3/W1 in Table 5; Fig 4, bottom). CK leakage resulted in no difference between the control group and any other group (Fig 4, bottom).

In hearts arrested with the cardioplegic solution containing diltiazem or ryanodine, the change in AF during the preischemic treatment before the cardioplegic arrest (W2/W1 in Table 6 or Table 7) was significantly greater than in the control group at an ischemic temperature of 37°C. The postischemic recovery of AF (W3/W1 in Table 6) was less in the 0 μmol/L diltiazem (plus 0.9 μmol/L phenylephrine) group than in the control group but was similar in the 0.5, 1.0, and 5.0 μmol/L diltiazem (plus 0.9 μmol/L phenylephrine) groups and the control group

(Fig 5A). There was a significant increase in CK leakage in the 0 μmol/L diltiazem (plus 0.9 μmol/L phenylephrine) group compared with the control group, but CK leakage decreased to the control level in the 0.5, 1.0, and 5.0 μmol/L diltiazem (plus 0.9 μmol/L phenylephrine) groups (Fig 5A). The postischemic recovery of AF (W3/W1 in Table 7) was significantly less in the 0 nmol/L ryanodine (plus 0.9 μmol/L phenylephrine) group than in the control groups, but AF in the 0.175, 1.750, and 8.750 nmol/L ryanodine (plus 0.9 μmol/L phenylephrine) groups recovered to a level similar to that in the control group (Fig 5B). There was a significant increase in CK leakage in the 0 nmol/L ryanodine (plus 0.9 μmol/L phenylephrine) group compared with the control group, but CK leakage decreased to the control level in the 0.175, 1.750, and 8.750 nmol/L ryanodine (plus 0.9 μmol/L phenylephrine) groups (Fig 5B).

Preischemic Treatment With an $\alpha+\beta$ -Adrenergic Stimulator

Global Ischemia and Reperfusion The change in AF during the pretreatment (W2/W1 in Table 8) was significantly greater than in the control group at both ischemic temperatures. At 37°C, the postischemic recovery of AF (W3/W1 in Table 8) was significantly less in the 16 and 80 nmol/L groups than in the control group (Fig 6, top). CK leakage in the 80 nmol/L group was significantly greater than that in the control group (Fig 6, top). At 20°C, the postischemic recovery of AF (W3/W1 in Table 8) was significantly less in the 80 nmol/L group than in the control group (Fig 6, bottom). There was no difference in CK leakage between groups (Fig 6, bottom).

Discussion

Intracellular calcium overload is, in general, a key mechanism of ischemia-reperfusion injury, and the positive inotropic action of catecholamines can be induced by an increase in intracellular calcium^{11,12}. Therefore, there is a possibility that preischemic treatment with catecholamines may aggravate calcium overload during ischemia and reperfusion and then accelerate ischemia-reperfusion injury. Our previous studies were able to confirm the dose-dependent acceleration of ischemia-reperfusion injury induced by preischemic treatment with either a phosphodiesterase inhibitor (milrinone) or a β -adrenergic stimulator (isoproterenol)^{3,4}. Most of the catecholamines such as dopamine, epinephrine, norepinephrine, and

Table 6 Changes in Indices of Cardiac Function During Phenylephrine Treatment and After Ischemia in Hearts Arrested With St Thomas' Hospital Cardioplegic Solution Containing Various Concentrations of Diltiazem (Global Ischemia and Reperfusion)

Temperature during ischemia (°C)	Phenylephrine (μmol/L)	Diltiazem (μmol/L)	Aortic flow		Coronary flow		Heart rate		Aortic pressure	
			W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)
37	Control	0	101.5±1.6	68.0±3.2	104.4±3.0	94.3±2.8	94.6±3.5	91.3±5.5	102.0±1.2	93.9±0.8
	0.9	0	118.7±2.1*	49.3±3.5*	121.2±3.8*	89.4±2.9	97.5±2.9	92.6±2.4	119.7±3.3*	91.0±1.2
	0.9	0.5	123.3±3.4*	69.8±3.6	124.5±2.1*	105.1±5.4	97.6±0.8	97.5±5.1	115.7±1.3*	91.3±2.0
	0.9	1.0	130.2±3.7*	78.8±1.4	133.4±2.0*	101.6±3.3	103.9±0.9	105.9±3.3	113.9±1.5*	91.3±1.4
	0.9	5.0	123.7±1.7*	64.7±2.9	123.0±4.1*	93.1±4.4	96.0±4.0	95.0±2.1	113.9±1.6*	94.6±1.7

Values are expressed as mean±standard error of the mean. **p*<0.05 vs the control group. W1, the control value measured during the first working perfusion; W2, the value measured during the second working perfusion; W3, the value measured during the third working perfusion. *n*=6.

Table 7 Changes in Indices of Cardiac Function During Phenylephrine Treatment and After Ischemia in Hearts Arrested With St Thomas' Hospital Cardioplegic Solution Containing Various Concentrations of Ryanodine (Global Ischemia and Reperfusion)

Temperature during ischemia (°C)	Phenylephrine (μmol/L)	Ryanodine (nmol/L)	Aortic flow		Coronary flow		Heart rate		Aortic pressure	
			W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)
37	Control	0	101.5±1.6	68.0±3.2	104.4±3.0	94.3±2.8	94.6±3.5	91.3±5.5	102.0±1.2	93.9±0.8
	0.9	0	118.7±2.1*	49.3±3.5*	121.2±3.8*	89.4±2.9	97.5±2.9	92.6±2.4	119.7±3.3*	91.0±1.2
	0.9	0.175	128.7±5.4*	73.5±4.5	124.1±4.0*	101.6±2.8	93.2±1.4	91.3±2.7	121.9±2.2*	97.7±2.6
	0.9	1.750	126.8±3.1*	76.2±2.1	133.1±4.7*	99.0±7.0	96.1±3.1	98.5±4.0	119.6±2.0*	96.3±1.8
	0.9	8.750	132.6±2.2*	64.4±5.9	129.4±3.7*	95.5±3.3	95.4±1.4	91.4±3.9	120.0±1.0*	94.8±1.9

Values are expressed as mean±standard error of the mean. **p*<0.05 vs the control group. W1, the control value measured during the first working perfusion; W2, the value measured during the second working perfusion; W3, the value measured during the third working perfusion. *n*=6.

Table 8 Changes in Indices of Cardiac Function During Adrenaline Treatment and After Ischemia (Global Ischemia and Reperfusion)

Temperature during ischemia (°C)	Adrenaline concentration (nmol/L)	Aortic flow		Coronary flow		Heart rate		Aortic pressure	
		W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)	W2/W1 (%)	W3/W1 (%)
37	Control	96.1±1.1	73.5±2.7	99.5±2.1	102.1±5.0	96.5±1.2	96.9±2.8	99.4±0.7	95.0±1.4
	16	102.1±3.2*	52.2±5.1*	115.1±3.8*	87.4±3.9*	99.8±2.7	90.9±4.1	110.4±1.8*	87.8±2.9*
	80	120.2±5.1*	26.9±5.9*	142.3±4.6*	88.4±2.9*	112.1±4.4*	90.5±2.9	122.3±1.8*	87.9±1.5*
20	Control	102.7±1.7	67.3±2.3	100.1±1.6	88.2±3.0	95.2±1.5	97.4±2.0	104.0±2.5	95.3±3.9
	16	120.4±4.9*	57.8±4.3	130.1±2.9*	95.0±5.8	97.6±1.3	95.4±2.4	110.7±3.1	88.7±2.1
	80	131.2±7.3*	28.9±7.3*	141.3±4.0*	75.3±15.9	107.1±1.7*	76.5±15.5	128.6±5.8*	69.0±13.9*

Values are expressed as mean±standard error of the mean. **p*<0.05 vs the control group. W1, the control value measured during the first working perfusion; W2, the value measured during the second working perfusion; W3, the value measured during the third working perfusion. *n*=6.

Fig 5. Postischemic recovery of aortic flow (AF) and creatine kinase (CK) leakage after normothermic (37°C) ischemia in hearts treated with phenylephrine during the second 20-min working perfusion and arrested with St Thomas' Hospital cardioplegic solution containing diltiazem (A) or ryanodine (B). Values are expressed as mean±standard error of the mean. **p*<0.05 vs the control group. Open columns: recovery of AF; hatched columns: CK leakage.

Fig 6. Postischemic recovery of aortic flow (AF) and creatine kinase (CK) leakage after normothermic (37°C) or hypothermic (20°C) ischemia in hearts treated with epinephrine during the second 20-min working perfusion and arrested with St Thomas' Hospital cardioplegic solution. Values are expressed as mean $\pm$ standard error of the mean. * $p < 0.05$ vs the control group. Open columns: recovery of AF; hatched columns: CK leakage.

dobutamine routinely used in a clinical setting have not only β -adrenergic effects but also α -adrenergic effects. In our previous study⁴, however, several treatments such as diltiazem, ryanodine, and extracellular calcium handling did not yield any beneficial effects in protection of the myocardium pretreated with high doses of milrinone and isoproterenol. Therefore, we studied the effects of preischemic treatment not only with β stimulator but also with α and $\alpha + \beta$ -adrenergic stimulator at different doses and investigated the effects of diltiazem and ryanodine on ischemia-reperfusion injury induced by the moderate dose of α or β -adrenergic stimulator.

Our results demonstrated that (1) at an ischemic temperature of 37°C, isoproterenol had a beneficial effect at a low dose (8.0 nmol/L) but a harmful effect at a high dose (40.0 nmol/L), and phenylephrine and epinephrine had a harmful effect at any dose; (2) at an ischemic temperature of 20°C, isoproterenol and epinephrine had a harmful effect at a high dose (40.0 nmol/L and 80 nmol/L, respectively); (3) diltiazem and ryanodine abolished the harmful effect of isoproterenol or phenylephrine. Although the results of diltiazem and ryanodine study were not consistent with our previous report⁴, it should be noted that the dose of isoproterenol used in this study was lower than in our previous study. Preischemic treatment with a high dose of isoproterenol may result in lethal ischemia-reperfusion injury that does not respond to any calcium handling.

The results of this study indicate several important points about the effect of catecholamines, including that: (1) a high-dose β -adrenergic stimulator may induce lethal myocardial injury regardless of the ischemic temperature; (2) a low-dose β -adrenergic stimulator may exert a beneficial effect on ischemia-reperfusion injury; (3) there may be a temperature dependency in the effect of an α -adrenergic stimulator on ischemia-reperfusion injury; (4) the deleterious effect of catecholamines could be involved in intracellular calcium handling through the sarcolemmal calcium channel or the sarcoplasmic reticular

calcium channel.

Do Catecholamines Induce Myocardial Injury without Ischemia?

Some investigators reported the deleterious effect of catecholamines upon the myocardium¹³⁻¹⁵ and suggested involvement of some mechanisms in the harmful effects generated by catecholamines. Singal et al¹⁶ reported the role of free radicals in catecholamine-induced cardiomyopathy. Opie et al¹⁷ applied the imbalance of oxygen demand-supply ratio in the endocardium to explain the mechanism of catecholamine-induced cell injury. However, as shown in the present study, neither isoproterenol (40.0 nmol/L) nor phenylephrine (1.5 μ mol/L) decreased any index of cardiac function in time-matched controls. Thus, at least in the aerobic perfusion alone (without ischemia), neither a β -adrenergic stimulator nor an α -adrenergic stimulator seems to have a harmful effect upon the myocardium.

Mechanism of Enhanced Ischemia-Reperfusion Injury as a Result of Preischemic Catecholamine Treatment

It has been shown that there are several major pathways of calcium entry into the cytosol as follows, transsarcolemmal calcium influx via the calcium channel and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, and calcium release from the sarcoplasmic reticulum^{13,14,18}. It is well established that β -adrenergic stimulation activates adenylate cyclase through GTP binding protein (Gs), resulting in elevation of intracellular cyclic AMP. A protein kinase activated by cyclic AMP, called protein kinase A, is thought to phosphorylate the sarcolemmal calcium channel protein, increasing its calcium permeability. There is some evidence in the literature suggesting that cyclic AMP depletion during myocardial ischemia may protect the myocardium against ischemia-reperfusion injury. Szilvassy et al¹⁹ have investigated mechanisms responsible for a beneficial effect of the overdrive pacing-induced preconditioning and found that cyclic AMP increased during overdrive pacing, but the increase in cyclic AMP was attenuated during the overdrive pacing repeated after 10- to 60-min intervals. More importantly, Vegh et al²⁰ revealed that, if the heart rate is increased with a pacing before ischemia, the heart exhibited a greater postischemic functional recovery compared with the heart not paced before ischemia. According to those studies, it seems likely that cyclic AMP depletion as a result of excess cyclic AMP production, maintaining cyclic AMP concentration at a lower level during ischemia, might contribute to a protective effect against ischemia-reperfusion injury. On the other hand, the intracellular signaling pathways of α -adrenergic stimulation in the myocardial cell are less clear than those in the smooth muscle cell. It has been confirmed so far that the positive inotropism of α -adrenergic stimulation may be mediated by the activation of phospholipase C through GTP binding protein (Gp)^{11,12,18,21}. Activated phospholipase C produces inositol triphosphate and diacylglycerol from phosphatidylinositol bisphosphate. Diacylglycerol activates protein kinase C and inositol triphosphate induces calcium release from the sarcoplasmic reticulum. Protein kinase C, the activity of which is modulated by cytosolic calcium concentration, is thought to increase the sensitivity of contractile protein to free calcium ion. Although it is still controversial whether the inotropism of α -adrenergic stimulation is mediated by the

increase in intracellular calcium concentration or the increase in myofibrillar sensitivity to calcium;²² it is likely that the inotropic action during α -adrenergic stimulation becomes a trigger of myocardial contracture during ischemia and reperfusion. Recently, several studies have reported that α -adrenergic stimulation activates the Na^+/H^+ exchanger, possibly through protein kinase $\text{C}_2^{22,23}$ and that the activation of the Na^+/H^+ exchanger plays an important role in the generation of ischemia-reperfusion injury induced by calcium influx through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger.²⁴⁻²⁶ In the present study, the result that either diltiazem or ryanodine, when added to the cardioplegic solution, prevented ischemia-reperfusion injury enhanced by preischemic catecholamine treatment obviously indicates that the harmful effect of α -adrenergic stimulation may be related to sarcolemmal calcium influx and sarcoplasmic reticular calcium release.

Mechanism of Protective Effect of a Low-Dose β -Adrenergic Stimulator

In the present study, preischemic isoproterenol treatment exhibited greater functional recovery in the post-ischemic period at a low dose but not at a high dose. These results appear to indicate that, during myocardial ischemia, a low concentration of cyclic AMP would serve as a cardioprotective factor against ischemia-reperfusion injury, whereas a high concentration of cyclic AMP would serve as a cardiotoxic factor. It has been shown in some experiments that the treatment with exogenous dibutyl cyclic AMP could prevent tissue edema induced by phosgene or ischemia-induced injury;^{27,28} suggesting that an increase in cyclic AMP may improve postischemic functional recovery because myocardial edema is one of the important factors responsible for cardiac dysfunction after ischemia.²⁹⁻³² Also, it has been pointed out in skin materials that a low concentration of isoproterenol could serve as a potent stimulus of cyclic AMP production in the endothelial cells, which might inhibit edema formation.³³

Temperature Dependency in the Effect of Preischemic Catecholamine Treatment

Most of the earlier studies dealing with effects of catecholamine upon ischemia-reperfusion injury have mainly employed normothermic ischemia.³⁴ In such studies, arrhythmogenic effects of catecholamines during ischemia or reperfusion have been to a large extent investigated from the point of view of physicians. Surgeons' interest in the effect of preischemic catecholamine treatment would be concentrated exclusively on the post-ischemic functional recovery after hypothermic global ischemia, because an infusion of cold cardioplegic solution with topical cooling of the heart is a typical method of myocardial protection in open heart surgery. Therefore, it would be quite important from a surgical viewpoint to determine the difference in the effect of preischemic adrenergic stimulation between normothermic and hypothermic ischemia. Because intracellular signaling systems are mediated by enzymatic reaction, efficacy of adrenergic stimulation would be affected by temperature. As a result of a low enzymatic reaction, the effect of catecholamines may be abolished by hypothermia. In the present study, if a catecholamine such as isoproterenol or epinephrine was used for preischemic treatment at a high dose, the treatment led to a deterioration in the postischemic recovery after both normothermic and hypothermic ischemia.

Phenylephrine, even if used for preischemic treatment at any dose, did not affect the postischemic recovery after hypothermic ischemia, although phenylephrine caused it to deteriorate after normothermic ischemia. These results indicate that β -adrenergic stimulation (ie isoproterenol) as well as $\alpha + \beta$ -adrenergic stimulation (ie epinephrine) altered the postischemic recovery after hypothermic ischemia, whereas α -adrenergic stimulation (ie phenylephrine) did not, suggesting that substantial intracellular signaling from β -adrenergic stimulation, instead of α -adrenergic stimulation, may exert a detrimental effect during hypothermic ischemia. Further investigation into the role of temperature dependency in the harmful effect of adrenergic stimulation on the postischemic recovery will be necessary.

Clinical Relevance of the Present Study

The results from the present study tell us that attention should be paid when catecholamines are used before open-heart surgery in a clinical setting. Catecholamine administration is one of the essential treatments for obtaining stable hemodynamics for patients before heart surgery. It is likely that catecholamine is used before the heart is harvested for transplantation from donor patients. Our results suggest that neither a β -adrenergic nor an $\alpha + \beta$ -adrenergic stimulator should be used at a high dose before open-heart surgery and that a β -adrenergic stimulator, if it is used at a low dose, may be cardioprotective against ischemia-reperfusion injury.

Calcium antagonists including verapamil have been used as cardioplegic additives in clinical settings.^{35,36} It should be noted, however, that cardioprotective effects of calcium antagonists are temperature dependent. Indeed, our previous studies with the same experimental model^{8,37} showed cardioprotective effects in normothermic conditions, but the effects were abolished in hypothermic conditions. Consistent with our previous studies, cardioprotective effects of diltiazem on ischemia-reperfusion injury accelerated by a β -adrenergic stimulator were abolished in hypothermic conditions. In clinical studies, use of calcium antagonists in cardioplegic solution is still controversial because of significant risks such as a prolonged period of pacemaker dependency and postoperative cardiac dysfunction.^{35,36} Clinical use of calcium antagonists may be unsuitable for attenuation of ischemia-reperfusion injury accelerated by inotropic agents especially in hypothermic conditions. Further experimental studies such as measurement of intra-cellular calcium level at different temperatures are necessary to investigate the mechanism of temperature dependency of calcium antagonists.

Concluding Comments

The effects of preischemic treatment with several adrenergic compounds upon myocardial protection were investigated using an isolated working rat heart model. Our results suggest that, before open-heart surgery or heart preservation, neither a β -adrenergic nor an $\alpha + \beta$ -adrenergic stimulator should be used at a higher dose because of a harmful effect of preischemic β -adrenergic stimulation on the postischemic recovery. The harmful effect is presumably due to mechanisms related to intracellular calcium movement through the sarcolemmal L-type calcium channel or the sarcoplasmic reticular

calcium channel.

References

- Griffith BP, Hardesty RL: Multiple cadaveric organ procurement for transplantation with emphasis on the heart. *Surg Clin North Am* 1986; **66**: 451–457
- Iwai A, Sakano T, Uenishi M, Sugimoto H, Yoshioka T, Sugimoto T: Effects of vasopressin and catecholamines on the maintenance of circulatory stability in brain-dead patients. *Transplantation* 1989; **48**: 613–617
- Yau TM, Weisel RD, Mickle DAG, Ivanov J: Cardioplegia for the dysfunctional ventricle. In: Engelman RM, Levitsky S, editors. A textbook of cardioplegia for difficult clinical problems. New York: Futura, 1991: 83–94
- Komai H, Yamamoto F, Tanaka L, Ichikawa H, Shibata T, Koide A, et al: Harmful effects of inotropic agents on myocardial protection. *Ann Thorac Surg* 1991; **52**: 927–933
- Shimada Y, Yamamoto F, Ohashi T, Kumada Y, Kagisaki K, Ishikawa T: Harmful effect of inotropic agents to the myocardial preservation (abstract). *J Cardiovasc Surg* 1991; **32**(Suppl): 31
- Hearse DJ, Braimbridge MV, Jynge P: Models and markers for the investigation of myocardial tissue damage. In: Protection of the ischemic myocardium: cardioplegia. New York: Raven Press, 1981: 50–92
- Gerhardt W: Methods of enzymatic analysis III. Weinheim: Verlag Chemie, 1983: 510–518
- Yamamoto F, Yamamoto H, Yoshida S, Ichikawa H, Takahashi A, Tanaka K, et al: The effects of several pharmacologic agents upon postischemic recovery. *Cardiovasc Drugs Ther* 1991; **5**: 301–308
- Steen PA, Tinker JH, Pluth JR, Barnhost DA, Tarhan S: Efficacy of dopamine, dobutamine and epinephrine during emergence from cardiopulmonary bypass in man. *Circulation* 1978; **57**: 378–383
- Sato Y, Matsuzawa H, Eguchi S: Comparative study of effects of adrenaline, dobutamine and dopamine on systemic hemodynamics and renal blood flow in patients following open heart surgery. *Jpn Circ J* 1982; **46**: 1059–1072
- Akera T: Pharmacological agents and myocardial calcium. In: Langer GA, editor. Calcium and the heart. New York: Raven Press, 1990: 299–331
- Katz AM: Receptors, coupling proteins, and second messengers. In: Katz AM, editor. Physiology of the heart. New York: Raven Press, 1992: 274–302
- Rona G: The significance of factors modifying the development of isoproterenol induced myocardial necrosis. *Am Heart J* 1963; **66**: 389–395
- Nirmala C, Puvanakrishnan R: Isoproterenol-induced myocardial infarction in rats: functional and biochemical alterations. *Med Sci Res* 1994; **22**: 575–577
- Chvapil M, Owen JA: Effect of zinc on acute and chronic isoproterenol induced heart injury. *J Mol Cell Cardiol* 1977; **9**: 151–159
- Singal PK, Kapur N, Dhillion KS, Beamish RE, Dhalla NS: Role of free radicals in catecholamine induced cardiomyopathy. *Can J Physiol Pharmacol* 1982; **60**: 1390–1397
- Opie LH, Walpoth B, Barsacchi R: Calcium and catecholamine: relevance to cardiomyopathies and significance in therapeutic strategies (abstract). *J Mol Cell Cardiol* 1985; **17**(Suppl 2): 21–24
- Opie LH: Receptors and signal transduction. In: Opie LH, editor. The Heart. New York: Raven Press, 1991: 147–175
- Karmazyn M, Moffat MP: Role of Na^+/H^+ exchange in cardiac physiology and pathophysiology: mediation of myocardial reperfusion injury by the pH paradox. *Cardiovasc Res* 1993; **27**: 915–924
- Scholz W, Albus U: Na^+/H^+ exchange and its inhibition in cardiac ischemia and reperfusion. *Basic Res Cardiol* 1993; **88**: 443–455
- Taylor CW, Merritt JE: Receptor coupling to polyphosphoinositide turnover: parallel with the adenylate cyclase system. *Trends Pharmacol Sci* 1986; **7**: 238–242
- Karmazyn M, Moffat MP: Na^+/H^+ exchange and regulation of intracellular Ca^{2+} (letter). *Cardiovasc Res* 1994; **27**: 2079–2080
- Iwakura K, Hori M, Watanabe Y, Kitabatake A, Cragoe Jr EJ, Yoshida H, Kamada T: α_1 -Adrenoreceptor stimulation increases intracellular pH and Ca^{2+} in cardiomyocytes through Na^+/H^+ and $\text{Na}^+/\text{Ca}^{2+}$ exchange. *Eur J Pharmacol* 1990; **186**: 29–40
- Pierce GN: The role of sodium-proton exchange in ischemic/reperfusion injury in the heart. *Am J Cardiovasc Pathol* 1992; **4**: 91–102
- Szilvassy Z, Ferdinandy P, Bor P, Jakab I, Lonovics J, Koltai M: Ventricular overdrive pacing-induced anti-ischemic effect: a conscious rabbit model of preconditioning. *Am J Physiol* 1994; **266**: H2033–H2041
- Vegh A, Szekeres L, Parratt JR: Transient ischemia induced by rapid cardiac pacing results in myocardial preconditioning. *Cardiovasc Res* 1991; **25**: 1051–1053
- Seibert AF, Thompson WJ, Taylor A, Wilborn WH, Barnard J, Haynes J: Reversal of increased microvascular permeability associated with ischemia-reperfusion: Role of cAMP. *J Appl Physiol* 1992; **72**: 389–395
- Kennedy TP, Michael JR, Hasty D, Sciuto AM, Hopkins C, Lazar R, et al: Dibutyl cAMP, aminophylline, and beta-adrenergic agonists protect against pulmonary edema caused by phosgene. *J Appl Physiol* 1989; **67**: 2542–2552
- Tranum-Jensen J, Janse MJ, Fiolet JWT, Krieger WJG, d'Almoncourt CN, Durrer D: Tissue osmolality, cell swelling, and reperfusion in acute regional myocardial ischemia in the isolated porcine heart. *Circ Res* 1981; **49**: 364–381
- Rubboli A, Sobotka PA, Euler DE: Effect of acute edema on left ventricular function and coronary vascular resistance in the isolated rat heart. *Am J Physiol* 1994; **267**: H1054–H1061
- Steenbergen C, Hill ML, Jennings RB: Volume regulation and plasma membrane injury in aerobic, anaerobic, and ischemic myocardium in vitro: effects of osmotic cell swelling on plasma membrane integrity. *Circ Res* 1985; **57**: 864–875
- Yeh T, Parmar JM, Rebeyka IM, Lofland GK, Allen EL, Dignan RJ, et al: Limiting edema in neonatal cardiopulmonary bypass with narrow-range molecular weight hydroxyethyl starch. *J Thorac Cardiovasc Surg* 1992; **104**: 659–665
- Warren JB, Wilson AJ, Loi RK, Coughlan ML: Opposing roles of cyclic AMP in the vascular control of edema formation. *FASEB J* 1993; **7**: 1394–1400
- Schömig A, Richardt G: The role of catecholamines in ischemia. *J Cardiovasc Pharmacol* 1990; **16**: S105–S112
- Jamieson WRE: Cardioplegic additives: the importance of optimization of dose. In: Roberts AJ, editor. Myocardial protection in cardiac surgery. New York: Marcel Dekker, 1987: 321–362
- Christakis GT, Weisel RD, Mickle DAG, Ivanov J, Madonik MM, Benak AM, et al: Calcium antagonists for myocardial protection. In: Roberts AJ, editor. Myocardial protection in cardiac surgery. New York: Marcel Dekker, 1987: 413–433
- Yamamoto F, Manning AS, Braimbridge MV, Hearse DJ: Cardioplegia and slow calcium-channel blockers: Studies with verapamil. *J Thorac Cardiovasc Surg* 1983; **86**: 252–261