Effects of Acylcarnitine-Transferase Inhibitors on Adenine Nucleotide Metabolism and Ischemic Tissue Injury in Isolated Perfused Rat Heart

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SUMMARY

The ability of irreversible acylcarnitine-transferase inhibitors, sodium 2[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate (POCA) and 2-tetradecyl-glycidic acid (TDGA), to reduce myocardial ischemic injury was studied in Langendorff-perfused hearts exposed to ischemia (zero mmHg) followed by aerobic reperfusion (60 mmHg). Rat hearts were pretreated with either POCA (15 mg/kg) or TDGA (5 mg/kg) s.c. 120 min before the perfusion. Treated hearts showed a decreased release of creatine kinase and lactate on reperfusion after 30 min ischemia. POCA-treated hearts showed significantly higher ATP concentrations than control hearts on reperfusion. POCA also improved the maximum recovery of the pressure-rate product but with a significant delay. During the ischemic period, though, POCA decreased the ATP concentration at a rate three times that of controls during the first 10 min. No further reductions were observed for up to 30 min of ischemia. TDGA also showed a reduction of ATP. Thus, the observation that POCA stimulated ATP synthesis and reduced creatine kinase release on reperfusion after ischemia suggests that this agent provides some protection to the ischemic myocardium. However, during ischemia, it is likely that the depletion of ATP concentration induced by POCA resulted in delayed recovery of mechanical function on reperfusion.

Additional Indexing Words:

Acylcarnitine-transferase inhibitors Free fatty acids oxidation Myocardial energy metabolism Perfused rat heart

MYOCARDIAL ischemia releases catecholamines which stimulate lipolysis and oxidation of free fatty acids (FFA).^{1),2)} This process is believed to be one of the causes for the development of ischemic injury because of an increase in oxygen consumption³⁾ and/or inhibition of adenine nu-

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cleotide translocase.⁴⁾ Even though little is known about the role of the accumulation and oxidation of FFA and acyl CoA on ischemic injury, the therapeutic significance of a reduction of FFA oxidation has already been provided using agents that act by various mechanisms. Oxfenicine (S-4-hydroxyphenyl glycine),⁵⁾ TDGA⁶⁾ and POCA⁷⁾ inhibit FFA oxidation at the level of acylcarnitine-transferase in mitochondrial inner membrane, resulting in the stimulation of glucose oxidation associated with the activation of pyruvate dehydrogenase.⁸⁾ The latter two agents inhibit acylcarnitine-transferase in an irreversible manner.⁶⁾

Oxfenicine reportedly improves recovery of mechanical function and reduced ischemic damage in rat heart⁹⁾ and in canine heart.¹⁰⁾ Furthermore, Pearce et al, using the technique of pyridine nucleotide surface fluorescence photography, showed that addition of oleate to hypoxic heart increased the size and number of anoxic areas and that subsequent addition of TDGA caused the anoxic areas to disappear.¹¹⁾ However, Seitelberger et al observed that POCA exerted no beneficial effects on regional function in the underperfused canine myocardium, although FFA uptake was reduced significantly.¹²⁾

This study was conducted to determine: (a) if inhibition of endogenous FFA oxidation by irreversible acylcarnitine-transferase inhibitors (POCA and TDGA) leads to improved recovery of mechanical function and reduction of enzyme leakage in ischemic, reperfused rat hearts and (b) if a switch from preferential endogenous FFA oxidation to carbohydrate oxidation improves energy metabolism during ischemia.

MATERIALS AND METHODS

Animals:

Male Sprague-Dawley rats weighing 350–400 g were deprived of food overnight but were allowed free access to water. They were anesthetized with pentobarbital (45 mg/kg, i.p.). Immediately before the excision of the heart, heparin (100 IU, Novo, Denmark) was injected into the femoral vein.

Measurement of long-chain fatty acid oxidation:

The heart was homogenized in 9 vol of 0.25 M sucrose. An aliquot of homogenate (0.1 ml) was incubated at 37° for 60 min in 0.9 ml Krebs-Henseleit bicarbonate (KHB) buffer (pH 7.4) containing 0.5 μ Ci [1-¹⁴C] palmitate, 2 mM ATP, 0.16 mM CoA, 2 mM L-carnitine and 0.1% bovine serum albumin. After the reaction was stopped by addition of 0.2 ml 2 M HClO₄, the CO₂ evolved was absorbed by 0.2 ml Scintilamine-OH (Dotite, Kumamoto). The radioactivity was determined using appropriate quench

corrections. Protein was measured by the method of Lowry et al.¹³⁾

Heart perfusion:

The perfused heart preparation used a modified method of Langendorff, which included cannulation of the aorta and a constant perfusion pressure of 60 mmHg.¹⁴⁾ The perfusion medium was made up of KHB buffer containing 11 mM glucose, equilibrated with a gas mixture of 95% O₂+5% CO₂. The perfusion medium was filtered through a 1.6 μ m filter. The perfusate was not recycled in a flow-through system. The temperature of the perfusate and perfused heart was maintained at 37° with double-layered water jackets.

Time sequence of experiments:

POCA (15 mg/kg) or TDGA (5 mg/kg) was injected s.c. 120 min before the start of the perfusion. The dosage of drugs and time interval used were estimated by the activation of pyruvate dehydrogenase in rat heart (data not shown). These drugs were dissolved in ethanol and 10% Cremophor ER solution (Nakarai Chemicals, Kyoto).

Initially, the hearts were perfused for 15 min (the control period) with oxygenated KHB buffer and then they were submitted to ischemia (zero mmHg) up to 60 min. After ischemia, aerobic perfusion was restored again (the reperfusion period). Samples of the coronary effluent were collected every two minutes, except for the first 5 min after the start of the reperfusion, during which the whole effluent was collected for analysis of creatine kinase and lactate. The coronary flow was monitored by timed collection of perfusate. For the determination of intracellular concentrations of ATP, ADP and AMP, hearts were quick-frozen at appropriate times with aluminum clamps pre-cooled in liquid nitrogen and then pulverized. Dry weights of the hearts were determined by drying tissue powder at 80° for 36 h.

Mechanical function:

Intraventricular pressures, peak left ventricular pressure (LVP) and left ventricular end-diastolic pressure (LVEDP), were measured by inserting a needle through the apex of the heart into the left ventricle. Heart rate (HR) was measured with a frequency counter from the left ventricle pressure records. The product of (LVP-LVEDP)×HR was used as an index of mechanical function.

Analytical methods:

Adenine nucleotide, ATP, ADP and AMP^{15),16)} were enzymatically determined. Concentration was expressed as μ mol/g dry tissue wt. Creatine kinase was measured by the method of Urdal and Stromme.¹⁷⁾ Lactate was measured enzymatically.¹⁸⁾

Reagents:

ATP, CoA, L-carnitine and reagents for the determination of adenine

nucleotides, creatine kinase and lactate were obtained from Boehringer-Mannheim-Yamanouchi, Tokyo. [1-¹⁴C] palmitate was from New England Nuclear. POCA and TDGA were prepared by K. Niigata and N. Itoh at the Chemical Division of our laboratories. Other reagents were of analytical grade from commercial sources.

Statistics:

Analysis of differences was performed using the Student's t-test or the Newman-Keuls test.

RESULTS

Inhibition of oxidation of palmitate in rat heart after POCA or TDGA pretreatment in vivo:

When fasted rats were injected s.c. with POCA (15 mg/kg) or TDGA (5 mg/kg) 120 min before *in vitro* experiments, the ability of their heart homogenate to oxidize [1-¹⁴C] palmitate to ¹⁴CO₂ was markedly diminished (Table I). Thus, inhibitory effects of both agents on FFA oxidation *in vivo* were well preserved under the *in vitro* conditions.

Effects of POCA and TDGA on changes in creatine kinase, lactate and coronary flow during 20 min reperfusion after 30 min ischemia:

Table II shows the effects of POCA and TDGA on creatine kinase and lactate leakage from perfused rat hearts during the 20 min period of reperfusion after 30 min ischemia. Rat hearts pretreated with either POCA or TDGA released less creatine kinase and lactate (POCA: 47.3% and 45.9%, TDGA: 70.2% and 67.2%, respectively) than did hearts from vehicle treated control rats. On the other hand, POCA and TDGA increased coronary flow by about 50% ($p < 0.05$). These results indicated that the reduction of creatine kinase and lactate release induced by these two agents was not due to poor washout of creatine kinase and lactate by coronary flow.

Table I. Inhibition of Oxidation of Palmitate in Heart Homogenate after Pretreatment with POCA or TDGA in Vivo

	Oxidation of [1- ¹⁴ C] palmitate	
	dpm/mg protein	Inhibition (%)
Control (5)	12,231 ± 373	0
POCA (6)	5,004 ± 278*	59.1 ± 2.3*
TDGA (6)	5,327 ± 300*	56.4 ± 2.5*

CO₂ formation from [1-¹⁴C] palmitate was measured as described in the Methods. Results are means ± SE. Numbers of observations are shown in parentheses.

* $p < 0.05$, compared with control.

Effects of POCA on the recovery of the pressure-rate product during reperfusion after 20 or 30 min ischemia:

As shown in Fig. 1A, maximum recoveries of product of [peak left ventricular pressure (LVP)—left ventricular end-diastolic pressure (LVEDP)] $\times$ heart rate (HR) on reperfusion after 20 min ischemia were attenuated by

Table II. Effects of POCA and TDGA on Changes in Creatine Kinase, Lactate and Coronary Flow during 20 Min Reperfusion after 30 Min Ischemia

	Creatine kinase (U/min)	Lactate (μ mol/min)	Coronary flow (ml/min)
Control (7)	1.71 ± 0.09	3.94 ± 0.28	9.13 ± 0.58
POCA (6)	$0.90 \pm 0.20^*$	$2.13 \pm 0.33^*$	$14.12 \pm 1.28^*$
TDGA (6)	$0.51 \pm 0.10^*$	$1.29 \pm 0.10^*$	$13.48 \pm 0.95^*$

POCA or TDGA was injected subcutaneously 120 min before the start of the perfusion. Samples of the coronary effluent were collected every 2 min except the first 5 min after the start of the reperfusion, during which the whole effluent was collected. The coronary flow was monitored by timed collection of perfusate. Results are means $\pm$ SE. Numbers of observations are shown in parentheses.

* $p < 0.05$, compared with control.

Fig. 1. Effect of POCA on the recovery of the pressure-rate product during reperfusion after 20 min (A) or 30 min (B) ischemia.

POCA (15 mg/kg, ●) or vehicle (○) was injected s.c. 120 min before the start of the perfusion. Hearts were perfused for a 15 min control period, followed by differing periods of ischemia. Subsequently, hearts were reperfused for 30 min under non-ischemic conditions. Each point represents mean $\pm$ SE. Numbers of observations are shown in parentheses.

* $p < 0.05$, compared with control at the same interval.

about 29% compared with the value before ischemia (at -15 min, $p < 0.05$). Furthermore, hearts exposed to 30 min of ischemia showed only 38.4% recovery of the pressure-rate product compared with the value before ischemia (Fig. 1B). On the other hand, after 20 min of ischemia, the pressure-rate product of POCA-treated hearts recovered significantly to their pre-ischemic values at the end of 30 min reperfusion, even though POCA increased the pressure-rate product by 21% under aerobic conditions before ischemia (at -15 min, $p < 0.05$). However, there was no recovery of the pressure-rate product during the first 5 min of reperfusion.

As shown in Fig. 1B, POCA produced a recovery of the pressure-rate product to pre-ischemia control levels. The results in Fig. 1 showed that POCA improved the maximum recovery of the pressure-rate product on reperfusion after 20 min of ischemia and also delayed the recovery during the first 5 min of reperfusion.

Effects of POCA on changes in ATP, ADP and AMP concentrations of hearts exposed to differing periods of ischemia:

To investigate the possible relationship between the delayed recoveries of the pressure-rate product and adenine nucleotide concentration in hearts treated with POCA, the effects of POCA on ATP, ADP and AMP concentrations were examined in hearts as a function of the duration of the ischemic period (Fig. 2).

Myocardial ischemia (10–60 min) caused a progressive decrease in the ATP concentration, with concomitant increases in ADP and AMP concentrations in the control heart. However, ischemia longer than 20 min resulted in a significant reduction of the total concentration of adenine nucleotides (Table III). On the other hand, after 10 min of ischemia, POCA decreased both the ATP concentration from the control value of 16.3 ± 0.8 to 3.7 ± 0.4 $\mu\text{mol/g}$ dry wt (Fig. 2, $p < 0.05$) and the total adenine nucleotide concentration from 24.8 ± 0.6 to 13.0 ± 0.7 $\mu\text{mol/g}$ dry wt (Table III, $p < 0.05$). No further reductions in ATP concentrations were observed in hearts of POCA treated rats. TDGA also reduced ATP concentrations under the same ischemic conditions (10 min: 7.5 ± 0.8 , 20 min: 2.5 ± 0.1 $\mu\text{mol/g}$ dry wt). These results suggested that the lower concentrations of ATP observed in ischemic hearts treated with POCA corresponded with a delayed recovery of the pressure-rate product.

Effects of reperfusion on the recovery of ATP, ADP and AMP concentrations of POCA-treated hearts exposed to 30 min ischemia:

To further investigate the relationship between energy states and improved maximum recoveries of pressure-rate product in hearts treated with POCA, we measured myocardial ATP, ADP and AMP concentrations on

Fig. 2. Effects of POCA on changes in ATP, ADP and AMP concentrations of hearts exposed to differing periods of ischemia.

POCA was injected s.c. 120 min before the start of the perfusion. The hearts were perfused for a 15 min control period, followed by different periods of ischemia. For the determination of adenine nucleotide, hearts were quick-frozen at appropriate times of ischemia with aluminum clamps pre-cooled in liquid nitrogen. Each column represents mean $\pm$ SE. Numbers of observations are shown in parentheses.

* $p < 0.05$, compared with control at the same interval.

† $p < 0.05$, compared with the value before ischemia.

Table III. Effect of POCA on Changes in Total (ATP+ADP+AMP) Concentrations Induced by Ischemia

	Period of ischemia ($\mu\text{mol/g dry wt}$)				
	0 min	10 min	20 min	30 min	60 min
Control	27.3 $\pm$ 1.0	24.8 $\pm$ 0.6	19.7 $\pm$ 1.6†	15.8 $\pm$ 0.7†	8.9 $\pm$ 0.5†
POCA	27.5 $\pm$ 0.4	13.0 $\pm$ 0.7*†	10.6 $\pm$ 0.5*†	11.6 $\pm$ 0.5*†	6.2 $\pm$ 0.6*†

The experimental procedure and numbers of observations are explained in Fig. 2. Results are means $\pm$ SE.

* $p < 0.05$, compared with control at the same interval.

† $p < 0.05$, compared with the value before reperfusion.

reperfusion after 30 min ischemia. Before the start of reperfusion (zero time), the ATP concentration and the total concentration of adenine nucleotides observed in POCA-treated hearts were lower than in control hearts (Fig. 3 and Table IV). In contrast to the energy states during ischemia, POCA pretreatment resulted in ATP synthesis at higher rates than control during the first 10 min of reperfusion, the total concentration of adenine nucleotides also recovered above the control value. Thus, POCA-treated hearts showed much higher ATP concentrations than control hearts at 10 min (Fig. 3). This effect of POCA on ATP synthesis was also observed at 20 min of reperfusion. However, after 40 min of reperfusion, there was no significant difference between control and POCA treated rats (see Fig. 3 and Table IV). Thus, it is very likely that POCA reduced the tissue damage due to reperfusion after ischemia through the improvement of adenine nucleotide metabolism.

Fig. 3. Effects of reperfusion on the recovery of ATP, ADP and AMP concentrations of POCA-treated hearts exposed to 30 min ischemia.

POCA was injected 120 min before the start of the perfusion. After 30 min ischemia, hearts were reperfused in non-ischemic conditions. For the determination of adenine nucleotide, hearts were quick-frozen at appropriate times of reperfusion. Each column represents mean $\pm$ SE. Numbers of observations are shown in parentheses.

* $p < 0.05$, compared with control at the same interval.

† $p < 0.05$, compared with the value before reperfusion.

Table IV. Effects of Reperfusion and POCA on Changes in Total (ATP+ADP+AMP) Concentrations Induced by 30 Min Ischemia

	Period of reperfusion ($\mu\text{mol/g dry wt}$)			
	0 min	10 min	20 min	40 min
Control	15.8 ± 0.7	14.7 ± 0.8	14.7 ± 0.6	16.5 ± 1.0
POCA	$11.6 \pm 0.5^*$	$15.6 \pm 0.1^\dagger$	$15.7 \pm 0.5^\dagger$	$17.5 \pm 0.3^\dagger$

The experimental procedure and numbers of observations are explained in Fig. 3. Results are means $\pm$ SE.

* $p < 0.05$, compared with control at the same interval.

† $p < 0.05$, compared with the value before ischemia or reperfusion.

DISCUSSION

Using a Langendorff rat heart preparation, we examined the effects of POCA and TDGA, irreversible acylcarnitine-transferase inhibitors, on damage induced by ischemia of variable duration. The results indicated that POCA-treated hearts showed about a 60% inhibition of FFA oxidation and recovered stable mechanical functions (pressure-rate product) after 20 min ischemia. Hearts perfused without this agent failed to recover to pre-ischemic values (Table I and Fig. 1). From the studies of Pearce et al¹¹⁾ with working rat heart preparations, the addition of 1 mM oleate to the perfusate resulted in a reversible decrease in left ventricular pressure development and aortic output under hypoxic conditions. They also showed that perfusion with TDGA (50 μM) prevented this deleterious effect of oleate. Therefore, it is highly possible that FFA oxidation is one of the causes for the deleterious effects of ischemia as well as hypoxia on mechanical failure.

Current studies have suggested that the deleterious effects of ischemia are characterized by early falls in cellular concentrations of high energy phosphates (ATP and creatine phosphate).^{19),20)} At much later stages, this damage is evidenced by a major leakage of intracellular enzymes (e.g., creatine kinase, lactate dehydrogenase) and by a myocardial contracture.^{19),21)} If myocardium is reperfused after significant cellular damage has occurred, reperfusion itself accelerates tissue damage.

In accord with these current studies, POCA and TDGA reduced the ischemic injury, evidenced by a less intensive release of creatine kinase (47.3% and 70.2% reduction, respectively) (Table II). They also suppressed the release of lactate, indicating improvement of the unbalanced redox state induced by ischemia. Furthermore, the most striking effects of POCA during the reperfusion period was that this agent increased ATP synthesis at higher rates than control. As a result of this effect, POCA-treated hearts showed

significantly higher ATP concentrations than those in control hearts. Thus, it is very likely that POCA preserved cardiac cellular function.

A different picture emerged when the effects of POCA on ATP, ADP and AMP concentrations during ischemia were examined (Fig. 2). This treatment caused drastic reductions in both ATP and the total concentration of adenine nucleotides after 10 min of ischemia. POCA-pretreatment decreased ATP concentrations at three times the rate of the control group during the first 10 min of ischemia (Fig. 2). It is well established that depleted high energy phosphate stores (ATP and creatine phosphate) are associated with loss of contractile integrity and a rigor-like state, evidenced by a rise in end-diastolic pressure.²²⁾ Seitelberger et al¹²⁾ showed that POCA impaired regional myocardial contractility in underperfused canine myocardium. Therefore, it is highly possible that the delayed recovery of the pressure-rate product observed in POCA-treated hearts corresponded closely with contractile failure due to depleted ATP concentration.

Little is known about the underlying causes for myocardial ATP reduction induced by acylcarnitine-transferase inhibitors during ischemia. However, one possibility is that FFA are the principal myocardial energy substrate during the ischemic period and well-oxygenated conditions.²³⁾ The inhibition of endogenous FFA oxidation by POCA resulted in a shortage of substrate for ATP synthesis, even though the utilization of glucose and endogenous glycogen was enhanced. Pearce et al¹¹⁾ indicated that at high work load (400 beats/min), TDGA addition (10 μ M) resulted in decreased cardiac function. This change may reflect an inhibition of endogenous FFA oxidation, since 5 mM glucose was added into the perfusion medium as the only exogenous substrate. Thus, endogenous FFA are still an important substrate when energy demand is exceeded.

Another possibility is that the accumulation of FFA and acyl-CoA derivatives induced by acylcarnitine-transferase inhibitors during ischemia may inhibit ATP synthesis by the inhibition of adenine nucleotide translocase⁴⁾ or uncoupling effects of oxidative phosphorylation.²⁴⁾ Bachmann et al²⁵⁾ reported that mitochondria, isolated from the hearts dosed with TDGA for 4 weeks, showed progressive uncoupling of oxidative phosphorylation (decrease in ADP/O ratio), with concomitant changes in electron transfer reaction (oxygen consumption). However, it is apparent in this study that POCA did not display an uncoupling effect of oxidative phosphorylation at normoxic conditions, since this agent did not affect the ATP concentration before ischemia (Fig. 2).

REFERENCES

1. Karwatowska-Krynska E, Beresewicz A: Effect of locally released catecholamines on lypolysis and injury of the hypoxic isolated rabbit heart. *J Mol Cell Cardiol* **15**: 523, 1983
2. Manning A, Keogh J, Hearse DJ, Coltart J: Beta-blockade and ischemic injury: effects of partial agonist activity. *Cardiovasc Res* **14**: 619, 1980
3. Mjøs OD: Effect of free fatty acid on myocardial function and oxygen consumption in intact dogs. *J Clin Invest* **50**: 1386, 1971
4. Shrago E: The effect of long chain fatty acyl CoA esters on adenine nucleotide translocase and myocardial metabolism. *Life Sci* **22**: 1, 1978
5. Higgins AJ, Morville M, Burges RA, Blackburn KJ: Mechanism of action of oxfenicine on muscle metabolism. *Biochem Biophys Res Commun* **100**: 291, 1981
6. Tutwiler GF, Ryzlak MT: Inhibition of mitochondrial carnitine palmitoyl transferase by 2-tetradecylglycidic acid (McN-3802). *Life Sci* **26**: 393, 1980
7. Turnbull DM, Bartlett K, Younan SI, Sherratt HSA: The effects of 2[5-(4-chlorophenyl)-pentyl] oxirane-2-carbonyl-CoA on mitochondrial oxidations. *Biochem Pharmacol* **33**: 475, 1984
8. Tutwiler GF, Ho W, Mohrbacher RJ: 2-Tetradecylglycidic acid. *Methods in Enzymology* **72**: 533, 1981
9. Higgins AJ, Morville M, Burges RA, Gardiner DG, Page MG, Blackburn KJ: Oxfenicine diverts rat muscle metabolism from fatty acid to carbohydrate oxidation and protects the ischaemic rat heart. *Life Sci* **27**: 963, 1980
10. Burges RA, Gardiner DG, Higgins AJ: Protection of the ischaemic dog heart by oxfenicine. *Life Sci* **29**: 1847, 1981
11. Pearce FJ, Forster J, Deleeuw G, Williamson JR, Tutwiler GF: Inhibition of fatty acid oxidation in normal and hypoxic perfused rat hearts by 2-tetradecylglycidic acid. *J Mol Cell Cardiol* **11**: 893, 1979
12. Seitelberger R, Kraupp O, Winkler M, Brugger G, Raberger G: Effects of the acylcarnitine-transferase blocking agent sodium 2[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate (POCA) on metabolism and regional function in the underperfused canine myocardium. *J Cardiovasc Pharmacol* **7**: 273, 1985
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* **193**: 265, 1951
14. Neely JR, Revetto MJ: Techniques for perfusing isolated rat heart. *in* *Methods in Enzymology*, ed by Hardman JG, O'Mally BW, Vol 139, Academic Press, New York, p 43, 1974
15. Jaworek D, Gruber W, Bergmeyer HU: Adenosine-5'-triphosphate, determination with 3-phosphoglycerate kinase. *in* *Methods of Enzymatic Analysis*, ed by Bergmeyer HU, Academic Press, New York, p 2097, 1974
16. Jaworek D, Gruber W, Bergmeyer HU: Adenosine-5'-diphosphate and adenosine-5'-monophosphate. *in* *Methods of Enzymatic Analysis*, ed by Bergmeyer HU, Academic Press, New York, p 2127, 1974
17. Urdal P, Stromme JH: Effects of Ca⁺⁺, Mg⁺⁺ and EDTA on creatine kinase activity in cerebrospinal fluid. *Clin Chem* **25**: 147, 1979
18. Gutmann I, Wahlefeld AW: L-(+)-lactate, determination with lactate dehydrogenase and NAD. *in* *Methods of Enzymatic Analysis*, ed by Bergmeyer HU, Academic Press, New York, p 1464, 1974
19. Shikama H, Noshiro O, Ohta A, Ohata I: Effects of nicardipine on ischemic mechanical failure and tissue injury in isolated perfused rat heart. *Jpn Heart J* **26**: 813, 1985
20. Hearse DJ, Crome R, Yellow DM, Wyse R: Metabolic and flow correlates of myocardial ischaemia. *Cardiovasc Res* **17**: 452, 1983
21. Kloner RA, Braunwald E: Observations on experimental myocardial ischemia. *Cardiovasc*

Res **14**: 371, 1980

22. Takeo S, Sakanashi M: Possible mechanisms for reoxygenation-induced recovery of myocardial high-energy phosphate after hypoxia. *J Mol Cell Cardiol* **15**: 577, 1983
23. Oram JF, Suzanne L, Bennetch L, Neely JR: Regulation of fatty acid utilization in isolated perfused rat hearts. *J Biol Chem* **248**: 5299, 1973
24. Katz AM, Messineo FC: Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium. *Circ Res* **48**: 1, 1981
25. Bachmann E, Weber E, Zbinden G: Biochemical aspects of cardiotoxic effects of a novel type hypoglycemic agent in rats. *J Mol Cell Cardiol* **15** (suppl 1): 67, 1983