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Biochemical Changes of Myocardial Necrosis induced by Isoproterenol and Protective Effects of β-Blocker and Anti-inflammatory Drugs

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Biochemical Studies of myocardial lesions induced by isoproterenol (ISP) were performed on the changes of myocardial water content, high energy phosphate levels and plasma enzyme activities. Protective effects of propranolol and anti-inflammatory drugs on the biochemical shifts by ISP were also examined. Cardiac water content increased immediately after subcutaneous injection of ISP and reduced near the normal level after 3 days. Anaerobic glycolysis and depletion of high energy phosphates were observed immediately after an injection of ISP. These biochemical shifts returned to normal within 24 hr except adenosine triphosphate (ATP) and total adenine nucleotide levels which remained at lower levels than normal. Distinct indications of returns toward the normal values with the levels of ATP and total adenine nucleotides took place at the same time as water content reduced. Plasma creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (GOT) and lactic dehydrogenase (LDH) activities were markedly elevated after the treatment of ISP and did not reach to normal after 24 hr. Pretreatment with propranolol blocked the biochemical shifts by ISP, such as cardiac edema, depletion of myocardial adenine nucleotides and elevation of plasma LDH activity. Prednisolone or indomethacin pretreatment decreased the water content and plasma LDH activity, but did not show the influence on cardiac adenine nucleotide levels.

Introduction

Rona and his associates, in their extensive studies, demonstrated the efficacy of isoproterenol (ISP), a synthetic β-adrenergic compound, as a cardiotoxic agent in various species over a wide dose range.[2] The histological and histochemical studies of myocardial lesions induced by ISP have been investigated.[3]

Myocardial anoxia or ischemia leads to anaerobic glycolysis, acidosis, electrolyte shifts and edema in the heart tissue.[4] The significant decrease in the levels of myocardial adenosine triphosphate (ATP) and phosphocreatine in necrotic heart was also demonstrated.[5–8] However, few reports are available about biochemical changes occurred after an administration of ISP.

The present study was undertaken to know changes of myocardial high energy phosphate levels, water content in heart tissue and plasma enzyme concentrations, such as creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (GOT) and lactic dehydrogenase (LDH), at various periods following ISP treatment. An attempt was also made to examine the effects

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of a β-adrenergic blocking agent and anti-inflammatory drugs on the biochemical shifts by ISP.

Material and Method

Animals——Male Wistar rats weighing 190 to 220 g were used throughout the experiments. The animals were housed in individual cages and allowed free access to food and water. Each experimental group consisted of 5 animals.

Experimental Procedures——Three series of experiments were performed. In the first series, a single injection of ISP at 5 mg/kg was given subcutaneously. At various periods after the injection, blood pressure, heart rate, cardiac water content, myocardial glycogen, lactate, high energy phosphates and plasma enzymes such as CPK, GOT and LDH were quantitatively determined. Blood pressure was measured with plethysmographic tail method.[9] Heart rate was recorded with electrocardiograph (Nihonkoden MC-11) under the slight anesthesia with ether. For the analysis of water content, the hearts were removed quickly under anesthesia (pentobarbital, 50 mg/kg *i.p.*), washed with saline and dried. For the specimen of cardiac glycogen, lactate and phosphate compounds, the chest of rat was opened with positive pressure breathing under pentobarbital anesthesia, and the heart was carefully exposed and frozen in situ with a Wollenberger tongue[10] precooled in liquid nitrogen. This frozen muscle was kept under $-80°$ until analyses were started. For the quantitative determinations of plasma enzyme activities, plasma was prepared from blood which was harvested by heart puncture. The plasma samples were stocked under refrigeration until enzyme activities were measured. In the second series of experiments, ISP at a daily dosage of 5 mg/kg was given once a day for 4 days. Heart and plasma samples were successively harvested every day. Myocardial water content, high energy phosphate levels and plasma enzymes were analyzed respectively. In the third series, propranolol (10 mg/kg), prednisolone (10 mg/kg) or indomethacin (5 mg/kg) was given intraperitoneally 30 min before the subcutaneous injection of ISP (5 mg/kg). Heart and plasma samples were obtained 24 hr after the ISP treatment for the determinations. The control group received intraperitoneal injection of physiological saline 30 min before the ISP treatment.

Extraction Procedure and Chemical Analysis——For the quantitative determinations of myocardial glycogen, lactate and high energy phosphates, the frozen muscles were homogenized with 5 volumes of 6% cold perchloric acid in a Potter-Elvejem glass homogenizer which had been precooled with ice water containing NaCl. All treatments for the homogenization were performed in a room maintained at 5°. A portion of the homogenate was used to measure glycogen. The remaining homogenate was centrifuged for 10 min at 10^4 *g* at 0°. The clear supernatant fluid was decanted into a precooled test tube and neutralized to pH 7 by the addition of 2 M K_2CO_3 solution at 0°. An aliquot of the neutralized extract was used for the determination of lactate, ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate and inorganic phosphate. Glycogen was extracted from the homogenate with ethanol precipitation method[11] and analyzed by the anthrone method.[12] Lactate was determined by the method of Hohorst.[13] Creatine phosphate and ATP were measured by the method of Lamprecht, *et al.*[14,15] ADP and AMP were simultaneously measured with the method of Adam.[16] Inorganic phosphate was determined with the spectrophotometric method.[17] Total adenine nucleotides ($\sum$A.N.) were calculated by summing up ATP, ADP and AMP values. CPK, GOT and LDH in plasma were determined by the method of Nuttall,[18] Reitman[19] and Cabaud[20] respectively. Myocardial water content was determined by weighing a wet tissue sample and drying to a constant weight in an oven at 105 to 110°. The data related to heart muscle were expressed per g dry weight of the tissue. All experimental results were given as the mean $\pm$ standard error.

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Result

Changes of Heart Rate and Blood Pressure after Treatment of ISP

Heart rate increased rapidly for one hr to a maximum level persisting until 4 hr and showed a decreasing trend at 6 hr, but increased again until 24 hr, as shown in Fig. 1-A. Throughout this process, no significant change of systolic pressure was observed (Fig. 1-B).

Tissue Weight and Water Content Alterations in ISP-treated Heart Muscle

Both myocardial wet and dry weights showed an increasing trend and were significantly raised above the control level 24 hr after the treatment ($p<0.01$). (Fig. 2-B). Cardiac water content increased rapidly to a maximum level after 2 hr and gradually decreased until 8 hr, but was again elevated after 24 hr (Fig. 2-A). This level was maintained to the 2nd day but decreased markedly following the 3rd day (Fig. 5).

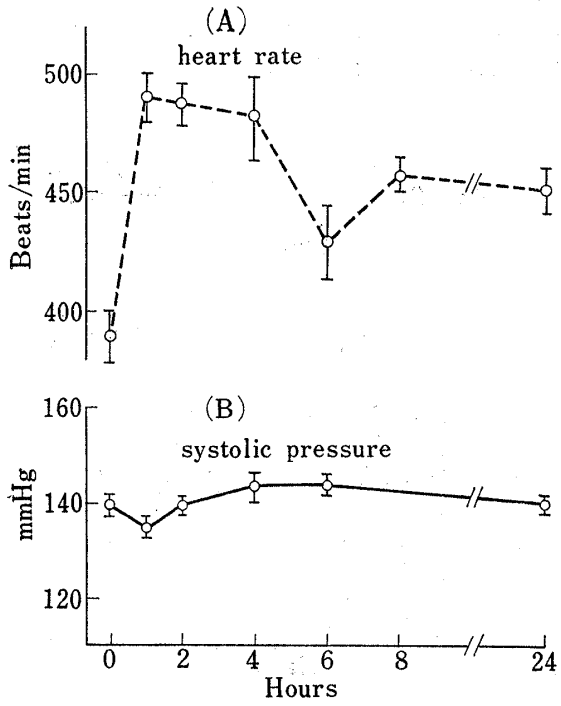

Fig. 1. Changes in Heart Rate and Systolic Blood Pressure after Subcutaneous Injection of ISP

The bars represent standard errors of the means.

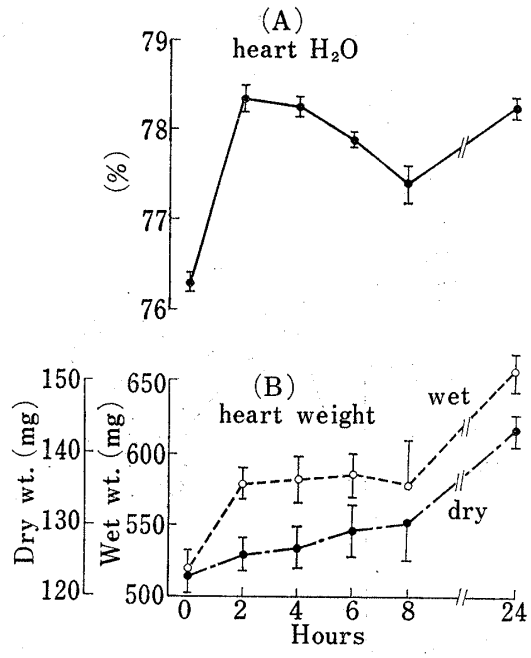

Fig. 2. Changes in Myocardial Water Content and Heart Weight after Treatment of ISP

Alterations of Cardiac Glycolysis and Phosphate Levels after ISP Treatment

Fig. 3 shows the changes of myocardial glycogen, lactate and phosphate levels after a single injection of ISP. Cardiac glycogen content decreased rapidly but began to increase after 2 hr and returned to the control value at 8 hr. Lactic acid level was elevated after 2 hr and returned to the initial value thereafter. Creatine phosphate level was markedly decreased to a half of the initial level after 2 to 4 hr and returned thereafter. On the other hand, the level of ATP diminished after 2 to 4 hr and slightly increased thereafter, but did not reach to the initial level over 24 hr. Associated with these changes, there were transient decreases of ADP and AMP, and a temporary increase in inorganic phosphate level over 24 hr. Consequently, the level of total adenine nucleotides was rapidly reduced after 2 hr and remained at the reduced level over 24 hr. Fig. 5 shows the alterations of myocardial ATP, total adenine nucleotides and water content over 7 days. Reductions in ATP and total adenine nucleotide

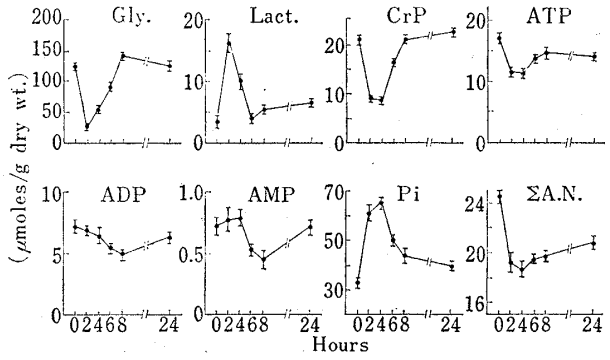

Fig. 3. Changes in Cardiac Glycogen, Lactate and Phosphate Compounds following Injection of ISP
ΣA.N., Total adenine nucleotides

contents after one and 2 days were returned to the initial values after 3 days. On the other hand, the increased water content after one and 2 days was sharply reduced near the initial level after 3 days. It was found that the changes of cardiac ATP and total adenine nucleotide levels were inversely proportional to that of myocardial water content.

Plasma Enzyme Levels after Treatment of ISP

Fig. 4 shows changes of plasma CPK, GOT and LDH activities over 24 hr. The activities of GOT and LDH increased until 4 hr, maintained the highest levels after 4 to 8 hr and reduced thereafter, but did not reach to the initial levels until 24 hr. CPK activity increased, reached a peak after 2 hr and gradually decreased thereafter.

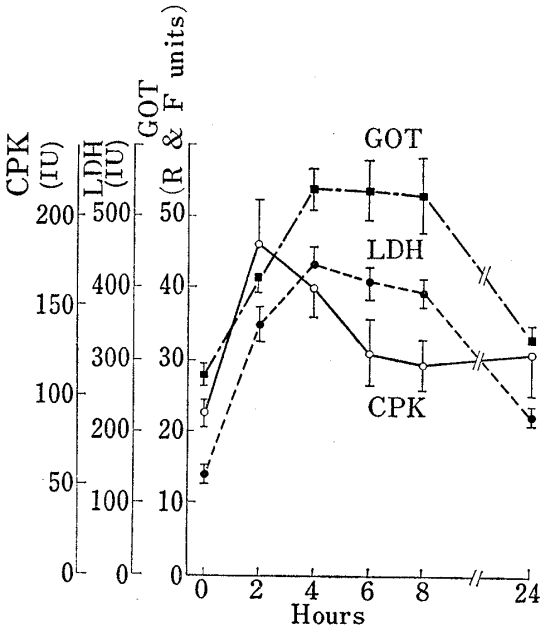

Fig. 4. Plasma Enzyme Changes after Injection of ISP

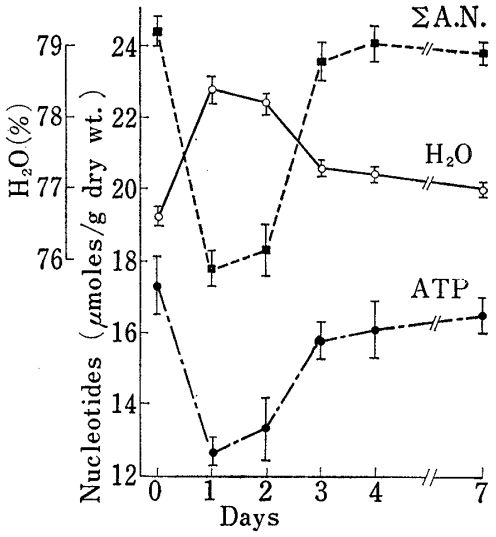

Fig. 5. High Energy Phosphate and Water Content in Heart Muscle after Treatment of ISP

Effect of Repeated Injections of ISP on Myocardial Water Content, High Energy Phosphate Levels and Plasma Enzyme Activities

The results of successive stages in biochemical changes after the repeated injections of ISP are indicated in Fig. 6. After a single injection of ISP, marked decreases of cardiac ATP and total adenine nucleotide levels, and an increase of water content were observed. Following the second injection of ISP, ATP and total adenine nucleotides reduced to some extent and water content increased slightly, but after the 3rd and the 4th treatments, the former did not decrease and the latter did not increase. It was noted that cardiac water content and adenine nucleotide concentrations were inversely proportionate. Creatine phosphate level in heart muscle, however, gradually decreased in consequence of the repeated administrations of ISP. Fig. 7 shows the result of changes in plasma enzyme levels such as CPK, GOT and LDH. The LDH activity increased and reached a maximum on the 3rd injection of ISP. After initial rises of CPK and GOT activities, there were subsequent falls during the

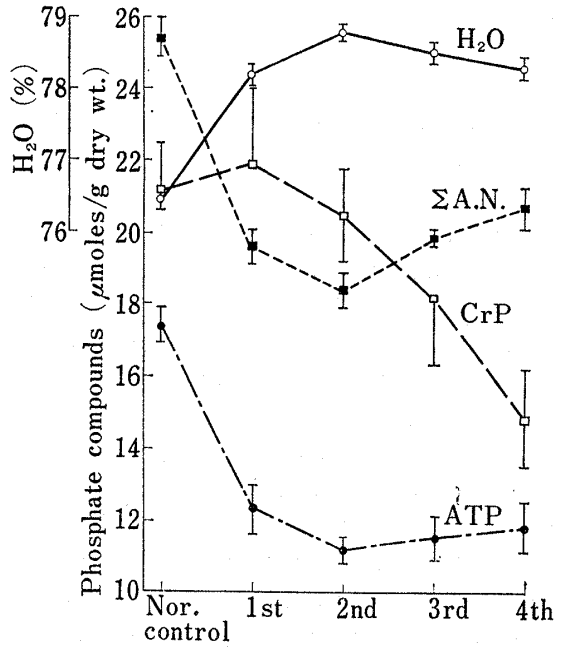

Fig. 6. Influence of Consecutive Injections of ISP on Myocardial High Energy Phosphates and Water Content

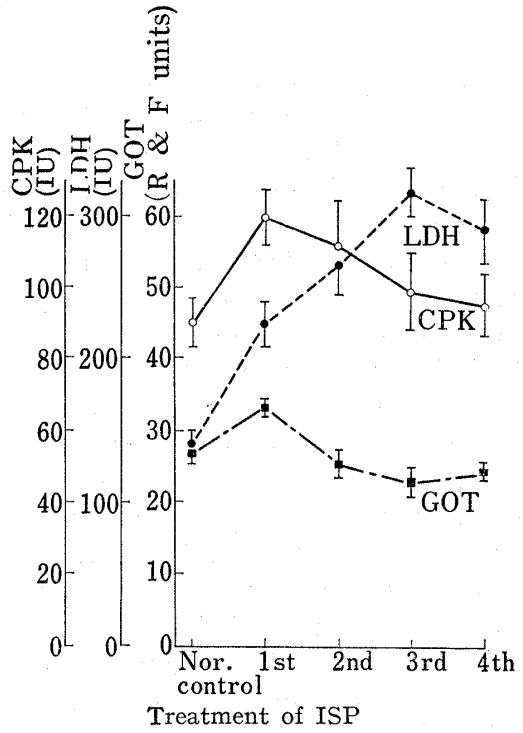

Fig. 7. Influence of Consecutive Injections of ISP on Plasma Enzyme Activities

stage of the 2nd to the 4th treatment of ISP

Effect of Propranolol, Prednisolone and Indomethacin on Myocardial Water Content, High Energy Phosphate Levels and Plasma LDH Activity following ISP Treatment

The above experiments confirmed that ISP induced an increase in myocardial water content, decreases of ATP and total adenine nucleotide levels, and the leakage of tissue enzymes to blood. Following experiments were designed to investigate protective effects of a β-blocker and anti-inflammatory drugs on the biochemical shift in heart muscles by ISP. As shown in Table I, the pretreatment with propranolol significantly protected the changes of the water content, ATP and total adenine nucleotide levels, and plasma LDH activity after ISP. On the other hand, the pretreatment with prednisolone or indomethacin significantly reduced cardiac water content and plasma LDH activity compared with those after ISP alone. ATP and total adenine nucleotide levels, however, were not protected statistically ($p>0.05$), although the mean values were higher than those of ISP treatment alone.

Table I. Effect of Pretreatment with Propranolol, Prednisolone and Indomethacin on Myocardial Water Content, Adenine Nucleotide Levels and Plasma LDH Activity following Treatment of ISP

Treatment	Heart			Plasma LDH (IU)
	H_2O (%)	ATP (μmoles/g.d.w.)	Σ.A.N. (μmoles/g.d.w.)	
Normal control	76.4±0.07	17.3±0.3	24.4±0.2	198±14
ISP	78.2±0.11	11.4±0.5	17.4±0.4	309±17
ISP+propranolol	76.9±0.07[a)]	15.8±0.5[a)]	22.7±0.4[a)]	215±13[a)]
ISP+prednisolone	77.7±0.05[a)]	12.5±0.3	18.6±0.5	214±18[a)]
ISP+indomethacin	77.8±0.03[b)]	12.7±0.5	18.8±0.6	195±11[a)]

Propranolol (10 mg/kg), prednisolone (10 mg/kg) or indomethacin (5 mg/kg) was given intraperitoneally 30 minutes before subcutaneous injection of ISP.
a) Differs from ISP values with $p<0.01$.
b) Differs from ISP values with $p<0.05$.

Discussion

Our experimental results described here indicate that ISP produces prompt myocardial edema and significant decreases in ATP and total adenine nucleotides. In the stage of repair in myocardial edema, there were distinct indications of return toward normal values for cardiac ATP and total adenine nucleotide levels. Though pretreatment with β-blocker, propranolol, inhibited both biochemical shifts in myocardial adenine nucleotides and cardiac edema induced by ISP, anti-inflammatory drugs such as prednisolone and indomethacin reduced the latter but did not influence the former.

Rona and co-workers found that ISP produced infarct-like diffused necrosis in heart muscles.[2] The important role of hemodynamics in the production of myocardial lesions by ISP has been shown by the strong inotropic and chronotropic actions of ISP with its resultant greater oxygen demand to the heart muscles.[21] In addition, it has been demonstrated that the reduction of systemic blood pressure by means of peripheral vasodilation may contribute to the production of myocardial necrosis by ISP.[21] In our result about blood pressure, however, no significant change was observed after the treatment of ISP. The reduction of systemic blood pressure might occur in the large dosage of ISP than that of our experiment.

The increase in total tissue water content immediately after ISP injection was observed in our experiments. The electron microscopical observations of early myocardial alterations are consistent with hypoxic pathogenesis such as mitochondrial swelling after the treatment of ISP.[22,23] Our biochemical observation supports the electron microscopical studies mentioned above.

Myocardial dry weight was increased over 24 hr after ISP, suggesting an enhancement of protein synthesis in heart muscles. Zbinden and Moe reported that protein synthesis after ISP injection showed an increasing trend, as measured by valine-^{14}C incorporation.[24]

Wexler and Kittinger demonstrated that there was a dramatic rise and fall in serum CPK, GOT and LDH following ISP-induced myocardial infarction in rat, and the degree of rise and fall in serum enzyme activities was commensurate with the extent of the myocardium infarcted.[25–27] Our results on plasma enzyme activities after ISP were well consistent with those of Wexler and Kittinger.

In the early stage after the ISP treatment, there was an intensified glycogen depletion and an increase of lactate, suggesting an inhibition of aerobic glycolytic metabolism. Moreover, there was a decreased accumulation of high energy phosphate compounds as a result of mechanical overload. After the acute effects of ISP, myocardial glycogen, lactate and phosphate compounds except ATP and total adenine nucleotides returned to the initial values.

In the later stage (later than 8 hr after ISP injection), myocardial degeneration and necrosis become histologically demonstrable, and the basic and earliest lesions would appear to be hyaline necrosis of the muscle fibers.[28] By our experiments, decreases of ATP and total adenine nucleotides, and an increase of the water content in heart muscles were observed in this stage. It was demonstrated by Kako that there was a significant decrease of cardiac ATP after two consecutive injections of ISP.[7] In the case of consecutive injections of ISP at a daily dosage of 5 mg/kg, the elevated water content and the reduced ATP and total adenine nucleotide levels in heart muscles remained at their shifted levels so far as we examined. On

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the other hand, cardiac creatine phosphate level decreased gradually and LDH activity in plasma increased by repeated ISP. A decrease of cardiac creatine phosphate and an increase of plasma LDH activity with the consecutive injections of ISP may reflect the development of degeneration in myocardial cell membranes.

Three days after a single ISP injection, there was a distinct indication of return toward normal values for water content, and ATP and total adenine nucleotide levels in heart muscles, suggesting toward rapid progression of healing. However, complete return to the control value in water content was not observed within 7 days. It was reported by Lehr that 3 as well as 5 days after the injection of large amount of adrenergic amines, the inorganic phosphate and potassium contents of myocardium were still significantly below normal, whereas the sodium content returned into the physiological range.[29] On microscopical examination by Kahn and co-workers, marked myocytolysis already occurred by 48 hr and, by 5 to 6 days, the necrotic muscle fibers were resorbed and were replaced by fibrous tissue.[28]

Myocardial necrosis produced by ISP have been inhibited by β-adrenergic blocking agents from biochemical and histological points of view.[30-33] The effect of a β-adrenergic blocking agent, propranolol, on the biochemical shifts by ISP was confirmed by our experiments. Moreover, the protective effect of anti-inflammatory drugs such as prednisolone and indomethacin was examined by us. Prednisolone and indomethacin were effective on the changes of cardiac water content and plasma LDH activity, whereas not effective on the change of myocardial ATP and total adenine nucleotide levels. Prednisolone and indomethacin probably protected against myocardial edema and degeneration of cell membranes produced by ISP but did not facilitate energy metabolism in myocardial tissue.

It has been demonstrated that both hemodynamic and biochemical effects of catecholamines may cause the production of myocardial necrosis.[34,35] From our results, early changes of the myocardial water content and adenine nucleotide levels by ISP might contribute to the initiation of the failure of cellular function.

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