THE EFFECTS OF β -BLOCKING AGENTS ON MITOCHONDRIAL FUNCTION IN ISCHEMIC MYOCARDIUM

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We investigated the protective effects of β -adrenergic blocking agents on ischemia-induced mitochondrial dysfunction in 42 anesthetized mongrel dogs. The animals, divided into 6 groups of 7 dogs each, were premedicated with either saline for the controls, or D, L-propranolol (0.5 mg/kg), D-propranolol (0.5 mg/kg), D, L-acebutolol (2.5 mg/kg), D, L-pindolol (0.1 mg/kg), or L-isoproterenol (0.2 μ g/kg/min, for 10 min). Myocardial mitochondria were prepared from both the normal and the ischemic areas after 30 min of coronary ligation. The concentration of long-chain acyl-CoA was measured enzymatically. Respiratory control index, ADP/O, and the rate of oxygen consumption in State III of mitochondria were also measured. In the control group, acyl-CoA in ischemic mitochondria increased significantly compared with that in normal mitochondria, and mitochondrial dysfunction was observed. Administration of L-isoproterenol further increased acyl-CoA level and accelerated the dysfunction, whereas premedication with β -blocking agents reduced the elevation of acyl-CoA level and prevented the dysfunction. Premedication with D-propranolol had no effect on mitochondria. Since the accumulation of acyl-CoA interferes with normal mitochondrial function, these results suggest that the protective effects of β -blocking agents are based, at least in part, on the prevention of the accumulation of acyl-CoA esters.

SINCE myocardial oxygen consumption is increased by augmentation of cardiac contractility by catecholamines,¹ it is thought that the elevated circulating catecholamine level in acute ischemia² could contribute to myocardial damage. Indeed, the effect of β -adrenergic blocking agents in decreasing the amount of ischemia and necrosis after experimental coronary ligation is well recognized.³⁻⁷ Recently, not only propranolol, but many other β -adrenergic block-

ing agents have been employed and widely used for the treatment of ischemic heart disease. Opie et al⁸ observed a relationship between the increase in free fatty acids induced by β -receptor stimulation and the subsequent damage to ischemic myocardium. We have also demonstrated that acyl-CoA, established as an intermediate in fatty acid metabolism, accumulates in the ischemic heart mitochondria and plays an important role in ischemia-induced mitochondrial dysfunction.⁹ Thus, in the present study, we investigated in animal experiments the relationship between the effects of β -adrenergic blocking agents (D, L-propranolol, D, L-acebutolol, D, L-pindolol) on acyl-CoA metabolism and their known protective effects on the ischemic myocardium. In addition,

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TABLE I INDEXES OF MITOCHONDRIAL FUNCTION IN EACH GROUP

		St. III O ₂ (n atoms/mg/min)	RCI	ADP/O
Control group	N	313 $\pm$ 19	4.01 $\pm$ 0.23	1.91 $\pm$ 0.08
	I	238 $\pm$ 25#	2.59 $\pm$ 0.39#	1.21 $\pm$ 0.22#
D,L-Propranolol group	N	320 $\pm$ 21	4.05 $\pm$ 0.22	1.89 $\pm$ 0.03
	I	265 $\pm$ 19*	3.30 $\pm$ 0.25**	1.77 $\pm$ 0.06**
D-Propranolol group	N	314 $\pm$ 14	4.06 $\pm$ 0.19	1.90 $\pm$ 0.10
	I	235 $\pm$ 21	2.60 $\pm$ 0.24	1.29 $\pm$ 0.17
D,L-Acebutolol group	N	316 $\pm$ 22	4.13 $\pm$ 0.12	1.92 $\pm$ 0.03
	I	267 $\pm$ 20*	3.35 $\pm$ 0.23**	1.82 $\pm$ 0.05**
D,L-Pindolol group	N	310 $\pm$ 24	4.03 $\pm$ 0.21	1.86 $\pm$ 0.02
	I	269 $\pm$ 19*	3.36 $\pm$ 0.24**	1.80 $\pm$ 0.04**
L-Isoproterenol group	N	301 $\pm$ 13	4.08 $\pm$ 0.13	1.85 $\pm$ 0.03
	I	203 $\pm$ 13**	2.67 $\pm$ 0.12	1.01 $\pm$ 0.11

Results are expressed as mean $\pm$ SD.

N = normal area, I = ischemic area, # = $p < 0.01$ vs. normal area in the control group, * = $p < 0.05$, ** = $p < 0.01$ vs. ischemic area in the control group.

we investigated the effects of D-propranolol and the β -adrenergic stimulating drug L-isoproterenol.

METHODS

D, L-Propranolol and D-propranolol were supplied by ICI Pharma. D, L-Acebutolol and D, L-pindolol were purchased from Rhone-Poulenc-Chugai and Shionogi Pharmaceutical Co., respectively. Other chemicals of reagent grade were purchased from Sigma Chemical Co.

Forty-two mongrel dogs of either sex weighing between 8–12 kg were anesthetized with sodium pentobarbital (50 mg/kg) injected intraperitoneally. Under artificial respiration, the chest was opened at the 4th or 5th intercostal space on the left side, and the heart was suspended in a pericardial cradle after incision of the pericardium. The left anterior descending coronary artery (LAD) was dissected free immediately distal to the first diagonal branch and ligated 10 min after the infusion of the drugs or isotonic saline into a cannulated femoral vein. The dogs were divided into 6 groups of 7 animals each: Control group, isotonic saline (5 ml/kg); D, L-propranolol group, 0.5 mg/kg; D-propranolol group, 0.5 mg/kg; D, L-acebutolol group, 2.5 mg/kg; D, L-pindolol group, 0.1 mg/kg; and L-isoproterenol group, 0.2 μ g/kg/min was infused

for 10 min and then LAD was ligated. Each drug was administered for 5 min except as indicated for isoproterenol. In each group, hearts were removed 30 min after LAD ligation. Heart mitochondria from the normal and the ischemic areas were prepared according to Hatefi's method,¹⁰ and their respiratory control index (RCI), ADP/O, and the rate of oxygen consumption in State III (St. III O₂) were measured immediately after the preparation, using an oxygen electrode and a closed cell. The incubation medium contained 0.3M mannitol, 10 mM KCl, 10 mM potassium phosphate, pH 7.4, 2.5 mM MgCl₂ and 0.25 mM EDTA in a total volume of 2.8 ml. Respiration was initiated by addition of 0.1 ml of mitochondrial suspension (10–15 mg protein/ml), and then 0.1 ml of succinate (0.2 M) as substrate and 0.05 ml of ADP (10 mM) were added subsequently. RCI was taken as the ratio between the rate of oxygen consumption after and before addition of ADP. St. III O₂ was calculated from the mitochondrial oxygen consumption in n atoms of oxygen consumed per mg of mitochondrial protein per min during State III respiration. ADP/O calculated from the ratio of μ moles of ADP phosphorylated to atoms of oxygen consumed. To measure the concentration of long-chain acyl-CoA in mitochondria, duplicated 1.0 ml samples of mitochondria (10–15 mg

protein/ml) were used for this assay.¹¹ To each sample, 0.5 ml of 12% (v/v) HClO₄ was added, and the precipitate was washed with 2 volumes of H₂O and suspended in 1.0 ml of 10 μ M dithiothreitol. This mixture was brought to pH 11.5 by the addition of 1N KOH. Long-chain acyl-CoA esters in the mixture were hydrolyzed to free CoA by incubation for 10 min at 55°C. Resulting free CoA was determined according to a DTNB method.¹² For one sample, the absorbance at 412 nm was measured after reacting with DTNB. For the other sample, free CoA derived from acyl-CoA was converted to acetyl-CoA by addition of enough D, L-acetyl carnitine and carnitine acetyltransferase before the reaction with DTNB. The concentration of long-chain acyl-CoA in mitochondria was calculated from the difference of the absorbance between the two samples.

Statistical analysis was done by Student's *t*-test.

RESULTS

Table I shows St. III O₂ of mitochondria prepared from the non-ischemic and the ischemic areas in each group. In the control group, St. III O₂ of mitochondria from the ischemic area was 238 ± 25 (n atoms/mg protein/min) (mean $\pm$ SD), which was significantly lower than that of mitochondria from the non-ischemic area (313 ± 19). In D, L-propranolol group, St. III O₂ of mitochondria from the ischemic area was 265 ± 19 . Although this value is significantly lower than that of mitochondria from the non-ischemic area, it is apparent that D, L-propranolol prevented much of the damage caused by ischemia in the control group. In the D-propranolol group, St. III O₂ of mitochondria from the ischemic area was 235 ± 21 , indicating that this compound exerted no protective effect on mitochondrial function. In D, L-acebutolol group and D, L-pindolol group, St. III O₂ of mitochondria from the ischemic area were 267 ± 20 and 269 ± 19 , respectively. These values are significantly reduced compared with those of mitochondria from the non-ischemic area. Nevertheless, it is apparent that these mitochondria were protected when compared with St. III O₂ of mitochondria from the ischemic area in the control group. In isoproterenol group, St. III O₂ of mitochondria from the ischemic area was 203 ± 13 , i.e., severely decreased compared with that of mitochondria from the ischemic area in the control group.

TABLE II CONCENTRATIONS OF LONG-CHAIN ACYL-CoA IN MITOCHONDRIA IN EACH GROUP

	Acyl-CoA (n mol/mg protein)	
	Normal Area	Ischemic Area
Control group	0.19 ± 0.03	$0.85 \pm 0.08\#$
D, L-Propranolol group	0.17 ± 0.02	$0.65 \pm 0.04^{**}$
D-Propranolol group	0.23 ± 0.03	0.89 ± 0.05
D, L-Acebutolol group	0.16 ± 0.02	$0.65 \pm 0.02^{**}$
D, L-Pindolol group	0.17 ± 0.01	$0.64 \pm 0.03^{**}$
L-Isoproterenol group	0.16 ± 0.02	$1.20 \pm 0.05^{**}$

Results are expressed as mean $\pm$ SD.

= $p < 0.01$ vs. normal area in the control group, ** = $p < 0.01$ vs. ischemic area in the control group.

In the control group, RCI of mitochondria from the ischemic area was decreased significantly compared with that of mitochondria from the non-ischemic area (Table I). In the D, L-propranolol group, RCI of mitochondria from the ischemic area was decreased significantly compared with that of mitochondria from the non-ischemic area, but damage to these mitochondria was less by this criterion than to mitochondria from the ischemic area in the control group. D-Propranolol, in contrast, exerted no protective effect upon the mitochondria in the ischemic area. In the D, L-acebutolol and D, L-pindolol groups, RCI of mitochondria from the ischemic area were 3.35 ± 0.23 , and 3.36 ± 0.24 , respectively. The values indicate significant protection by these compounds compared to mitochondria prepared from the ischemic area in the control group. In the isoproterenol group, RCI of mitochondria from the ischemic area was 2.67 ± 0.12 , i.e., almost equal to that of mitochondria from the ischemic area in the control group. In the D, L-propranolol, D, L-acebutolol and D, L-pindolol groups, the decrease of ADP/O of mitochondria from the ischemic area was prevented significantly compared with that of mitochondria from the ischemic area in the control group (Table I). In the D-propranolol group and the isoproterenol group, the decrease of ADP/O was almost equal to that in the control group, respectively.

Table II shows the concentration of long-chain

acyl-CoA from the non-ischemic and the ischemic areas in each group. In the control group, the concentration of long-chain acyl-CoA in mitochondria from the ischemic area increased significantly (0.85 ± 0.08 n mol/mg protein) compared with that from the non-ischemic area (0.19 ± 0.03). In D, L-propranolol, D, L-acebutolol, and D, L-pindolol groups, the increase in long-chain acyl-CoA level in mitochondria from the ischemic area was prevented significantly but only partially with the respect to mitochondria from the ischemic area of the control group. There was no significant difference between the D-propranolol group and the control group with respect to the concentration of long-chain acyl-CoA esters in mitochondria from the respective ischemic areas. In the isoproterenol group, the level of long-chain acyl-CoA in mitochondria from the ischemic area (1.20 ± 0.05) was significantly greater than that of the control group.

DISCUSSION

Alhquist, in 1948, proposed the division of the sites of adrenergic action into α and β -receptor groups.¹³ The concept of receptors for adrenergic action was first proved by the agent discovered by Powell and Slater,¹⁴ in 1958, which specifically blocked the β -receptors. Furthermore, propranolol was shown by Black et al.¹⁵ to be a new adrenergic β -receptor antagonist which could be used clinically. Thereafter, the β -adrenergic blocking agents have been tried in a variety of clinical conditions.¹⁶⁻²⁰ The observations were presented that the administration of propranolol decreased the infarct size following experimental coronary artery occlusion⁷ and improved cardiac metabolism in acute ischemia.⁴ Considering these studies, it is obvious that the β -adrenergic blocking drugs exert protective effects against ischemia-induced myocardial damage. Sobel et al.¹ demonstrated that catecholamines administered *in vivo* induced uncoupling of oxidative phosphorylation of subsequently isolated mitochondria. Kjekshus and Mjøs²¹ reported that isoproterenol further increased ST segment elevation in acute myocardial ischemia. These experimental studies have shown that β -blocking drugs protect ischemia-induced myocardial damage but, in converse, increased sympatho-adrenal discharge in acute ischemia results in deleterious effects on the myocardium. Moreover, Opie,²² in his review, has stressed the relationship between high levels of free fatty acids due to catecholamines

and their "toxic" cardiac effects. In this study, we demonstrated that premedication with D, L-propranolol, D, L-acebutolol or D, L-pindolol partially prevented ischemia-induced heart mitochondrial dysfunction, whereas premedication with L-isoproterenol exacerbated the dysfunction. Previously, we observed²³ in dogs that high levels of free fatty acids due to administration of lipid emulsion induced heart mitochondrial dysfunction and that this dysfunction was caused by the excess accumulation of long-chain acyl-CoA in mitochondria. The latter compounds are known to be potent inhibitors of ATP-ADP translocation across the mitochondrial inner membrane.²⁴ Carnitine protects against mitochondrial dysfunction by preventing the acyl-CoA accumulation.²³ Moreover, we have reported⁹ that accumulation of long-chain acyl-CoA was at least one of the causative factors in mitochondrial dysfunction in ischemic myocardium. In addition, we have presented the correlation formula,

$$f(\text{St. III O}_2) = -126 \times \text{Acyl-CoA} + 343$$

between mitochondrial function represented by St. III O₂ and the levels of acyl-CoA in mitochondria. In the present study, the concentration of long-chain acyl-CoA in mitochondria from the ischemic area in the D, L-propranolol group was a value of 0.65 and there was a good correspondence between St. III O₂: 261 calculated by the formula described above and that in mitochondria from the ischemic area in the D, L-propranolol group: 265. Similarly, the concentrations of long-chain acyl-CoA in mitochondria from the ischemic areas in the D-propranolol, D, L-acebutolol, D, L-pindolol or L-isoproterenol groups corresponded to the respective values of 0.89, 0.65, 0.64, and 1.20. Calculated St. III O₂ (respective values, 231, 261, 262, and 192) corresponded well those observed in this study (respective values of 235, 267, 269, and 203). Considering the fact that catecholamines are well known to increase free fatty acids,²² these results suggest that catecholamines further increase long-chain acyl-CoA in ischemic mitochondria, and that one of the protective effects of β -blocking agents depends on the inhibition of acyl-CoA accumulation in ischemic mitochondria. β -Blocking agents are classified into subtypes according to whether they are with or without membrane stabilizing activity (MSA) and intrinsic sympathomimetic activity (ISA).²⁵ D, L-Propranolol has MSA and no ISA, D, L-pindolol has ISA and

no MSA, whereas D, L-acebutolol has both MSA and ISA. In the present experiment, ischemia-induced mitochondrial dysfunction was not prevented in the D-propranolol group, however, it was prevented in the D, L-propranolol, D, L-pindolol, and D, L-acebutolol groups. These results strongly suggest that protective effects of β -adrenergic blocking agents against ischemia-induced mitochondrial dysfunction depends on blockade of β -adrenergic stimuli regardless of their MSA or ISA.

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