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INHIBITION OF THE MITOCHONDRIAL Mg^{2+}-ATPase BY PROPRANOLOL

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Abstract—The *in vitro* effects of propranolol, a commonly used β-adrenergic blocker, on the membrane structure and function of rat heart mitochondria were investigated. It was found that the respiratory control and oxidative phosphorylation of the isolated mitochondria decreased concomitantly when the drug was added to the assay medium. At the concentration higher than 1.0×10^{-4} M, propranolol significantly inhibited the State 3 respiration but had little effect on the State 4 respiration of the mitochondria. On the other hand, the drug exhibited noncompetitive inhibitions toward the Mg^{2+}-ATPase activity of submitochondrial particles and purified enzyme preparations at the concentrations ranging from 3.0×10^{-4} to 1.5×10^{-3} M. The inhibitory constants of propranolol toward the enzyme activity in submitochondrial particles and in the purified preparation were estimated to be 6.7×10^{-4} and 1.4×10^{-3} M, respectively. However, the drug did not show significant effect on the activity of any of the enzyme complexes of the mitochondrial respiratory chain. It is thus concluded that propranolol impairs the mitochondrial respiration and oxidative phosphorylation mainly through its inhibition of the Mg^{2+}-ATPase activity of the mitochondria. This effect of propranolol may explain, at least partly, its depression effects on the cardiac functions of the animal.

Propranolol, a β-adrenergic blocking agent, has been known for some time to depress myocardial functions and cause heart failure in patients with some heart diseases [1]. Although these clinical observations have been well documented in the literature [2–6], the reason for it has remained obscure. The attribution of the depression effect of this drug to its β-adrenergic blocking action has been ruled out, since Parmley and Braunwald [2] and Nayler *et al.* [7] have shown that the negative inotropic effect of a series of β-adrenergic blockers including propranolol, is not proportional to their β-blocking potency. The inhibitory effect of propranolol on the energy metabolism of the cardiac cells could be a possible cause of the depression. The drug could affect the energy-dependent cellular functions or the energy generating system of the cardiac cells. In fact, Honig [8] has reported that several β-adrenergic antagonists significantly reduce the myofibrillar ATPase activity in dog heart muscle. Spann *et al.* [9] and Chandler *et al.* [10] also demonstrated that the impaired cardiac muscle contractility is closely related to the decrease of the myofibrillar ATPase activity. It was then found that propranolol and some other β-blocking drugs inhibit the calcium uptake [11–13], and Ca^{2+}-ATPase activity [14, 15] of the sarcoplasmic reticulum of the cardiac cells. Recently, Meltzer and Kassir [16] further demonstrated that propranolol exerts competitive inhibition on the calmodulin-activated Ca^{2+}-ATPase of human erythrocyte membranes.

On the other hand, there have been much evidence to suggest that the depression effect of propranolol and many other β-adrenergic blocking drugs is due to their effects on the membrane of the cardiac cells [17–20]. Huunan-Seppala [21] has further demonstrated that propranolol specifically binds to the mitochondrial membrane of the cell. Since the integrity of the mitochondrial membrane can be easily assessed by the examination of the respiratory control and the ADP:O ratio of the mitochondria [22], we have made use of these two indices to study the effects of propranolol on the membrane structures and function of rat heart mitochondria. Moreover, mitochondrial respiration and oxidative phosphorylation occur in the inner membrane of the mitochondria and provide more than 90% of the energy required by the cells, we have thus used the heart mitochondria to investigate propranolol effect on the energy transduction system of the cardiac cells. In order to specifically locate the action site of propranolol on the mitochondrial membrane, we have examined the effects of the drug on each of the five electron transfer complexes [23] of the respiratory chain. Finally, we did some enzyme kinetic studies on the enzyme that was affected by the drug to elucidate its mechanism of action.

MATERIALS AND METHODS

Rat heart mitochondria were prepared by the method of Vercesi *et al.* [24] with some modifications. Sprague–Dawley rats, which were purchased from the animal center of National Taiwan University, Taipei, were sacrificed by decapitation. The hearts were quickly excised and thoroughly washed with ice-cold 0.25 M sucrose solution. Connective tissues and fats were trimmed off the hearts, which were then minced into small pieces with a pair of sharp,

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chilled scissors. The finely minced tissues were gently homogenized with a loose-fit Potter–Elvehjem homogenizer into the buffer containing 0.25 M sucrose, 0.5 mM EGTA*, 3 mM Hepes, pH 7.2 (SEH buffer) at the ratio of 10 ml per g wet weight of heart. The homogenate was then centrifuged at 800 *g* for 10 min. The supernatant thus obtained was centrifuged at 9500 *g* for 10 min. The pellet was collected and washed twice with SEH buffer by repeating the above centrifugations. The final mitochondrial pellet was suspended in a minimal amount of SEH buffer. The entire procedure was carried out at 4°. Usually 10–15 mg of the mitochondria was obtained from 1 g of rat heart. The respiratory control ratio was usually higher than 5.0 and 3.0 for glutamate–malate-supported and succinate-supported respiration, respectively.

Submitochondrial particles were prepared by sonicating rat heart mitochondria with a polytron (Kinematic, Switzerland) in 50 mM Na–K-phosphate buffer, pH 7.4, over ice water. The sonication was carried out at the power output scale of 4 for 10 min, separated by 1–2 min intervals to reduce sample heating. The suspension was then centrifuged at 136,000 *g* for 30 min in the Beckman L5-50 ultracentrifuge. The firmly packed pellet was suspended in 50 mM Na–K-phosphate buffer, pH 7.4, by gentle homogenization. The yield was usually 40–50% of the protein of the starting mitochondria.

The Mg^{2+}-ATPase of rat heart mitochondria was isolated from submitochondrial particles and purified according to the method described by Vogel [25].

The succinate-supported and glutamate–malate-supported respiration rate of the mitochondria were determined polarographically in an oxygen monitor equipped with a Clark oxygen electrode (Yellow Springs Instrument Co.) as described by Estabrook [26]. The assay mixture was composed of 0.125 M sucrose, 50 mM KCl, 6 mM $MgCl_2$, 20 μM cytochrome *c*, 5 mM Hepes, and 10 mM Na–K-phosphate, pH 7.2. When succinate was used as substrate 20 μM rotenone was included in the assay medium. Usually 0.4–0.8 mg were incubated in the reaction chamber containing 2 ml of assay mixture at 23° for 3 min before assay. The reaction was initiated by addition of 30 μl of 0.6 M succinate or glutamate plus malate. The substrate-induced respiration was followed in a strip chart recorder for about 2 min, and then a suitable amount of ADP (usually 400 nmole) was introduced by a Hamilton syringe to the assay system to stimulate the respiration to State 3 [27]. After the expenditure of ADP the respiration resumes to State 4. The respiratory control ratio was measured as the ratio between the respiration rate of State 3 and State 4. The oxidative phosphorylation of the mitochondria was measured as the ADP:O ratio, which is the ratio between the amount of ADP added and the oxygen consumed in State 3. In the case of inhibition experiments, the mitochondria were incubated with various concentrations of propranolol in the reaction chamber at 23° for 3 min before assay.

Succinate-cytochrome *c* reductase activity was determined spectrophotometrically following the reduction of exogenous cytochrome *c* by succinate at 550 nm upon addition of succinate to submitochondrial particles [28]. Cytochrome oxidase activity was assayed polarographically by the method of Yonetani [29]. The NADH dehydrogenase activity was determined by following the reduction of DCIP at 600 nm upon addition of NADH to the assay system [30].

The Mg^{2+}-ATPase activity was assayed by the method of Schuster *et al.* [31]. The assay mixture, in a total volume of 1 ml, contained 0.2 M sucrose, 3 mM $MgCl_2$, 2 mM phosphoenolpyruvate, 0.5 mM NADH, 50 mM Hepes, pH 7.2, and 20 units each of pyruvate kinase and lactate dehydrogenase. When submitochondrial particles were used for assay 2 μM rotenone was included in the assay system to avoid the interference of the NADH dehydrogenase activity with the Mg^{2+}-ATPase activity. In a typical assay, an aliquot of the submitochondrial particles (30–50 μg) was incubated with the assay mixture at 23° for 3 min, and then a suitable amount of ATP was added to the system to initiate the reaction, which was spectrophotometrically followed at 340 nm for 6–8 min. For inhibition experiments, the enzyme preparation was incubated with a desired concentration of propranolol at 23° for 3 min before assay.

Protein concentration was determined by the Lowry method [32], and bovine serum albumin was used as the standard ($A^{280}_{1\%} = 6.6$).

Propranolol, pyruvate kinase, lactate dehydrogenase, sucrose, phosphoenolpyruvate, cytochrome *c*, Hepes, ATP, ADP, EGTA, NADH, and DCIP were all purchased from Sigma Chemical Co (St. Louis, MO). All the other chemicals and reagents were obtained commercially in the highest purity available.

RESULTS

The respiration of the inact mitochondria is intimately controlled by the availability of ADP in the assay medium. In the rat heart mitochondria prepared as described above there was very little ADP and the respiration initiated by succinate or glutamate plus malate was very slow. Addition of a suitable amount of ADP to the assay mixture usually induced an approximately three- and six-fold increases in the rate of succinate-supported and glutamate–malate-supported respiration, respectively (Fig. 1). This indicates that the isolated mitochondria were quite intact with respect to their membrane structure [24, 26]. However, in the presence of 1.0×10^{-3} M propranolol the respiratory control ratio decreased to 1.8 and 3.6, respectively (Fig. 1). On the other hand, the ADP:O ratio for intact mitochondria was usually close to 2.0 and 3.0 for succinate-supported and glutamate–malate-supported respiration, respectively (Fig. 1). At the concentration of 1.0×10^{-3} M, propranolol decreased the ADP:O ratio to 1.0 and 2.1 for the two systems, respectively (Fig. 1). These effects of

* Abbreviations used: EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-piperazineethane sulfonic acid; DCIP, 2,6-dichlorophenolindophenol.

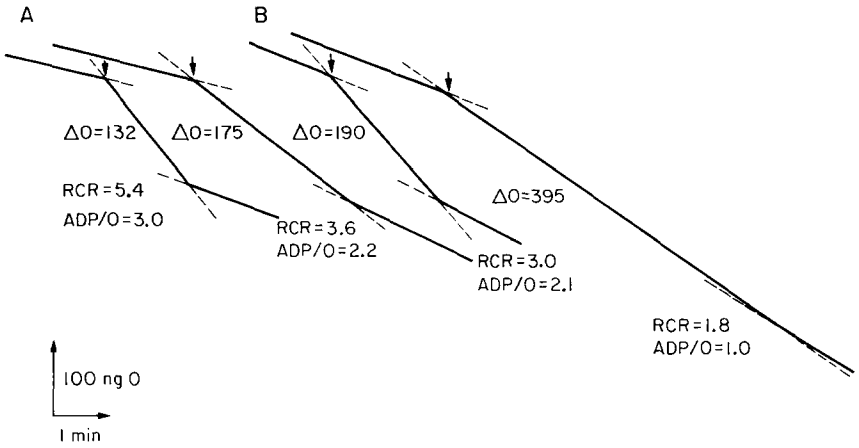

Fig. 1. Effect of propranolol on the respiratory control and oxidative phosphorylation of rat heart mitochondria. The assay system, in a total volume of 2 ml, contained 0.125 M sucrose, 50 mM KCl, 6 mM $MgCl_2$, 20 μM cytochrome *c*, 5 mM Hepes, and 10 mM phosphate buffer, pH 7.2. In each assay 0.5 mg mitochondria in 20 μl SEH buffer was added to the reaction chamber and incubated at 23° for 3 min before addition of the substrate. (a) illustrates the respiration supported by 9 mM each of glutamate and malate (left trace) and by 9 mM each of glutamate and malate in the presence of 1.0×10^{-3} M propranolol (right trace), respectively. (b) illustrates the respiration supported by 9 mM succinate (left trace) and by 9 mM succinate in the presence of 1.0×10^{-3} M propranolol (right trace), respectively. The number of oxygen atoms consumed (ng) in State 3 was indicated along each trace. The arrow in each trace indicates where 400 nmole of ADP was added to the assay system. The respiratory control ratio (RCR) and the ADP:O ratio for each trace was measured by the procedures described in Materials and Methods.

propranolol on rat heart mitochondria were dose-dependent, as shown in Tables 1 and 2. It was noticed that propranolol, at concentrations above 5.0×10^{-4} M, significantly inhibited the State 3 respiration but had little effect on the State 4 respiration (Tables 3 and 4). These results indicate that propranolol does not act like an uncoupler, such as 2,4-dinitrophenol [33] or some general anesthetics [34], which increase the State 4 respiration but only slightly affect the State 3 respiration.

We then tried to look for the respiratory enzyme(s) in the mitochondria that is inhibited by propranolol to account for the decrease of the State 3 respiration. Among the five electron transfer complexes only complex V [23] of the mitochondria was found to be sensitive to the drug. The Mg^{2+}-ATPase activity of this enzyme complex was significantly inhibited by propranolol, as shown in Fig. 2. However, the drug showed no measurable inhibition of the electron transfer activity of NADH dehydrogenase, succinate-cytochrome *c* reductase, and cytochrome oxidase of the mitochondria.

Since the inhibitory effect of propranolol toward the mitochondrial respiration has been located on the Mg^{2+}-ATPase from the above experiments, we then did some inhibition kinetic studies to pursue the action mechanism of this drug. Because the Mg^{2+}-ATPase has been known to protrude from mitochondrial inner membrane toward the matrix [33], we made the inhibition studies with the submitochondrial particles to avoid the complications of the latent ATPase activity of the mitochondria. By use of a series of varying fixed concentrations of the substrate in the presence of different drug con-

Table 1. Effect of propranolol on succinate-supported mitochondrial respiration and oxidative phosphorylation

Concentration of propranolol (M)	Respiratory control ratio	Decrease (%)	ADP:O ratio	Decrease (%)
0	3.0	0	2.1	0
8.5×10^{-5}	2.9	3.3	1.9	13.6
1.7×10^{-4}	2.8	6.7	1.7	22.7
3.4×10^{-4}	2.7	10.0	1.3	40.9
6.8×10^{-4}	2.5	16.7	1.1	59.1
1.0×10^{-3}	1.8	40.0	1.0	68.2

The respiratory control ratio was measured according to Chance and Williams [27], and the ADP:O ratio was determined by assuming the solubility of oxygen in the assay medium to be 250 μM [26]. The experimental conditions were detailed in Materials and Methods.

Table 2. Effect of propranolol on glutamate-malate-supported mitochondrial respiration and oxidative phosphorylation

Concentration of propranolol (M)	Respiratory control ratio	Decrease (%)	ADP:O ratio	Decrease (%)
0	5.4	0	3.0	0
2.5×10^{-4}	4.9	9.3	2.7	10.0
5.1×10^{-4}	4.0	25.9	2.6	13.3
1.0×10^{-3}	3.6	33.3	2.2	26.7
2.0×10^{-3}	1.8	66.7	0.6	80.0

The experimental conditions are described in Materials and Methods, and the solubility of oxygen in the assay medium was assumed to be 250 μM for the measurement of the ADP:O ratio [26].

Table 3. Effect of propranolol on succinate-supported State 3 and State 4 respiration of rat heart mitochondria

Propranolol concentration (M)	State 3 respiration (μmole/hr/mg)	Decrease (%)	State 4 respiration (μmole/hr/mg)	Decrease (%)
0	9.80	0	3.32	0
8.5×10^{-5}	9.77	0.6	3.66	0
1.7×10^{-4}	9.14	7.3	3.14	5.4
3.4×10^{-4}	9.42	4.3	3.32	0
6.8×10^{-4}	7.14	27.4	3.42	0
1.0×10^{-3}	5.94	39.6	3.14	5.4

The respiration rate of both States was calculated by assuming the solubility of oxygen in the assay medium to be 250 μM at 23° [26]. The experimental conditions are described in Materials and Methods.

Table 4. Effect of propranolol on glutamate–malate-supported State 3 and State 4 respiration of rat heart mitochondria

Propranolol concentration (M)	State 3 respiration (μmole/hr/mg)	Decrease (%)	State 4 respiration (μmole/hr/mg)	Decrease (%)
0	4.80	0	0.90	0
2.5×10^{-4}	4.98	0	1.02	0
5.1×10^{-4}	4.02	16.2	0.96	0
1.0×10^{-3}	3.00	37.5	0.78	13.3
2.0×10^{-3}	1.08	77.5	0.66	26.7

The experimental conditions are detailed in Materials and Methods, and the respiration rate of both States was calculated by assuming the solubility of oxygen in the assay medium to be 250 μM at 23° [26]

Table 5. Effect of propranolol on the kinetic constants of the purified Mg^{2+}-ATPase of rat heart mitochondria

Propranolol concentration (M)	V_{max}	K_m (mM)	$\frac{K_m}{V_{max}}$	K_i (mM)
0	11.73	0.34	2.9	—
3.4×10^{-4}	9.66	0.34	3.5	1.60
6.8×10^{-4}	7.42	0.35	4.7	1.44
1.0×10^{-3}	6.50	0.35	5.3	1.26

The kinetic constants were obtained from the data points of Fig. 4. V_{max} was expressed in the unit of μmole/min/mg. K_i was slightly decreased at the higher drug concentrations.

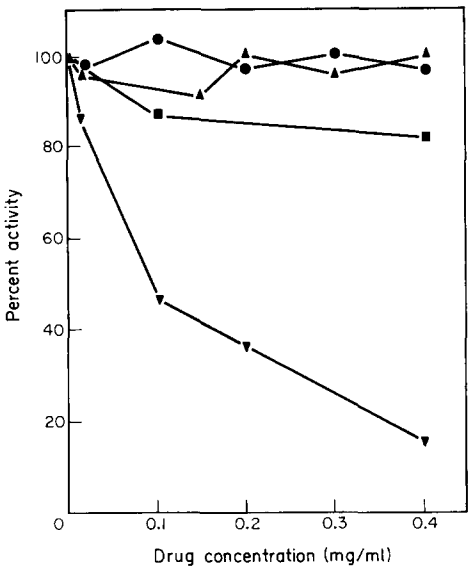

Fig. 2. Effects of propranolol on various electron transfer activities of rat heart mitochondria. The activity of NADH dehydrogenase (■), succinate-cytochrome *c* reductase (●), cytochrome oxidase (▲), and the Mg^{2+}-ATPase activity (▼) was assayed respectively by the methods described in Materials and Methods. In each assay the submitochondrial particles were incubated with the desired concentration of the drug at 23° for 3 min before addition of the substrate.

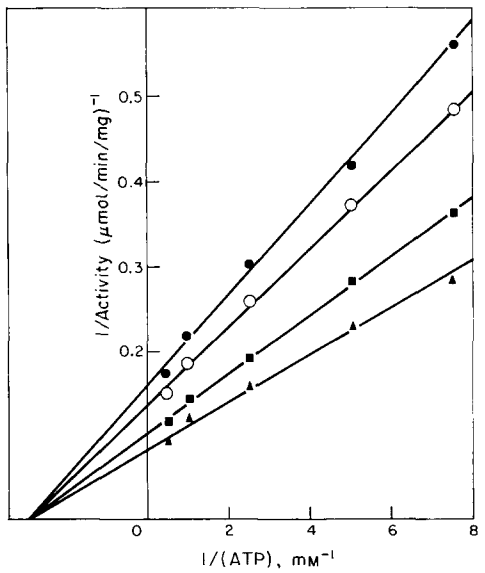

Fig. 4. Double reciprocal plot of propranolol inhibition of the purified Mg^{2+}-ATPase activity. The assay system was the same as that of Fig. 3 except that 10 μl of the purified Mg^{2+}-ATPase (about 6 μg) was used instead and no rotenone was added in the assay mixture. In the control experiments (▲) and each of the inhibition experiments, the ATP concentration varied from 0.13 to 2.0 mM. The final concentration of propranolol in the assay medium was 3.4×10^{-4} M (■), 6.8×10^{-4} M (○), and 1.0×10^{-3} M (●), respectively in the inhibition experiments.

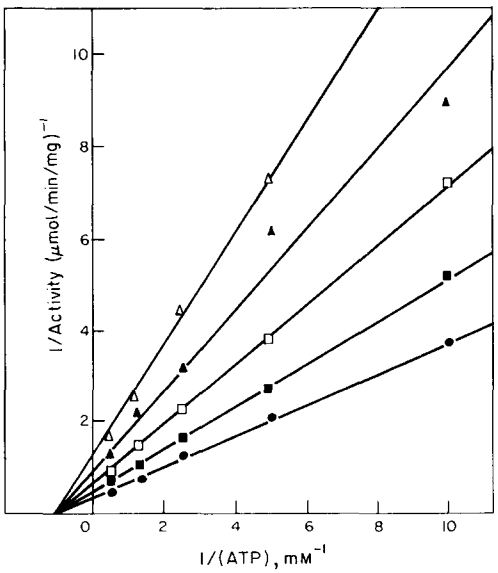

Fig. 3. Double reciprocal plot of propranolol inhibition of the Mg^{2+}-ATPase activity of the submitochondrial particles. In the control experiments (●), 20 μl of submitochondrial particles (about 30 μg) were incubated at 23° for 3 min before addition of ATP (0.1–2.0 mM), and the absorbance change at 340 nm was followed in a Perkin–Elmer lambda-5 spectrophotometer for 6–8 min. In the inhibition experiments, an identical amount of the submitochondrial particles was preincubated at 23° for 3 min in the assay medium containing propranolol at the final concentration of 3.4×10^{-4} M (■), 6.8×10^{-4} M (□), 1.0×10^{-3} M (▲), and 1.4×10^{-3} M (△), respectively.

centrations we found that propranolol is a non-competitive inhibitor of the mitochondrial Mg^{2+}-ATPase, as shown in Fig. 3. The inhibition constant was estimated to be 0.67 mM. Because the Mg^{2+}-ATPase activity is a partial reaction of the complex V, which is still associated with the membrane fragments in the submitochondrial particles, we can not differentiate from the above experiments whether propranolol directly acts on the enzyme molecule or exerts its inhibition through some secondary effects. In order to answer this question we further investigated the inhibitory action of propranolol on the purified Mg^{2+}-ATPase. The purified enzyme contained 5 bands as checked by SDS-polyacrylamide gel electrophoresis and had a specific activity of about 20 units (data not shown). These properties are comparable to those reported by the literature [25]. This purified Mg^{2+}-ATPase was found to be oligomycin-insensitive in contrast to the enzyme in submitochondrial particles [33]. This indicates that the purified Mg^{2+}-ATPase was not bound to mitochondrial inner membrane anymore, and was suited for studying the direct action of propranolol on the enzyme. By use of the purified Mg^{2+}-ATPase we did another set of inhibition experiments, and the results, as shown in Fig. 4, reveal the same inhibition pattern as that obtained with the submitochondrial particles. The inhibition constant as calculated from the plot was 1.4 mM, which is higher than that obtained with the Mg^{2+}-ATPase in the submitochondrial particles. The various kinetic constants of propranolol inhibition of the purified Mg^{2+}-ATPase are listed in Table 5. It can be seen that V_{max}

decreased proportionately to the increase of the drug concentration, whereas the K_m value of the substrate was not changed by the drug.

DISCUSSION

The integrity of the mitochondrial membrane is easily monitored by the respiratory control and the ADP:O ratio of the mitochondria in an oxygen monitor. Mitochondria are thus very suitable for studying the effects of biologically active compounds, such as drugs and toxins, on the structure and functions of the biological membrane [22]. Moreover, mitochondria provide most of the energy for the cell and thus are the right material for studying the drug effects on the energy metabolism of the cell. With these considerations in mind, we have employed rat heart mitochondria to study the effects of propranolol on the energy transduction system of the cardiac cells and try to provide a mechanism to account for the depression effect of the drug on cardiac functions.

The respiratory control ratio was about 3.0 and the ADP:O ratio was near 2.0 for succinate-supported respiration, and the values were about 6.0 and 3.0 for glutamate–malate-supported respiration of the mitochondria prepared by the procedure used in this study. These indices were concomitantly decreased when propranolol was added to the assay medium, and both effects were dose-dependent (Tables 1 and 2). It was noted that the drug did not affect the State 4 respiration but significantly inhibited the State 3 respiration when the drug concentration was above 5.0×10^{-4} M (Tables 3 and 4). These results indicate that propranolol does not behave like an uncoupler but rather act like an inhibitor of the mitochondrial respiration. However, there are two possibilities for propranolol to reduce the State 3 respiration, one is to inhibit the activity of the electron transport chain and the other is to inhibit the activity of the proton-translocating Mg^{2+}-ATPase of the mitochondria. If the former were true then propranolol should have shown inhibitory effect on at least one of the electron transfer complexes of the respiratory chain. The results as shown in Fig. 2 ruled out this possibility and clearly indicated that it was the inhibition of Mg^{2+}-ATPase activity that caused the decrease of the State 3 respiration. The inhibition of Mg^{2+}-ATPase activity by propranolol was noncompetitive (Fig. 3) and irreversible by either dialysis or addition of surplus ATP (data not shown).

In order to determine specifically whether propranolol acts directly on the Mg^{2+}-ATPase or indirectly through the membrane phase of the enzyme complex, we did inhibition studies with the purified enzyme preparation. The results, as shown in Fig. 4, unequivocally demonstrated that the drug indeed inhibits the Mg^{2+}-ATPase by directly acting on the enzyme molecule. This rules out the possibility that propranolol inhibits the State 3 respiration by interacting with the oligomycin-sensitivity conferring protein [33], since the purified Mg^{2+}-ATPase is not associated with that protein anymore but yet still sensitive to propranolol. It is possible that the drug specifically binds to the enzyme molecule and changes the tertiary structure of the protein and causes a dose-dependent denaturation of the enzyme. Since propranolol bears no structural similarity to ATP, it is comprehensible that the inhibition of the drug on Mg^{2+}-ATPase is noncompetitive (Figs. 3 and 4).

Propranolol is one of the β-blocking drugs whose negative inotropic effects have been repeatedly attributed to the so-called membrane stabilization effects [17–19]. However, these effects are usually poorly-defined and sometimes misused as recently pointed out by Smith [20], and Hong and Chiang [36]. We believe that the membrane stabilization activity of propranolol [18] should be defined according to the particular type of action observed. In this paper, we have clearly demonstrated that propranolol impairs mitochondrial functions mainly through its inhibition of the Mg^{2+}-ATPase activity of the complex V of the mitochondrial inner membrane. This action is quite different from the uncoupling effect of some general anesthetics [34]. Moreover, the effect of propranolol on mitochondrial respiration and oxidative phosphorylation can be easily rationalized with the chemiosmotic theory [35]. At State 3 respiration, mitochondria actively pump protons across the inner membrane and build up an electrochemical gradient which is supposed to be utilized by the Mg^{2+}-ATPase complex to make ATP. When propranolol is present the Mg^{2+}-ATPase activity is inhibited and thus the proton gradient generated by respiration can not be consumed and will then feed-back inhibit the active respiration of State 3. At the concentration lower than 2.0×10^{-3} M, propranolol inhibition of the Mg^{2+}-ATPase activity is not complete and thus the State 4 respiration is still able to proceed as the control (cf. Fig. 1, and Tables 3 and 4).

In clinical treatments, the plasma levels of propranolol associated with β-adrenergic receptor blockade in man are approximately 4.0×10^{-8} M, and as high as 4.0×10^{-7} M has been reported [37–39]. These concentrations are well below the concentration (10^{-5}–10^{-4} M) of the drug required to exert biochemical effects on animal heart mitochondrial functions as shown in this study. However, it has been shown that plasma concentrations of propranolol in animals may be well below the concentration of the drug in certain tissues, including lung and heart [40]. In patients overdosed with propranolol the plasma levels of the drug can reach as high as 10^{-5} M [41]. Therefore, the drug concentration in the heart will be higher than enough to damage the mitochondrial functions of the cardiac cell. On the other hand, the concentration of propranolol required for local anesthetic effects in isolated animal and human cardiac muscle preparation is between 10^{-5} and 10^{-4} M [42, 43]. This concentration is similar to the concentration of propranolol required to impair the mitochondrial structure and functions (Tables 1–4). At the same concentration ranges, propranolol also inhibits many other energy-dependent functions of animal cardiac cells [8–15]. These cascade effects of propranolol may be negligible when the patients or animals receive only a low dose of the drug for β-adrenergic receptor blockade. However, when the patients or animals take propranolol for a long period of time or on a very high dosage at a time, the drug may

be accumulated in heart and some other tissues at severely high concentrations that in turn cause the aforementioned cascade effects to damage the cardiac functions. The biochemical effects of propranolol on animal heart mitochondrial respiration and oxidative phosphorylation may play an important role in the well-known non-receptor-mediated membrane action of the drug. These effects of propranolol, which occur only above certain concentrations, may explain the well-documented depression effect of the drug on the cardiac functions of the animal.

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