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ALTERED ENERGY COUPLING IN RAT HEART MITOCHONDRIA FOLLOWING *IN VIVO* TREATMENT WITH PROPRANOLOL

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Abstract—Effects of acute and chronic treatment with propranolol on oxidative phosphorylation in rat heart mitochondria were examined. Acute propranolol treatment resulted in inhibition of coupled respiration with pyruvate + malate and succinate as substrates. Chronic treatment resulted in decreased state 3 respiration rates with all the substrates employed. The net effect of propranolol treatment was decreased ATP-phosphorylation rates suggesting that this was possibly one of the modes of its cardiodepressant activity. Additionally, chronic propranolol treatment brought about a decrease in the content of cytochrome $c + c_1$ in heart mitochondria. Estimation of propranolol concentrations in serum, whole tissue homogenate and heart mitochondria indicated that although the mitochondria accumulated the highest amount of the drug, the intramitochondrial concentration of the drug was one or two orders of magnitude lower than that which is required to bring about inhibition of respiration under *in vitro* conditions. Besides, the concentrations reached under acute and chronic treatment conditions were almost comparable. The results, therefore, suggest that the action of the drug *in vivo* may involve more intricate mechanisms than those observed under *in vitro* conditions.

Propranolol—the well-known β-adrenergic receptor antagonist—is widely used for the treatment of hypertension [1, 2], cardiac arrythmias and angina pectoris [3], prevention of myocardial infarction [4, 5] and several other clinical conditions [6, 7]. The drug is believed to act by blocking the β-adrenoceptors of the heart, thus protecting it from adrenergic stimulation which causes oxygen wastage and ATP and creatinine phosphate depletion [8, 9]. The negative inotropic cardiodepressant activity of propranolol has been explained partly on the basis of its action *in vitro* on sarcoplasmic reticulum [10, 11] and mitochondrial function in the heart [12–15] and has also been repeatedly attributed to its so-called membrane stabilization effects [16–18]. Interestingly it has been shown that the negative inotropic effect of a series of β-adrenergic blockers including propranolol is not proportional to their β-blocking potency [19, 20].

It has been reported that several β-adrenergic antagonists significantly reduce the myofibrillar ATPase activity in dog heart muscle [21], inhibit calcium uptake [22] and Ca^{2+}-ATPase activity [23, 24] of the sarcoplasmic reticulum of the cardiac cell. The decrease in the myofibrillar ATPase activity is closely related to the depression in cardiac muscle contractility. Inhibition of the energy generating system of the cardiac cells could, therefore, result in depressed myocardial functions [25]. Thus, it was observed that under *in vitro* conditions propranolol inhibited coupled as well as uncoupled respiration in heart mitochondria [12–15]. Huunan-Seppala has further demonstrated that propranolol specifically binds to the mitochondrial membrane in rat heart [26].

The *in vitro* studies [12–15, 21–24] however, cannot be extrapolated directly to the *in vivo* effect of this drug on mitochondrial function particularly since the concentrations of drug employed (10^{-5}–10^{-4} M) are several fold higher than those reached in plasma of patients undergoing clinical treatments [27]. An examination of the *in vivo* effects of propranolol on the cellular energy generating system i.e. mitochondrial energy coupling is therefore warranted. The present communication summarizes the findings of such investigations. The effects of both acute and chronic treatments of rats with propranolol were studied since it has been found in clinical practice that while propranolol exerts immediate effects, it is also administered for prolonged periods as a protective measure. Graded amounts of propranolol were administered to the rats in the present studies in order to examine the dose-response of cardiac mitochondrial function to the drug action.

MATERIALS AND METHODS

Chemicals. Sodium salts of succinic acid, L-glutamic acid, pyruvic acid, L-malic acid, ascorbic acid, adenosine diphosphate (ADP) and rotenone, mannitol, DL-propranolol hydrochloride, ethyleneglycol-bis-(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA), 3[*N*-Morpholino], propane sulfonic acid (MOPS), ethylene diaminetetraacetic acid (EDTA) and Triton X-100 were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). *N,N,N′,N′*-Tetramethyl-*p*-phenylenediamine (TMPD) was from British Drug Houses (Poole, U.K.). All other chemicals used were of the highest purity grade available commercially.

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All solvents were double-distilled prior to use.

Animals. Male albino rats of Wistar strain weighing between 220–250 g were used and were administered DL-propranolol hydrochloride (made up in physiological saline) intraperitoneally (i.p.) at the doses of 2.5, 5.0 or 10.0 mg/kg body weight [28]. The acute treatment consisted of a single injection and the animals were killed 1 hr later. For chronic treatment, the animals received propranolol injections for five consecutive days and were killed 1 hr after the last injection. The control animals received only the saline vehicle.

Isolation of mitochondria and study of oxidative phosphorylation. The animals were killed by decapitation and their hearts were quickly collected in chilled (0–4°) isolation medium consisting of 0.225 M mannitol, 0.075 M sucrose and 1 mM EDTA, pH 7.4 [29]. The tissue was washed repeatedly with the isolation medium to free it from adhering blood, minced into small pieces (1.5 × 1.5 mm) and homogenized using a Potter-Elvehjem type glass–teflon homogenizer to obtain 10% (w/v) homogenate. The isolation of mitochondria was essentially as described by Doussiere *et al.* [30] and Katyare and Billimoria [31]. Mitochondria were washed once by resedimenting and were finally suspended in the isolation medium at a concentration of *ca.* 13 mg protein/mL. All operations were carried out at 0–4° using a Sorvall RC5C refrigerated centrifuge.

Measurements of oxidative phosphorylation were carried out as reported earlier [31, 32] at 25° in a medium (total volume 1.3 mL) consisting of 225 mM sucrose, 20 mM KCl, 1 mM EGTA, 10 mM MOPS buffer pH 7.4 and 5 mM potassium phosphate buffer, pH 7.4 employing a Clark-type oxygen electrode [31, 33]. Glutamate (10 mM), succinate (10 mM), pyruvate (10 mM) + malate (1 mM) and ascorbate (10 mM) + TMPD (0.1 mM) were used as the substrates. With succinate and ascorbate + TMPD, 1.0 μM rotenone was included in the reaction medium. Approximately 1–3 mg of mitochondrial proteins were used per experiment depending on the substrate employed. ADP-phosphorylation rates were calculated assuming total phosphorylation of the added ADP [14, 31, 34].

Quantification of cytochrome contents. The contents of the cytochromes in Triton X-100 solubilized mitochondria were quantified essentially by following the procedure described earlier [32]. Calculation of cytochromes content was as reported previously [32].

Determination of propranolol content in serum and tissue samples. Propranolol content in serum and tissues was measured essentially as described by Shand *et al.* [27] with some modifications. Thus, 0.5 mL aliquots of serum, homogenate or mitochondrial suspension (approximately 5–7 mg protein) were alkalinized with 1.0 mL of 1.0 N NaOH and extracted into 5.0 mL of *n*-heptane containing 1.5% (v/v) isoamyl-alcohol. The tubes were vortexed vigorously and centrifuged at 2000 rpm for 10 min. Four mL of the organic phase was then extracted into 4.0 mL of 0.1 N HCl. The tubes were vortexed vigorously and centrifuged. The upper organic phase was removed and discarded using a Pasteur pipette and the fluorescence of the acid phase was measured in a Hitachi 203 fluorescence spectrophotometer (excitation λ: 290 nm; emission λ: 340 nm). Appropriate blanks were used for serum, homogenate and mitochondria. The blanks did not display any detectable fluorescence. In separate experiments it was found that the recovery of a range of added propranolol concentrations from serum and tissue samples using the above procedure was approximately 80%; varying amounts of tissue, serum or mitochondrial proteins were employed for the recovery experiments.

Glassware used for the determination of propranolol by fluorimetry was soaked overnight in 3.0 N HCl, rinsed five times with tap water and five times with distilled water prior to use.

A standard curve was constructed using pure DL-propranolol hydrochloride in the range 0 to 50 ng/mL and the amounts of propranolol in the samples were read off against the standard.

Protein determinations were according to Lowry *et al.* [35] with crystalline bovine serum albumin used as the standard.

Results are given as mean ± SEM of the number of independent observations indicated in individual tables. Student's *t*-test was employed to evaluate the significance of differences between the means.

RESULTS

Effects of acute propranolol treatment on oxidative phosphorylation

These data are summarized in Table 1.

Thus with glutamate as the respiratory substrate, the ADP/O ratios did not change following propranolol treatment. However, the respiratory rates exhibited tendency towards decrease at higher (5.0 and 10.0 mg) drug concentrations. Consequently, the ADP-phosphorylation rates were found to be decreased significantly. This is illustrated with data on 10.0 mg dose (Table 1). For pyruvate + malate state 3 respiration rate decreased by 16%, with a parallel decrease in the ATP synthesis rate. The latter, however, was not statistically significant. Almost similar effects were seen even at 5.0 mg dose (data not given). A lower dose of 2.5 mg had no effect. The trend was practically the same for succinate, with a 10.0 mg dose showing 11% decrease in state 3 respiration and a tendency towards decreased ADP-phosphorylation. Acute propranolol treatment did not influence oxidative phosphorylation with ascorbate + TMPD substrate pair (Table 1).

Effects of chronic propranolol treatment on oxidative phosphorylation

Following chronic treatment with propranolol, state 3 respiration rate with glutamate decreased progressively in a dose-dependent manner from 18–58% which was reflected in a similar (19–53%) decrease in the ATP-synthesis rate. The state 4 respiration rates recorded even a greater decrease of 46–79% (Table 2). The trend was similar for pyruvate + malate oxidation where the state 3 respiration rates and ADP-phosphorylation rates decreased from 37–45% and 40–49%. However, the state 4 respiration rates were unaffected (Table 3).

Table 1. Effects of acute propranolol treatment on oxidative phosphorylation in rat heart mitochondria

Substrate	Propranolol treatment (mg)	ADP/O ratio	Rate of respiration (nmol O_2/min/mg protein) + ADP	Rate of respiration (nmol O_2/min/mg protein) − ADP	ADP phosphorylation rate (nmol/min/mg protein)
Glutamate	0.0 (24)	3.06 ± 0.29	42.7 ± 2.5	9.3 ± 1.7	261.0 ± 14.8
	10.0 (12)	3.08 ± 0.25	37.2 ± 2.7 NS	6.6 ± 1.7 NS	225.0 ± 8.6*
Pyruvate + malate	0.0 (30)	2.82 ± 0.10	77.8 ± 2.7	12.2 ± 1.4	433.0 ± 16.9
	10.0 (12)	2.94 ± 0.16	65.5 ± 2.1†	10.3 ± 1.8 NS	387.9 ± 23.0 NS
Succinate	0.0 (20)	1.26 ± 0.07	160.8 ± 6.4	82.4 ± 8.8	394.9 ± 20.7
	10.0 (28)	1.33 ± 0.05	142.9 ± 4.6*	73.4 ± 4.4 NS	373.6 ± 14.6 NS
Ascorbate + TMPD	0.0 (8)	0.43 ± 0.02	185.8 ± 12.2	99.5 ± 9.1	160.9 ± 8.4
	10.0 (16)	0.46 ± 0.07	193.3 ± 5.3 NS	103.3 ± 3.7 NS	179.1 ± 5.1 NS

Doses of propranolol are expressed as mg/kg body weight. The animals were administered a single injection (i.p.) and killed 1 hr later. Oxidative phosphorylation was measured polarographically at 25° in a total volume of 1.3 mL. Respiration medium and other experimental details are given in Materials and Methods. Results are given as mean ± SEM of the number of independent observations indicated in the parentheses.

* $P < 0.05$, † $P < 0.002$ compared to control; NS, not significant.

Table 2. Effect of chronic propranolol treatment on oxidative phosphorylation in rat heart mitochondria using glutamate as the substrate

Propranolol treatment (mg)	ADP/O ratio	Rate of respiration (nmol O_2/min/mg protein) + ADP	Rate of respiration (nmol O_2/min/mg protein) − ADP	ADP-phosphorylation rate (nmol/min/mg protein)
0.0 (24)	3.05 ± 0.21	44.2 ± 2.1	16.0 ± 2.5	252.8 ± 7.6
2.5 (18)	2.86 ± 0.12	36.2 ± 1.8†	4.9 ± 0.8‡	204.3 ± 7.7‡
5.0 (10)	2.85 ± 0.17	31.3 ± 1.7‡	8.6 ± 1.9*	176.7 ± 11.5‡
10.0 (10)	3.00 ± 0.29	18.7 ± 1.0‡	3.4 ± 1.0‡	118.2 ± 7.7‡

The animals were administered propranolol injections (i.p.) for five consecutive days and were killed 1 hr after the last injection. Other experimental details are as described in Table 1 and in Materials and Methods. The results are given as mean ± SEM of the number of observations indicated in parentheses.

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ compared to control.

Table 3. Effect of chronic propranolol treatment on oxidative phosphorylation in rat heart mitochondria using pyruvate + malate as the substrate

Propranolol treatment (mg)	ADP/O ratio	Rate of respiration (nmol O_2/min/mg protein) + ADP	Rate of respiration (nmol O_2/min/mg protein) − ADP	ADP phosphorylation rate (nmol/min/mg protein)
0.0 (14)	2.94 ± 0.16	84.2 ± 2.4	13.1 ± 2.7	496.7 ± 33.6
2.5 (8)	2.77 ± 0.08	52.9 ± 3.4*	11.2 ± 0.8 NS	295.6 ± 22.4*
5.0 (10)	2.47 ± 0.20	50.5 ± 3.4*	10.9 ± 2.4 NS	253.2 ± 21.7*
10.0 (24)	2.97 ± 0.10	46.1 ± 1.9*	7.7 ± 0.6 NS	269.8 ± 15.7*

Experimental details are as described in Table 2 and in Materials and Methods. The results are given as mean ± SEM of the number of observations indicated in parentheses.

* $P < 0.001$ compared to control. NS, not significant.

When succinate was employed as the respiratory substrate, the effect was seen only at the highest concentration of the drug, amounting to 21% decrease in both the state 3 respiration rate and ATP synthesis rate (Table 4). For ascorbate + TMPD decrease in the state 3 respiration rate (34% decrease) was noted only at the highest dose of the drug. The ADP/O ratios were somewhat low.

Table 4. Effect of chronic propranolol treatment on oxidative phosphorylation in rat heart mitochondria using succinate as the substrate

Propranolol treatment (mg)	ADP/O ratio	Rate of respiration (nmol O_2/min/mg protein) + ADP	− ADP	ADP-phosphorylation rate (nmol/min/mg protein)
0.0 (12)	1.28 ± 0.06	160.5 ± 4.8	82.9 ± 8.7	407.1 ± 15.4
2.5 (8)	1.28 ± 0.06	160.9 ± 6.3 NS	83.9 ± 7.0 NS	409.7 ± 22.1 NS
5.0 (10)	1.09 ± 0.05	155.6 ± 4.7 NS	88.4 ± 7.9 NS	338.4 ± 16.8*
10.0 (22)	1.27 ± 0.07	127.1 ± 4.4†	78.6 ± 4.3 NS	322.2 ± 20.6*

Experimental details are as described in Table 2 and in Materials and Methods. The results are expressed as mean ± SEM of the number of observations indicated in parentheses.
* $P < 0.01$, † $P < 0.001$ compared to control. NS, not significant.

Table 5. Effect of chronic propranolol treatment on oxidative phosphorylation in rat heart mitochondria using ascorbate + TMPD as the substrate

Propranolol treatment (mg)	ADP/O ratio	Respiration rate (nmol O_2/min/mg protein) + ADP	− ADP	ADP-phosphorylation rate (nmol/min/mg protein)
0.0 (13)	0.45 ± 0.01	210.3 ± 8.0	75.9 ± 3.5	190.3 ± 7.6
2.5 (14)	0.35 ± 0.01	210.4 ± 11.3 NS	82.9 ± 4.6 NS	147.3 ± 10.9†
5.0 (13)	0.39 ± 0.01	211.8 ± 6.1 NS	116.2 ± 4.1‡	163.0 ± 6.5*
10.0 (23)	0.40 ± 0.01	138.6 ± 4.7‡	85.0 ± 3.5 NS	110.9 ± 3.8‡

Experimental details are as described in Table 2 and in Materials and Methods. The results are given as mean ± SEM of the number of observations indicated in parentheses.
* $P < 0.02$, † $P < 0.01$, ‡ $P < 0.001$, compared to control. NS, not significant.

Table 6. Effect of propranolol treatment on intramitochondrial cytochrome contents

Propranolol treatment (mg)	Cytochrome content (pmol/mg protein) aa_3	b	$c + c_1$
Acute			
0.0 (8)	446.2 ± 20.0	290.6 ± 18.0	598.7 ± 20.0
2.5 (5)	452.8 ± 20.0 NS	272.1 ± 29.0 NS	575.2 ± 12.0 NS
5.0 (5)	416.4 ± 10.0 NS	241.8 ± 23.0 NS	608.2 ± 16.0 NS
10.0 (5)	429.5 ± 34.0 NS	256.6 ± 15.0 NS	580.7 ± 23.0 NS
Chronic			
0.0 (8)	455.2 ± 30.0	280.4 ± 18.0	611.2 ± 20.0
2.5 (5)	469.1 ± 20.0 NS	283.2 ± 10.0 NS	522.4 ± 23.0*
5.0 (5)	443.6 ± 15.0 NS	285.8 ± 18.0 NS	488.0 ± 20.0†
10.0 (5)	428.3 ± 38.0 NS	275.6 ± 24.0 NS	476.9 ± 25.0†

The doses of propranolol are expressed as mg/kg body weight. Experimental details are as described in Tables 1 and 2 and in Materials and Methods. The results are given as mean ± SEM of the number of observations indicated in parentheses.
* $P < 0.02$, † $P < 0.002$; NS, not significant.

Consequently, the ATP-synthesis registered a decrease from 14–42%. State 4 respiration rate increased transiently (53% increase) at 5.0 mg propranolol dose (Table 5).

Effect of propranolol treatment on intra-mitochondrial cytochromes content

As is to be expected, the acute treatment with propranolol did not have any effect on the cytochromes content in the heart mitochondria (Table 6). However, when the propranolol treatment was of a chronic nature, there was a significant reduction in the content of cytochromes $c + c_1$ (Table 6). The decrease was found to be in the range of 15–22%. The other classes of cytochromes i.e. aa_3 and b were unaffected by chronic propranolol treatment.

Propranolol concentration in tissues

The data on propranolol concentrations in serum, homogenate and mitochondria after acute and chronic treatment with the drug are given in Table

Table 7. Concentrations of propranolol in serum, homogenate and mitochondria following *in vivo* acute and chronic treatment of rats with the drug

Treatment (mg)	Serum (ng/mL)	Homogenate (ng/g tissue)	Mitochondria (ng/mg protein)
Acute			
0.0	ND	ND	ND
2.5	88.8 ± 3.56	643.8 ± 27.44	5.6 ± 0.24
5.0	96.5 ± 7.16	563.3 ± 31.76	6.3 ± 0.28
10.0	132.7 ± 4.32	609.0 ± 15.60	6.6 ± 0.49
Chronic			
0	ND	ND	ND
2.5	72.1 ± 3.54	593.8 ± 33.97	6.2 ± 0.20
5.0	97.8 ± 5.32	825.6 ± 73.88	7.1 ± 0.37
10.0	127.3 ± 7.23	778.8 ± 49.18	7.8 ± 0.30

The results are given as mean ± SEM of eight independent observations. Doses of propranolol are expressed as mg/kg body weight.
ND, not detectable.

7. It can be seen that in the serum, the propranolol content increased in both acute and chronic treatment groups in a dose-dependent manner. Interestingly, in both the treatment groups, comparable levels of propranolol concentrations were reached in serum for the given dose of propranolol and ranged from 72 to 133 ng/mL.

After acute propranolol treatment, the drug concentration in the whole tissue (heart homogenate) reached a more or less constant level (563 to 644 ng/g tissue) irrespective of the dose of propranolol employed. In the case of chronic treatment group, at the lowest dose employed, the concentration of propranolol in the homogenate was comparable to that for the acute treatment group. However, at the 5.0 and 10.0 mg doses, substantially higher levels of the drug were found to be present in the homogenate compared with the corresponding acute treatment groups (e.g. 779 to 826 ng/g tissue as against 563 to 644 ng/g tissue). It is noteworthy that in both the treatment groups the level of the drug present in the whole tissue were substantially higher (4.6–8.4-fold higher) compared with the serum.

The concentration of propranolol in the mitochondrial fraction ranged from 5.6–7.8 ng/g mitochondrial protein in the two treatment groups. Nevertheless, after chronic administration of propranolol, there was a tendency towards increased concentrations (15–26% increase) of the drug in the mitochondrial fraction with the increasing dose.

DISCUSSION

The present investigations were carried out with a view to finding out the immediate and long-term effects of propranolol on cardiac energy metabolism. This becomes relevant in view of the fact that the cardiodepressant activity of the drug is evident within minutes and also because the drug is given for prolonged periods for prevention of cardiac conditions such as myocardial infarction [4, 5]. The results of the present studies have brought out the subtle differences between immediate and long-term effects of propranolol treatment.

Thus, the major effects of acute propranolol treatment (Table 1) seemed to be a tendency towards decreased state 3 respiration rates/ADP-phosphorylation rates especially at the higher doses when glutamate, pyruvate + malate or succinate were used as the respiratory substrates. With ascorbate + TMPD, on the other hand, acute treatment with propranolol did not affect mitochondrial energy metabolism.

Chronic treatment with propranolol resulted in substantial reduction in state 3 respiration rates especially with NAD^+ linked substrates e.g. glutamate and pyruvate + malate. There was also a concomitant decrease in ADP-phosphorylation rates. When the substrate was succinate or ascorbate + TMPD, the treatment was effective in depressing state 3 respiration rates only at the highest dose. ADP-phosphorylation rates were also significantly diminished. Thus, in general, the chronic treatment was more effective in reducing respiratory activity and ADP-phosphorylation rates.

The difference between the chronic and acute treatments was also evident from the data on the intramitochondrial cytochromes contents. Thus, the acute propranolol treatment did not alter the cytochrome profiles. However, following the chronic treatment, cytochromes $c + c_1$ were significantly decreased. Therefore, it is possible that the early effects of propranolol treatment may ensue from specific and non-specific binding of propranolol to mitochondrial membrane, since it is known that in addition to the β-adrenergic sites, propranolol also binds in large proportion to other cellular sites possibly involving both lipid and protein domains causing membrane perturbations [36–39]. The long-term effects, on the other hand, may involve more intricate mechanisms such as compositional changes in the respiratory chain in terms of alterations in the cytochromes content as is evident from the data in Table 6.

Since the concentrations of propranolol in the whole tissue or mitochondria were generally on the higher side in the chronic treatment group (Table 7), it was of interest to calculate the intracellular

Table 8. Calculated concentrations of propranolol in serum, homogenate, mitochondria of rat heart following acute and chronic treatment

Treatment (mg)	Calculated propranolol concentration (μM)			
	Serum	Homogenate	Mitochondria	Cytosol
Acute				
2.5	0.30 ± 0.012	5.58 ± 0.22	15.66 ± 0.70	3.06 ± 0.15
5.0	0.33 ± 0.024	4.88 ± 0.25	17.69 ± 0.79	1.68 ± 0.20
10.0	0.45 ± 0.015	5.28 ± 0.14	18.68 ± 1.40	1.92 ± 0.18
Chronic				
2.5	0.24 ± 0.012	5.15 ± 0.31	17.47 ± 0.57	2.07 ± 0.11
5.0	0.33 ± 0.018	7.16 ± 0.57	20.03 ± 1.09	3.94 ± 0.32
10.0	0.43 ± 0.024	6.75 ± 0.44	22.09 ± 0.87	2.92 ± 0.14

The results are given as mean ± SEM of eight independent observations. Doses of propranolol are expressed as mg/kg body weight.

and intramitochondrial propranolol concentrations reached under the various treatment conditions. This was achieved by making certain basic assumptions e.g. it has been reported that in the rat heart, the amount of mitochondrial protein is about 65 mg per g tissue [40]. Based on this we tried to find out the total amount of propranolol associated with mitochondria and the cytosol. These values were then used to obtain the molar concentrations by using the mitochondrial and cytosolic water volume. Thus, it has been reported that the average aqueous volume of mitochondria is of the order of 1.2 μL/mg protein [41] and that water volume in the cell is about 5 times the mitochondrial water volume. Thus, the cytosolic aqueous volume would be about 4 times the mitochondrial volume. From calculations based on the above assumption it was apparent that a major portion ranging from 55–72% of the total cellular propranolol was associated with the mitochondria in both acute and chronic treatment groups and comparatively less propranolol was present in the cytosolic compartment (data not given). When actual molar concentrations were calculated based on mitochondrial water volume or cellular water volume (Table 8), in the whole tissue homogenate the concentration of the drug ranged from 4.9 to 7.2 μM while that in mitochondria was about 3–5 times higher. The concentration in the cytosol was indeed very low, about half that seen for the whole tissue. As against this, the concentrations in the serum were very, very low, about 10–20 times lower than those seen for whole tissue (Table 8).

It is thus clear that tissue such as heart, concentrated substantially high amounts of propranolol compared to the circulating levels of the drug; interestingly the highest concentrations within the tissue were found in the mitochondrial rather than the cytosolic fraction. Although the highest, the mitochondrial concentration still ranged from 16 to 22 μM (Table 8). These concentrations are at least one to two orders of magnitude lower than those employed for the *in vivo* studies. Thus, Bhayana *et al.* [14] found that the mitochondrial respiratory activity was inhibited at or around 0.3 mM concentrations of propranolol added under *in vitro* conditions. Similar findings were also reported by others [25]. Quinn and Crutcher [15] reported that the respiration in isolated rat heart mitochondria was inhibited only at 5–10 mM concentration of propranolol under *in vitro* conditions. Interestingly, even for sonic particles, the inhibitory concentrations were the same as those for the whole mitochondria [15]. Taken together, these results would suggest that for the *in vitro* studies, substantially higher concentrations than those reached under *in vivo* conditions are required. Besides, studies with the submitochondrial particles would seem to rule out the permeability barrier to the drug under *in vitro* conditions since the inhibitory concentrations for the whole mitochondria and the submitochondrial particles were of the same order of magnitude [15].

In our own studies, the intramitochondrial concentrations of the drug reached under both acute and chronic conditions were almost comparable. Nevertheless, the effects on respiratory activity in the two treatment groups were different. These results would therefore suggest that more intricate mechanisms are involved in the *in vivo* effects of the drug on energy metabolism in the mitochondria. Thus, in a simplistic way, the effects of acute treatment may ensue from a specific and nonspecific interaction of the drug with membranes [36–39] as discussed above. The effects of chronic treatment, on the other hand may be a consequence of altered cytochrome profiles e.g. decreased contents of cytochromes $c + c_1$ (Table 6). We have previously shown that cytochrome c is a rate-limiting step in regulating mitochondrial respiration [42].

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