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EFFECTS OF ACEBUTOLOL, PRACTOLOL AND PROPRANOLOL ON THE RAT HEART SARCOLEMMA*

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Abstract—The actions of acebutolol, practolol and propranolol on the rat heart sarcolemmal ATPase, adenylate cyclase and calcium binding activities were studied. None of these agents had any effect on the basal adenylate cyclase activity. Only propranolol at 2 mM or higher concentrations depressed calcium binding; this inhibitory effect was less pronounced at high concentrations of calcium. Propranolol (1–5 mM), but not practolol, markedly depressed Na^+-K^+ ATPase activity whereas acebutolol (3–5 mM) produced a slight but significant inhibition. Propranolol (1–5 mM), but not acebutolol or practolol, also inhibited the Ca^{2+} ATPase and Mg^{2+} ATPase activities. These results indicate sarcolemmal membrane as the site affected by propranolol and it is suggested that the cardiodepressant action of high doses of this agent may partly be due to changes in the sarcolemmal ATPase and calcium binding activities.

A well-known β-adrenergic receptor blocking agent, propranolol, has been demonstrated by various investigators to depress myocardial contractility and inhibit calcium accumulation by the heart microsomal and mitochondrial fractions [1–5]. On the other hand, acebutolol and practolol, which have been shown to exhibit greater cardioselectivity as β-adrenergic blocking agents in comparison to propranolol [6–8], were found to produce no appreciable effects on contractile force as well as calcium uptake by heart mitochondrial and microsomal fractions [5]. Although heart sarcolemma has been demonstrated to bind a considerable amount of calcium [9, 10], no information concerning the actions of different β-adrenergic blocking agents on this aspect is available in the literature. It should be mentioned here that propranolol has been reported to prevent the epinephrine-stimulated adenylate cyclase activity of heart sarcolemma [11, 12]; however, nothing is known about the effects of β-adrenergic blocking agents on the activities of other sarcolemmal enzymes such as Na^+-K^+ ATPase and Ca^{2+}/Mg^{2+} ATPase, which are considered to play an important role in the movements of different cations across the heart cell membrane [11, 13, 14]. It was therefore decided to examine the actions of propranolol on the heart sarcolemmal calcium binding, Na^+-K^+ ATPase and Ca^{2+}/Mg^{2+} ATPase activities. Both acebutolol and practolol were used for the purpose of comparison in this study.

MATERIALS AND METHODS

Male albino rats weighing 300–400 g were decapitated, the hearts quickly removed and the sarcolemmal fraction was isolated by the hypotonic-LiBr treatment method [13]. For this purpose, the ventricles were minced and homogenized in 20 vol. 50 mM Tris–HCl containing 1 mM EDTA, pH 7.4. The homogenate was passed through four layers of gauze and centrifuged at 1000 *g* for 10 min. The sediment was suspended in 20 vol. 10 mM Tris–HCl, pH 7.4, gently stirred for 30 min and centrifuged for 10 min at 1000 *g*. This procedure was repeated twice and the sediment thus obtained was extracted for 45 min with 0.4 M LiBr solution containing 10 mM Tris–HCl, pH 7.4. After centifugation at 1000 *g* for 10 min the pellet was washed and further extracted with 0.6 M KCl for 15 min. This suspension was centrifuged for 10 min at 1000 *g*, the sediment washed thoroughly and suspended in 1 mM Tris–HCl, pH 7.0. This procedure for isolating heart sarcolemmal membranes was carried out at 4°. Electron microscopic and marker enzyme examination of the rat heart fraction similar to that for the dog heart sarcolemma [13] revealed that these membrane vesicles contained minimal contamination due to major cytoplasmic constituents such as myofibrils, mitochondria and sarcoplasmic reticulum.

The ATP hydrolyzing activity of heart sarcolemma was studied by suspending 100–150 μg of membrane protein in a medium containing 50 mM Tris–HCl, pH 7.4, and 1 mM EDTA with or without 4 mM Mg^{2+} or Ca^{2+}. After incubating the membranes for 3 min at 37°, the reaction was started by the addition of 4 mM ATP and stopped 10 min later by the addition of 12% cold trichloroacetic acid. The P_i released in the clear supernatant was determined and the activities due to the presence of Ca^{2+} and Mg^{2+} were taken to be due to Ca^{2+} ATPase and Mg^{2+} ATPase activities respectively. The total ATP hydrolyzing activity of the heart membrane was determined in the same manner as described above except that the incubation medium contained 50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 4 mM $MgCl_2$, 120 mM NaCl and

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10 mM KCl. The difference between the total ATPase and Mg^{2+} ATPase activities was taken to be due to the Na^+-K^+ stimulated ATPase activity. The details of these methods for determining ATPase activities have been described elsewhere [11, 13]. The activities of Mg^{2+} ATPase and Na^+-K^+ ATPase were also determined in the presence of various concentrations of Mg ATP so that the ratio of Mg^{2+}:ATP was = 1. Likewise, the activity of Ca^{2+} ATPase was measured in the presence of various concentrations of Ca ATP by keeping the ratio of Ca^{2+}:ATP = 1. The results from such experiments were analyzed according to the method of Lineweaver and Burk [15].

In another set of experiments, mitochondrial and sarcoplasmic reticulum (microsomal) fractions were isolated by the procedures described previously [16] whereas myofibrillar fraction was isolated according to the method of Muir *et al.* [17]. The total ATPase activities of the mitochondrial and microsomal fractions (0.1–0.2 mg/ml) were determined in a medium containing 100 mM KCl, 20 mM Tris–HCl, pH 6.8, 2 mM $MgCl_2$, 2 mM ATP and 0.1 mM $CaCl_2$ at 37° [18]. On the other hand, the total ATPase activity of the myofibrillar fraction (0.5–1 mg protein/ml) was determined in a medium containing 10 mM histidine, pH 6.8, 5 mM sodium azide, 60 mM KCl, 3 mM $MgCl_2$ and either 0.1 mM $CaCl_2$ or 4 mM EGTA [19]. The myofibrillar ATPase in the presence of EGTA was taken to be due to the basal ATPase whereas the difference between the total and basal activities was taken to be due to the Ca^{2+} stimulated ATPase activity.

The P_i in the protein-free supernatant from all the ATPase reactions described above was determined by the method of Taussky and Shorr [20]. It should be mentioned that the 12% trichloroacetic acid used for stopping the ATPase reactions contained 50 mg of activated charcoal per ml because this treatment was necessary to remove the drug interference during colour development in the determination of P_i [21]. The protein concentration of all fractions was determined by the method of Lowry *et al.* [22]. The determination of adenylate cyclase activity was carried out by incubating about 100 μg of the sarcolemmal protein in a medium containing 50 mM Tris–HCl, pH 8.5, 8 mM caffeine, 5 mM KCl, 20 mM phosphoenolpyruvate, 2 mM cyclic AMP, 15 mM $MgCl_2$, 130 μg/ml pyruvate kinase and 0.4 mM [^{14}C]-ATP at 37° according to the procedure described elsewhere [11]. On the other hand, calcium binding by heart sarcolemma was carried out by incubating 0.2–0.3 mg membrane protein/ml in a medium containing 100 mM Tris–HCl, pH 7.4, and 1 mM $^{45}CaCl_2$ at 37° by a method described recently [10]. It should be mentioned here that all the fractions employed in this study were used within 1 hr of their isolation and any changes made in the incubation conditions for all reactions are described in the text.

Drug solutions were made fresh by dissolving the agents in distilled water and their pH was adjusted to 7.0. The drug effects were studied by incubating the cellular fractions in the absence or presence of different concentrations of drugs for 3 min before starting the reaction. All the enzymatic reactions were linear with respect to the protein concentrations and times of incubation employed in this study whereas results for calcium binding represent steady-state values. It should also be pointed out that the heart sarcolemmal preparations employed here do not possess ATP-dependent calcium binding or Ca^{2+} stimulated Mg^{2+} dependent ATPase activities [10]. Furthermore, the control values for different ATPases, adenylate cyclase and calcium binding activities observed in this study are comparable to those reported by numerous workers [10–14]. The results were analyzed statistically by employing the paired "*t*" test.

Table 1. Effects of different concentrations of acebutolol, practolol and propranolol on the adenylate cyclase and calcium binding activities of rat heart sarcolemma*

Drugs (mM)	Adenylate cyclase activity (pmoles cyclic AMP/mg protein/min)	Calcium binding (nmoles/mg protein
Control	221 ± 22	172 ± 20
Acebutolol:		
1	242 ± 20	164 ± 19
2	241 ± 17	176 ± 21
4	232 ± 18	161 ± 16
Practolol:		
1	245 ± 17	183 ± 18
2	234 ± 16	176 ± 21
4	230 ± 16	185 ± 17
Propranolol:		
1	213 ± 15	156 ± 15
2	211 ± 14	132 ± 14†
4	210 ± 13	103 ± 11†

* Each value is a mean ± S.E.M. of 6 experiments. The concentration of calcium in the incubation medium for determining calcium binding was 1 mM and the incubation time was 10 min.

† Significantly different ($P < 0.05$) from the control value.

RESULTS

In one set of experiments, the effects of different concentrations of acebutolol, practolol and propranolol on the basal adenylate cyclase and calcium binding activities of rat heart sarcolemma were examined and the results are given in Table 1. The adenylate cyclase activity was not affected by acebutolol, practolol and propranolol significantly ($P > 0.05$) whereas the calcium binding activity was significantly ($P < 0.05$) depressed by 2–4 mM propranolol only. In another set of experiments, the effects of 3 mM acebutolol, practolol and propranolol on calcium binding activity were studied by incubating the sarcolemmal fraction in the presence of different concentrations of calcium. The data in Table 2 indicate that unlike acebutolol and practolol, propranolol inhibited calcium binding at all concentrations of calcium. However, it should be noted that the inhibition produced by propranolol was about 50 and 20 per cent of the control calcium binding values at 0.12 and 2.4 mM calcium respectively. Thus it appears that the inhibitory effect of propranolol on calcium binding is antagonized by increasing the concentration of calcium in the medium. Since calcium binding with heart sarcolemma is almost a linear function of the calcium

Table 2. Effects of acebutolol, practolol and propranolol on calcium binding by rat heart sarcolemma in the presence of different concentrations of calcium*

Concentration of calcium (mM)	Calcium binding (nmoles/mg protein)			
	Control	Acebutolol (3 mM)	Practolol (3 mM)	Propranolol (3 mM)
0.12	27 ± 4	28 ± 5	29 ± 3	13 ± 2†
0.60	96 ± 12	90 ± 7	101 ± 9	54 ± 7†
1.20	197 ± 20	185 ± 15	208 ± 22	123 ± 17†
1.80	296 ± 31	278 ± 34	291 ± 26	197 ± 21†
2.40	439 ± 38	416 ± 33	448 ± 31	351 ± 29†

* Each value is a mean ± S.E.M. of 4 experiments. The incubation medium contained different concentrations of calcium as indicated and the time of incubation was 10 min.
† Significantly different ($P < 0.05$) from the control values.

concentrations employed in this study, the significance of such calcium binding is not clear at present except that a marked depressant effect of propranolol was seen at calcium concentrations (i.e. about 1.25 mM) which are normally present in the extracellular fluid.

The actions of different concentrations of acebutolol, practolol and propranolol on the Na^+-K^+

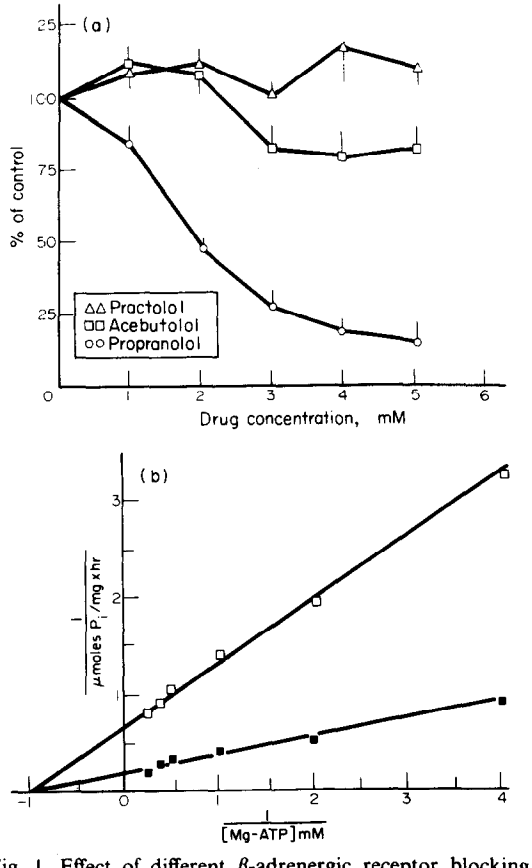

Fig. 1. Effect of different β-adrenergic receptor blocking agents on rat heart sarcolemmal Na^+-K^+ ATPase activity. (a) Dose-re-response for practolol (Δ), acebutolol (□) and propranolol (○). The results are expressed as per cent of the control activity (7.7 ± 0.6 μmoles Pi/mg protein/hr) determined in the absence of drugs. Each value is a mean ± S.E.M. of 6 experiments. (b) Lineweaver–Burk plot representing the Na^+-K^+ ATPase activities of heart sarcolemma in the absence (■) and presence (□) of 2 mM propranolol at different substrate (Mg ATP) concentrations. This graph is typical of 3 experiments.

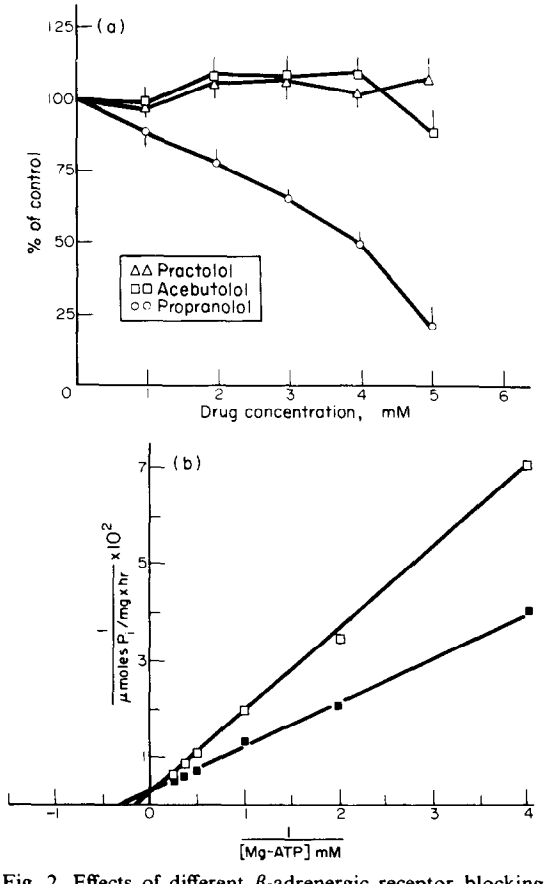

Fig. 2. Effects of different β-adrenergic receptor blocking agents on rat heart sarcolemmal Mg^{2+} ATPase activity. (a) Dose–response for practolol (Δ), acebutolol (□) and propranolol (○). The results are expressed as per cent of the control activity (27.5 ± 1.6 μmoles Pi/mg protein/hr) determined in the absence of drugs. Each value is a mean ± S.E.M. of 6 experiments. (b) Lineweaver–Burk plot representing the Mg^{2+} ATPase activities of heart sarcolemma in the absence (■) and presence (□) of 2 mM propranolol at different substrate (Mg ATP) concentrations. This graph is typical of 3 experiments.

ATPase activity of rat heart sarcolemma were investigated and the results are shown in Fig. 1a. Propranolol was found to produce a marked inhibitory effect on the Na^+-K^+ ATPase activity whereas practolol had no significant ($P > 0.05$) action. On the other hand, acebutolol depressed the Na^+-K^+ ATPase activity slightly but significantly at 3–5 mM concentrations. Propranolol (3 mM) was also found to inhibit the Na^+-K^+ ATPase activity at different substrate (Mg ATP) concentrations. From the Lineweaver-Burk plot of these data (Fig. 1b) it can be seen that propranolol depressed the maximal velocity of reaction (V_{max}) without changing the affinity of substrate (K_m) for this enzyme.

The results in Fig. 2a show that propranolol in 1–5 mM concentrations produced a dose dependent inhibitory effect on Mg^{2+} ATPase activity while acebutolol and practolol had no significant ($P > 0.05$) action. Lineweaver–Burk plot of the data by employing different substrate (MgATP) concentrations revealed that the inhibitory effect of propranolol (2 mM) was associated with an increase in the K_m value without any alterations in the V_{max} value for the Mg^{2+} ATPase (Fig. 2b). The Ca^{2+} ATPase activity of heart sarcolemma was also depressed by 1–5 mM propranolol but not by acebutolol or practolol (Fig. 3a). The inhibitory effect of propranolol on Ca^{2+} ATPase was apparent at different concentrations of substrate (Ca ATP) and was associated with an increase in the K_m value without any changes in the V_{max} value (Fig. 3b).

The possibility that the inhibitory action of propranolol on the ATPase activity is due to its complexation of added Ca^{2+} in the assay medium was investigated using 50 μM murexide in conjunction with Aminco-Chance dual wavelength spectrophotometry [23]. No change in absorbance of the Ca^{2+}-murexide or Ca ATP-murexide complex was noted on the addition of 1–5 mM propranolol. Thus the observed effect of propranolol on the ATPase activity cannot be ascribed to a reduction in the amount of substrate.

In order to demonstrate if the inhibitory effects of propranolol on the sarcolemmal ATPase activities are due to its β-adrenergic blocking activity, the effects of epinephrine, a well known β-adrenergic receptor agonist, were studied in the absence and presence of propranolol. The results in Table 3 indicate that epinephrine (0.15 mM) neither influenced different ATPase activities nor antagonized the inhibitory effects of propranolol (2 mM). Epinephrine in 0.25–0.5 mM concentrations was also ineffective in relieving the depressant action of 1 mM propranolol.

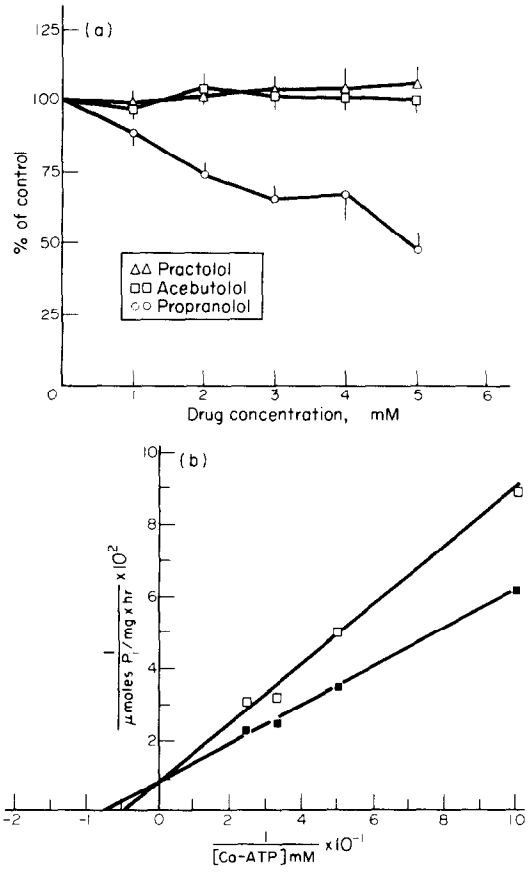

Fig. 3. Effects of different β-adrenergic receptor blocking agents on rat heart sarcolemmal Ca^{2+} ATPase activity. (a) Dose-response for practolol (△), acebutolol (□) and propranolol (○). The results are expressed as per cent of the control activity (31.0 ± 1.9 μmoles Pi/mg protein/hr) determined in the absence of drugs. Each value is a mean ± S.E.M. of 6 experiments. (b) Lineweaver–Burk plot representing the Ca^{2+} ATPase activities of heart sarcolemma in the absence (■) and presence (□) of 2 mM propranolol at different substrate (Ca ATP) concentrations. This graph is typical of 3 experiments.

The specificity of the site of action of acebutolol, practolol and propranolol was examined by studying the effects of these agents on the ATP hydrolyzing activities of mitochondrial, microsomal and myofibrillar fractions. It can be seen from Table 4 that acebutolol, practolol and propranolol significantly ($P < 0.05$) depressed the mitochondrial and microsomal ATPase activities while the myofibrillar

Table 3. Interaction of epinephrine (0.15 mM) and propranolol (2 mM) on the rat heart sarcolemmal ATPase activities*

Drugs	ATPase activity (μmoles P_i/mg protein/hr)		
	Na^+-K^+ ATPase	Mg^{2+} ATPase	Ca^{2+} ATPase
Control	7.8 ± 0.5	28.1 ± 1.5	30.5 ± 2.1
Propranolol	3.7 ± 0.4	19.2 ± 1.2	23.0 ± 1.8
Epinephrine	7.6 ± 0.6	28.4 ± 2.0	32.1 ± 2.9
Propranolol + epinephrine	3.5 ± 0.5	18.7 ± 1.3	23.4 ± 1.5

* Each value is a mean ± S.E.M. of 4 experiments.

Table 4. Effects of acebutolol, practolol and propranolol on the ATPase activities of rat heart mitochondria, heavy microsomes and myofibrils*

Drugs	ATPase activity (μmoles P_i/mg protein/hr)			
			Myofibrils	
	Mitochondria	Microsomes	Basal	Ca^{2+}-stimulated
Control	58.4 ± 5.8	89.4 ± 7.2	5.6 ± 0.4	16.2 ± 1.3
Acebutolol (3 mM)	41.0 ± 4.2†	62.0 ± 5.2†	4.9 ± 0.5	18.3 ± 1.7
Practolol (3 mM)	40.4 ± 4.0†	46.8 ± 5.4†	5.1 ± 0.3	17.9 ± 1.5
Propranolol (3 mM)	37.0 ± 3.8†	35.4 ± 1.8†	5.0 ± 0.4	15.6 ± 1.5

* Each value is a mean ± S.E.M. of 4 experiments.
† Significantly different ($P < 0.05$) from the control value.

ATPase activities were not affected by these agents significantly ($P > 0.05$).

DISCUSSION

In this study propranolol in 1–5 mM concentrations was found to decrease the heart sarcolemmal Na^+-K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase activities whereas significant depression in the calcium binding activity was seen at 2 mM or higher concentrations. Since the basal adenylate cyclase activity of heart sarcolemma was not affected by propranolol, it is unlikely that this agent exerts its depressant effect through some generalized mechanism. In this regard it should be noted that the inhibitory effects of propranolol on Mg^{2+} ATPase and Ca^{2+} ATPase were associated with increments in the K_m values whereas that on Na^+-K^+ ATPase was related to a decrease in the V_{max} value. Furthermore, depression of calcium binding by propranolol was more pronounced when the membrane fraction was incubated at lower concentrations of calcium. In addition, the degree of inhibitory effect of propranolol for Mg^{2+} ATPase was greater than that for Ca^{2+} ATPase but less than that for Na^+-K^+ ATPase (Figs 1–3). Such complex effects of propranolol on heart sarcolemma may be due to some non-specific action of this agent on the physio-chemistry of the membrane [24, 25].

Since propranolol inhibited mitochondrial and microsomal ATPase activities without any action on the myofibrillar ATPase activities, it can be argued that propranolol may affect the sarcolemmal ATPase activities in a manner similar to those in other membrane systems. It should be pointed out here that propranolol has been shown to inhibit the microsomal γ-$AT^{32}P$ reaction [26]. However, acebutolol and practolol, which significantly inhibited the microsomal and mitochondrial ATPase activities like that of propranolol [5], did not influence the sarcolemmal Mg^{2+} ATPase and Ca^{2+} ATPase activities whereas acebutolol decreased the sarcolemmal Na^+-K^+ ATPase activity slightly in high concentrations. Since acebutolol and practolol are more cardioselective than propranolol with respect to their β-adrenergic receptor blocking activity [6–8], the inhibitory effect of propranolol on sarcolemma cannot be attributed to its β-adrenergic blocking activity. This view is substantiated by the inability of epinephrine to relieve the depressant action of propranolol on the sarcolemmal ATPase activities. Furthermore, propranolol exerted its inhibitory effects on the sarcolemmal ATPase and calcium binding activities at 1 mM or higher concentrations whereas this agent in 50–100 μM concentrations has been shown to antagonize the stimulatory effects of epinephrine on the heart sarcolemmal adenylate cyclase activity [11, 12].

Since both acebutolol and practolol had no appreciable effects on myocardial contractility [5], the cardiodepressant action of propranolol cannot be explained by the antagonism of the action of endogenous catecholamines at β-adrenergic receptors. On the other hand, calcium has been reported to antagonize the interaction between propranolol and microsomal phospholipids [27] and it is well known that the cardiodepressant effect of propranolol is reversed by increasing the concentration of calcium in the perfusion medium. Our earlier suggestion that the negative inotropic effect of propranolol may partly be due to its action on sarcolemma [5] is consistent with the finding that propranolol decreased calcium binding by sarcolemma and this inhibitory effect was less pronounced at high concentrations of calcium in the medium. Since sarcolemmal Ca^{2+}/Mg^{2+} ATPase has been suggested to regulate the movement of calcium across the heart cell membrane [11, 14], the observed depression in this enzyme activity by propranolol would tend to reduce calcium influx. Although such a mechanism may be partly responsible for the cardiodepressant effect of propranolol, it should be noted that some investigators have failed to detect any inhibitory action of this agent on calcium influx [28]. It is also difficult to attribute the cardiodepressant action of propranolol to an inhibition of the sarcolemmal Na^+-K^+ ATPase because cardiac glycosides, which are its potent inhibitors, are associated with a positive inotropic action [29]. However, depression in the sarcolemmal Na^+-K^+ ATPase and Ca^{2+}/Mg^{2+} ATPase activities has been reported in failing hearts [30, 31] and it is possible that the observed effects of propranolol on sarcolemmal enzymes and contractile activity are of similar nature and significance.

Propranolol has been reported to decrease the contractile force of the isolated perfused rat heart preparations by 13, 68 and 99 per cent of the control value in 0.2, 0.5 and 1 mM concentrations respectively [5]. On the other hand, the results of this study indicate depression of the rat heart sarcolemmal ATPase and calcium binding activities at 1 mM or higher concentration of this agent. Propranolol in 0.5 and 1 mM concentrations was found to decrease the rat heart microsomal calcium uptake by 40 and 63 per cent and mitochondrial calcium uptake by 10 and 28 per cent respectively [5]. Thus it appears difficult to readily explain the cardiodepressant effects of propranolol on the basis of its actions on the sarcolemmal, mitochondria and sarcotubular membranes unless it is postulated that the sensitivity of these membrane systems to propranolol is decreased upon isolation as well as under the *in vitro* conditions. This is consistent with the observations that 100 to 150 times more concentration of catecholamines is required to activate adenylate cyclase in heart sarcolemmal and sarcotubular membranes under the *in vitro* conditions in comparison to that required for demonstrating the full positive inotropic effect [11, 12, 32, 33]. Alternatively, it is conceivable that the local concentration of propranolol in the vicinity of different membrane systems may become sufficiently high to inhibit various enzymatic activities when large doses of the drug are administered over a prolonged period for the treatment of certain cardiovascular dysfunctions. Whatever the exact subcellular mechanisms for the negative inotropic effect of propranolol may be, the results of this study indicate that this agent affects heart sarcolemma and suggest that this site of action may partly be of some significance in eliciting the cardiodepressant effect of propranolol.

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REFERENCES

1. B. Scales and D. A. D. McIntosh, *J. Pharmac. exp. Ther.* **160,** 261 (1968).
2. M. L. Hess, F. N. Briggs, E. Shinebourne and J. Hamer, *Nature, Lond.* **220,** 79 (1968).
3. A. M. Katz, D. I. Repke, M. Tada and S. Corkedale, *Cardiovasc. Res.* **8,** 541 (1974).
4. E. Noack and K. Greeff, *Naunyn-Schmiedeberg's Arch. Pharmak.* **266,** 410 (1970).
5. N. S. Dhalla and S. L. Lee, *Br. J. Pharmac.* **57,** 215 (1976).
6. B. Basil, R. Jordan, A. H. Loveless and D. R. Maxwell, *Br. J. Pharmac.* **48,** 138 (1973).
7. B. Basil, R. Jordan, A. H. Loveless and D. R. Maxwell, *Br. J. Pharmac.* **50,** 323 (1974).
8. A. J. Coleman and W. P. Leary, *Curr. Therap. Res.* **14,** 673 (1972).
9. J. R. Williamson, M. L. Woodrow and A. Scarpa, in *Basic Functions of Cations in Myocardial Activity* (Eds A. Fleckenstein and N. S. Dhalla), p. 61. University Park Press, Baltimore (1975).
10. N. S. Dhalla, M. B. Anand and J. A. C. Harrow, *J. Biochem.* **79,** 2011 (1976).
11. D. B. McNamara, J. N. Singh and N. S. Dhalla, *J. Biochem.* **76,** 603 (1974).
12. M. Tada, M. A. Kirchberger, J. M. Iorio and A. M. Katz, *Circ. Res.* **36,** 8 (1975).
13. D. B. McNamara, P. V. Sulakhe, J. N. Singh and N. S. Dhalla, *J. Biochem.* **75,** 795 (1974).
14. N. S. Dhalla, D. B. McNamara and M. B. Anand, in *The Sarcolemma* (Eds P. E. Roy and N. S. Dhalla), p. 1. University Park Press, Baltimore (1976).
15. M. Lineweaver and M. Burk, *J. Am. chem. Soc.* **56,** 658 (1934).
16. P. V. Sulakhe and N. S. Dhalla, *J. clin. Invest.* **50,** 1019 (1971).
17. J. R. Muir, A. Weber and R. E. Olson, *Biochim. biophys. Acta.* **234,** 199 (1971).
18. J. A. C. Harrow and N. S. Dhalla, *Biochem. Pharmac.* **25,** 897 (1976).
19. J. A. C. Harrow and N. S. Dhalla, *Can. J. Physiol. Pharmac.* **53,** 1058 (1975).
20. H. H. Taussky and E. Shorr, *J. biol. Chem.* **202,** 675 (1953).
21. F. Fuchs, E. W. Gertz and F. N. Briggs, *J. gen. Physiol.* **52,** 955 (1968).
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
23. S. Harigaya and A. Schwartz, *Circ. Res.* **25,** 781 (1969).
24. W. G. Nayler, *J. Pharmac. exp. Ther.* **153,** 479 (1966).
25. W. Lameijer and P. A. Van Zwieten, *Archs int. Pharmacodyn. Ther.* **210,** 306 (1974).
26. D. C. Pang and F. N. Briggs, *Biochem. Pharmac.* **22,** 1301 (1973).
27. R. L. Cross, B. A. Cross and J. H. Wang, *Biochem. biophys. Res. Commun.* **40,** 1155 (1970).
28. A. Silva-Garca and P. A. Van Zwieten, *J. Pharm. Pharmac.* **24,** 367 (1972).
29. A. Schwartz, G. E. Lindenmayer and J. C. Allen, *Pharmac. Rev.* **27,** 3 (1975).
30. C. W. Tomlinson, S. L. Lee and N. S. Dhalla, *Circ. Res.* **39,** 82 (1976).
31. N. S. Dhalla, J. N. Singh, E. Bajusz and G. Jasmin, *Clin. Sci. Mol. Med.* **51,** 233 (1976).
32. N. S. Dhalla, P. V. Sulakhe and D. B. McNamara, *Biochim. biophys. Acta* **323,** 276 (1973).
33. N. S. Dhalla, P. V. Sulakhe, R. L. Khandelwal and R. E. Olson, *Cardiovasc. Res.* **6,** 344 (1972).