Br. J. Pharmac. (1976), **57**, 215–221

COMPARISON OF THE ACTIONS OF ACEBUTOLOL, PRACTOLOL AND PROPRANOLOL ON CALCIUM TRANSPORT BY HEART MICROSOMES AND MITOCHONDRIA

N.S. DHALLA & S.L. LEE

Experimental Cardiology Laboratory, Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada R3E 0W3

1 The effects of acebutolol, practolol and propranolol (0.5–3 mM) on calcium uptake, calcium binding and ATPase activities of the rabbit and rat heart microsomal and mitochondrial fractions were investigated.

2 Dose-response and time course experiments revealed that propranolol greatly inhibited microsomal and mitochondrial calcium uptake whereas both acebutolol and practolol showed slight depressant effects.

3 The ATPase activities of microsomal and mitochondrial fractions were decreased by acebutolol, practolol and propranolol; however, the latter agent was more effective than the other two.

4 The inhibitory effects of acebutolol, practolol and propranolol on mitochondria and microsomes were not antagonized by adrenaline.

5 Propranolol decreased calcium binding by the microsomal fraction only, whereas acebutolol and practolol had no effect on microsomal or mitochondrial calcium binding.

6 The sensitivity of the rabbit heart subcellular fractions to the β-adrenoceptor blocking drugs was similar to that of the rat heart; however, the calcium uptake and ATPase activities of microsomes were more sensitive to propranolol than mitochondria in both species.

7 Perfusion of rat hearts with 0.2–1 mM propranolol decreased contractile force, and microsomal and mitochondrial fractions obtained from these hearts accumulated less calcium in comparison to the control. On the other hand, acebutolol and practolol (0.2–1 nM) had no appreciable effects on contractile force or subcellular fractions under similar conditions.

8 The negative inotropic effect of propranolol may partly be due to its inhibitory actions on calcium transport by subcellular organelles of the myocardium; the depressant action of propranolol on calcium transport is unlikely to be due to its β-adrenoceptor blocking property.

Introduction

The effects of some well known β-adrenoceptor blocking agents on subcellular membranes of the myocardium have been reported by various investigators. For example, propranolol has been shown to inhibit calcium transport and adenosine 5′-triphosphate (ATP) hydrolysis by the fragments of sarcoplasmic reticulum (microsomal fraction) (Scales & McIntosh, 1968; Hess, Briggs, Shinebourne & Hamer, 1968; White & Shinebourne, 1969; Shinebourne, White & Hamer, 1969; Solaro, Gertz & Briggs, 1972; Katz, Repke, Tada & Corkedale, 1974). This inhibitory effect of propranolol was reversed by catecholamines (Hess *et al.*, 1968; White & Shinebourne, 1969) while other investigators (Katz *et al.*, 1974) failed to confirm this finding. Thus it is not clear whether or not the depressant effect of propranolol on microsomal calcium transport is due to its β-adrenoceptor blocking activity. Although propranolol has also been shown to inhibit calcium uptake by mitochondria (Noack & Greeff, 1970; 1971), the interaction of propranolol with catecholamines has not been studied by employing these membranes. Furthermore, the data available in the literature do not permit any conclusion to be made concerning the comparative effects of propranolol on mitochondrial and microsomal calcium transport.

Recently acebutolol and practolol have been claimed to exhibit greater cardio-selectivity than propranolol with respect to their β-adrenoceptor blocking activity (Dohadwalla, Freedberg & Vaughan Williams, 1969; Coleman & Leary, 1972; Basil, Jordan, Loveless & Maxwell, 1973; 1974). However,

the actions of both acebutolol and practolol on calcium transporting properties of cardiac subcellular particles have not yet been described. In the present investigation, therefore, we have compared the effects of acebutolol, practolol and propranolol on calcium binding, calcium uptake and ATPase activities of the mitochondrial and microsomal fractions of the rat and rabbit hearts. The calcium transporting abilities of these subcellular fractions obtained from rat isolated hearts perfused with these β-adrenoceptor blocking agents were also examined to test if these subcellular effects of the drugs are associated with functional changes in the myocardium. It should be noted here that both sarcoplasmic reticulum and mitochondria are considered to regulate intracellular calcium and subsequently myocardial function (Harigaya & Schwartz, 1969; Dhalla, Sulakhe, Fedelesova & Yates, 1974), although their relative contribution during cardiac contraction and relaxation phases is far from clear.

Methods

Isolation of subcellular membrane fractions

Healthy albino rabbits and Sprague-Dawley rats were used. After killing the animals, the hearts were dissected out immediately, and rinsed thoroughly in a chilled 0.25 mM sucrose solution containing 1 mM disodium edetate (EDTA), at pH 7.0. The mitochondrial and heavy microsomal fractions were isolated by differential centrifugation according to the procedures described by Sulakhe & Dhalla (1971). In brief, the myocardium (ventricles) was homogenized in a medium containing 0.25 mM sucrose and 1 mM EDTA, pH 7.0; the mitochondrial fraction was obtained upon centrifugation at 10,000 *g* for 20 min whereas the heavy microsomal fraction was that pelleted upon spinning at 40,000 *g* for 40 minutes. Both mitochondrial and microsomal fractions were washed twice with homogenizing medium and 0.6 M KCl, pH 6.8 respectively and the final pellets of these subcellular membrane particles were suspended in 0.25 mM sucrose, pH 7.0. These membrane fractions were used immediately after isolation which was carried out at temperatures between 0° and 4°C.

Determination of calcium uptake and calcium binding

Both ATP-dependent Ca^{2+} uptake and Ca^{2+} binding by the isolated membrane fractions were determined by the Millipore filtration technique (Sulakhe & Dhalla, 1972). Calcium uptake by mitochondrial fraction was studied by incubating the membranes (0.1–0.3 mg protein) in a total volume of 1 ml reaction medium containing (mM): KCl 100, $MgCl_2$ 10, sodium succinate 5, KH_2PO_4 4, ATP 5, $^{45}CaCl_2$ 0.1, Tris-HCl 20, pH 7.0 at 37°C, whereas the microsomal Ca^{2+} uptake was studied by incubating the membrane fraction (0.02–0.05 mg protein) in 1 ml medium containing KCl 100, $MgCl_2$ 10, potassium oxalate 5, ATP 5, $^{45}CaCl_2$ 0.1, Tris-HCl 20, pH 7.0 at 37°C. ATP-dependent Ca^{2+} binding ability of the mitochondria or microsomes was studied by incubating the membranes (0.1–0.3 mg protein) in the same media as employed for calcium uptake experiments except that KH_2PO_4, succinate and potassium oxalate were omitted. The temperature of the reaction medium for calcium binding experiments was 25°C. The membrane fractions were pre-incubated for 2 to 3 min in the absence or presence of different drugs before starting the calcium accumulation reaction by the addition of $^{45}CaCl_2$. The reaction was terminated by Millipore filtration at various time intervals.

Drug solutions at desired concentrations were prepared fresh by dissolving the agents in deionized water and their pH was adjusted to 7.0.

Measurement of ATPase activities

Total ATPase activity of the mitochondrial and microsomal fractions was measured by incubation of the membrane fraction in the same reaction mixture as that used for the calcium binding experiments. The membrane fractions were pre-incubated for 3 min with or without drugs at 37°C before the reaction was started by adding ATP. The reaction was terminated by Millipore filtration at appropriate times. Inorganic phosphate in the protein-free filtrate was determined by the method described by Taussky & Shorr (1953) and the protein concentration was measured according to the method of Lowry, Rosebrough, Farr, & Randall (1951).

Perfusion of rat hearts

Perfusion of rat heart by the method of Langendorff is described elsewhere (Dhalla, Yates, Walz, McDonald & Olson, 1972). The control perfusion medium, Krebs-Henseliet solution containing 1.25 mM $CaCl_2$ was gassed with 95% O_2 and 5% CO_2, pH 7.2 at 37°C. The hearts were equilibrated by perfusing with the control medium for 10 min before starting perfusion with medium in the presence of drugs. The control hearts were perfused with Krebs-Henseliet solution only. The contractile force of the perfused hearts was monitored by employing a force-displacement transducer (FT.03) on a Grass polygraph (Dhalla *et al.*, 1972).

Results

Calcium binding and uptake by subcellular fractions

The rabbit and rat heart mitochondrial fractions in the presence of 4 mM inorganic phosphate (Pi)

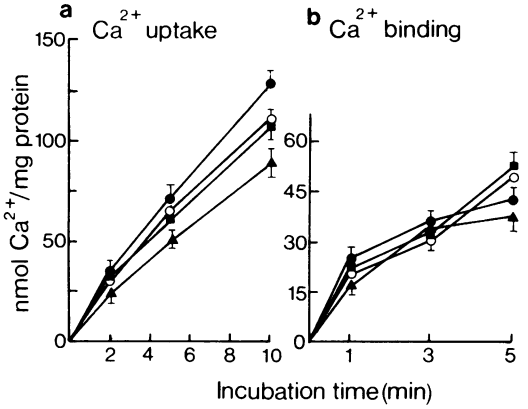

Figure 1 Effects of 3 mM acebutolol (O), 3 mM practolol (■) and 1 mM propranolol (▲) on (a) calcium uptake and (b) calcium binding activities of rat heart mitochondrial fraction at different intervals of incubation. (●) Control. Each value is a mean of 6 to 8 experiments. Vertical lines show s.e. mean.

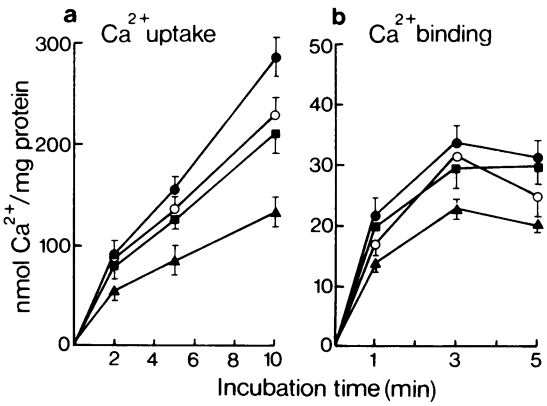

Figure 2 Effects of 3 mM acebutolol (O), 3 mM practolol (■) and 1 mM propranolol (▲) on (a) calcium uptake and (b) calcium binding activities of rat heart microsomal fraction at different intervals of incubation. ●, Control each value is a mean of 6 to 8 experiments. Vertical lines show s.e. mean.

(mitochondrial calcium uptake) accumulated 280 ± 18 and 132 ± 15 nmol Ca^{2+}/mg protein whereas the microsomal fractions in the presence of 5 mM potassium oxalate (microsomal calcium uptake) accumulated 610 ± 32 and 287 ± 27 nmol Ca^{2+}/mg protein in 10 min respectively. On the other hand, the rabbit and rat heart mitochondrial fractions in the absence of Pi (mitochondrial calcium binding) bound 65 ± 4 and 38 ± 3 nmol Ca^{2+}/mg protein whereas the microsomal fractions in the absence of oxalate (microsomal calcium binding) bound 44 ± 3 and

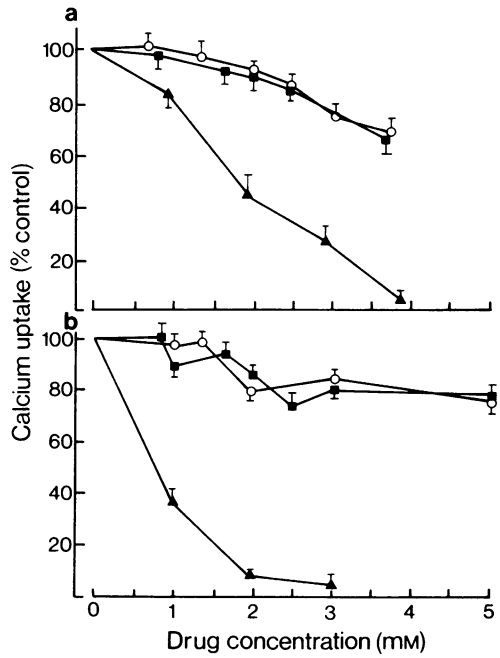

Figure 3 Effects of different concentrations of acebutolol (O), practolol (■) and propranolol (▲) on calcium uptake by (a) mitochondrial and (b) microsomal fractions of rabbit heart. The calcium uptake abilities of the subcellular fractions were determined for a period of 10 min and the results are expressed as % of the control values in the absence of drugs. Each value is a mean of 6 experiments. Vertical lines show s.e. mean.

33 ± 2 nmol Ca^{2+}/mg protein in 5 min respectively. The calcium binding and calcium uptake by the mitochondrial fractions, unlike microsomal fractions, were decreased by 65 to 85% by 5 mM sodium azide, a well known inhibitor of mitochondrial calcium transport (Sulakhe & Dhalla, 1971). Electron miscroscopic and marker enzyme studies (Sulakhe & Dhalla, 1971) revealed minimal cross contamination in the fractions employed in this study. It should be noted that our values for the calcium binding and uptake abilities by the rat and rabbit heart subcellular fractions are within the accepted range reported by different investigators and it is understood that the terms 'calcium binding' and 'calcium uptake' imply an arbitrary meaning for calcium accumulation in the absence and presence of permeant ions respectively.

Time course of drug effects on calcium binding and uptake

In one series of experiments, the calcium binding and calcium uptake abilities of subcellular fractions were

studied at different intervals of incubation with or without drugs. The results for rat heart are shown in Figures 1 and 2. Both acebutolol and practolol (3 mM) exerted a slight depressant action on mitochondrial and microsomal calcium uptake; however, this effect was significant ($P < 0.05$) only at 10 min of incubation with calcium (Figures 1 and 2). On the other hand, propranolol (1 mM) significantly decreased calcium uptake by mitochondrial and microsomal fractions at 2, 5 and 10 min of incubation. Calcium binding by mitochondria (Figure 1) was not significantly ($P > 0.05$) affected by acebutolol, practolol or propranolol whereas calcium binding by the rat heart microsomes (Figure 2) was significantly ($P < 0.05$) depressed by propranolol (1 mM) but not by acebutolol or practolol (3 mM). Similar results were obtained for the rabbit heart subcellular fractions. It should be pointed out that it is essential to adjust the pH of the drug solution to 7.0 before starting the incubation. For example, practolol, which is available as a free base, forms a highly alkaline solution and depressed mitochondrial and microsomal calcium uptake by about 70 to 82% at 3 mM concentration. This action of the alkaline solution of propranolol was found to be due to changes in the pH of the incubation medium rather than to the drug *per se*.

Dose-response of drugs on calcium uptake

The effects of different concentrations of acebutolol, practolol and propranolol were studied on the calcium uptake abilities of mitochondrial and microsomal fractions of rat and rabbit hearts. The results shown in Figure 3 and Table 1 indicate that both acebutolol and practolol at 2 to 3 mM concentrations decreased calcium uptake by both mitochondrial and microsomal fractions slightly but significantly ($P < 0.05$) whereas propranolol had a marked depressant action. The effectiveness of propranolol in decreasing calcium uptake by the subcellular fractions was qualitatively similar for both rat and rabbit hearts (Figure 3 and Table 1); however, mitochondrial calcium uptake was less sensitive to propranolol than microsomal calcium uptake in both species.

Effect of drugs on ATPase activity

In addition to accumulating calcium, both mitochondrial and microsomal fractions are known to hydrolyze ATP. The effects of drugs were therefore studied on ATPase activities of the subcellular fractions and the results for rabbit heart are given in Table 2. Both acebutolol and practolol (3 mM) decreased mitochondrial and microsomal ATPase activities significantly ($P < 0.05$) whereas a significant depressant effect of propranolol was apparent even at 1 mM. The mitochondrial ATPase was found to be less sensitive to propranolol than the microsomal ATPase of the rabbit heart. Similar results were also seen with rat heart subcellular fractions.

Table 1 Effects of different concentrations of acebutolol, practolol and propranolol on calcium uptake by rat heart mitochondrial and microsomal fractions

	% of control Ca^{2+} uptake*	
Drugs (mM)	*Mitochondria*	*Microsomes*
Acebutolol		
0.5	102 ± 5	99 ± 5
1.0	97 ± 4	92 ± 6
2.0	92 ± 3**	86 ± 4**
3.0	78 ± 6**	76 ± 5**
Practolol		
0.5	98 ± 8	100 ± 12
1.0	96 ± 2	94 ± 10
2.0	90 ± 3**	86 ± 4**
3.0	77 ± 3**	75 ± 5**
Propranolol		
0.5	91 ± 11	60 ± 12**
1.0	72 ± 12**	37 ± 10**
2.0	43 ± 8**	12 ± 3**
3.0	24 ± 6**	9 ± 2**

* Each value is a mean ± s.e. of 5 experiments. Calcium uptake in the absence (control) and presence of drugs was determined for a period of 10 min and the results are expressed as % of the control value.
** Significant inhibition ($P < 0.05$).

Table 2 Effects of different concentrations of acebutolol, practolol and propranolol on ATPase activity of rabbit heart mitochondrial and microsomal fractions

	*ATPase activity** (μmol Pi mg^{-1} min^{-1})	
Drugs (mM)	*Mitochondria*	*Microsomes*
Control	1.54 ± 0.10	1.60 ± 0.12
Acebutolol		
1	1.45 ± 0.16	1.33 ± 0.08
2	1.49 ± 0.13	1.29 ± 0.11
3	1.14 ± 0.09**	1.08 ± 0.10**
Practolol		
1	1.55 ± 0.15	1.35 ± 0.12
2	1.39 ± 0.08	1.39 ± 0.12
3	1.11 ± 0.11**	1.16 ± 0.08**
Propranolol		
1	1.22 ± 0.13**	1.04 ± 0.11**
2	0.95 ± 0.10**	0.76 ± 0.09**
3	0.74 ± 0.09**	0.55 ± 0.08**

* Each value is a mean ± s.e. of 5 to 6 experiments. The total ATPase activity was determined for a period of 5 minutes.
** Significant inhibition ($P < 0.05$).

Table 3 Contractile force and calcium uptake by mitochondrial and microsomal fractions obtained from rat hearts perfused for 10 min in the absence (control) or presence of different concentrations of acebutolol, practolol and propranolol

	Calcium uptake (%)*		*Contractile force (%)*
Drugs (mM)	*Mitochondria*	*Microsomes*	
Acebutolol			
0.2	104 ± 5	110 ± 7	102 ± 5
0.5	95 ± 3	115 ± 7	95 ± 6
1.0	91 ± 5	93 ± 8	91 ± 4
Practolol			
0.2	95 ± 3	92 ± 4	107 ± 2
0.5	95 ± 4	99 ± 7	109 ± 3
1.0	94 ± 4	99 ± 5	102 ± 4
Propranolol			
0.2	91 ± 6	89 ± 2**	87 ± 3**
0.5	85 ± 3**	70 ± 8**	32 ± 5**
1.0	67 ± 4**	56 ± 6**	0.8 ± 0.1**

* Each value is a mean ± s.e. of 4 experiments. Calcium uptake was studied for a period of 5 min and the results are expressed as % of the control.
** Statistically significant ($P < 0.05$).

Interaction of adrenaline with drugs on subcellular fractions

Adrenaline in concentrations from 10 to 150 μM was found to have no effect upon the abilities of mitochondrial and microsomal fractions to accumulate calcium as well as to hydrolyze ATP; however, higher concentrations of adrenaline were slightly inhibitory. The depressant actions of 1 mM propranolol, 3 mM acebutolol and 3 mM practolol on mitochondrial and microsomal calcium uptake (as shown in Figures 1 and 2) and ATPase activities (as shown in Table 2) were not antagonized by the addition of 10 to 150 μM adrenaline in the incubation medium.

Effect of drug perfusion on contractile force and subcellular fractions of rat heart

In order to test whether or not acebutolol, practolol and propranolol affect mitochondrial and microsomal calcium uptake *in vivo*, rat hearts were perfused with different concentrations of these agents for 10 minutes. The contractile force of these hearts was monitored, the subcellular fractions isolated and their abilities to accumulate calcium were examined. The results of this series of experiments are shown in Table 3. It was found that both acebutolol and practolol at 0.2 to 1 mM had no significant effect on the contractile force nor were there any appreciable changes in calcium transporting abilities of mitochondrial and microsomal fractions obtained from these drug-treated hearts. On the other hand, the hearts perfused with 0.2 to 1 mM propranolol showed a progressive depression in contractile force and yielded subcellular fractions which accumulated less calcium in comparison to those from the control hearts. The depression in calcium accumulating ability of the microsomal fraction from the propranolol-treated hearts was greater than that by the mitochondrial fraction. It should be pointed out that the concentrations of acebutolol, practolol and propranolol employed in this study completely prevented the positive inotropic effect of 0.5–1 μg adrenaline, which produced 65 to 90% increase in contractile force of the isolated perfused heart of the rat.

Discussion

In this study we have shown that propranolol inhibited heart microsomal calcium uptake in rat and rabbits. This finding is in agreement with observations reported by various investigators using dog and calf cardiac muscle preparations (Scales & McIntosh, 1968; Hess *et al.*, 1968; Shinebourne *et al.*, 1969; Katz *et al.*, 1974). Furthermore, our results concerning the depression of mitochondrial calcium uptake by propranolol with rabbit and rat hearts as measured by the Millipore filtration technique also confirm the findings of Noack & Greeff (1971) who used atomic absorption spectrophotometric measurements on the rat heart. In addition, we have shown that two other β-adrenoceptor blocking agents, acebutolol and practolol, decreased calcium uptake by mitochondrial and microsomal fractions; however, in contrast to propranolol, the depressant effect of these agents was seen in higher concentrations. Since acebutolol and practolol are more cardioselective β-adrenoceptor blocking agents than propranolol (Dohadwalla *et al.*, 1969; Coleman & Leary, 1972; Basil *et al.*, 1973; 1974), it is unlikely that the depressant effect of these agents is related to their β-adrenoceptor blockade. This view is supported by our observation that the mitochondrial and microsomal effects of acebutolol, practolol and propranolol were not antagonized by a β-adrenoceptor agonist, adrenaline. On the other hand, the inhibitory action of propranolol is considered to be some non-specific effect on the physico-chemistry of microsomal membranes (Lameijer & Van Zwieten, 1974) and has been shown to be antagonized by sucrose (Solaro *et al.*, 1972). The depression in microsomal calcium transport by propranolol has also been suggested to be due to inhibition of the ATPase activity (Scales & McIntosh, 1968) and of the γ-AT^{32}P reaction (Pang & Briggs, 1973). It is possible that the observed effects of acebutolol and practolol on microsomal and mitochondrial membranes are elicited through mechanisms similar to those for propranolol.

Calcium uptake by mitochondrial and microsomal fractions obtained from hearts perfused with different concentrations of propranolol was found to be less than the control value; mitochondrial calcium uptake decreased to a lesser degree in comparison to the microsomal calcium uptake. Furthermore, microsomal calcium uptake was depressed to a greater extent than mitochondrial calcium uptake when the subcellular fractions were incubated with propranolol. This difference in the sensitivity of mitochondrial and microsomal calcium uptake to propranolol cannot be attributed to the difference in experimental conditions employed in this study because propranolol was found to decrease microsomal calcium binding, unlike mitochondrial calcium binding, under similar conditions of incubation. Furthermore, mitochondrial ATPase was less sensitive to propranolol than microsomal ATPase. These differences may be due to different chemical compositions of mitochondrial and microsomal membranes with respect to their lipid and protein contents. It can also be seen from the data reported by Nayler (1966) that propranolol was more effective in inhibiting microsomal than mitochondrial lipid-facilitated transport of calcium.

Although rat heart subcellular fractions transported less calcium in comparison to the rabbit heart fractions, the degree of depression in calcium uptake

due to propranolol in both species was qualitatively similar. It is noteworthy that we have been able to show decreased ability for Ca^{2+} uptake in subcellular fractions obtained from hearts depressed to varying degrees upon perfusion with different concentrations of propranolol. On the other hand, acebutolol and practolol, which unlike propranolol, did not depress myocardial contractility significantly in the concentrations employed in this study, had no appreciable action on the calcium transporting abilities of mitochondrial and microsomal fractions obtained from these drug-treated hearts. Thus it is likely that changes in calcium transport properties of heart mitochondria and sarcoplasmic reticulum due to propranolol may represent the subcellular basis of its negative inotropic action. Since mitochondria were less sensitive to propranolol than microsomes, it appears that sarcoplasmic reticulum may play a major role in this mechanism. It should also be noted here that the present study does not rule out the possibility of propranolol acting on the sarcolemma which is also known to transport calcium like other membrane systems of the myocardium.

This work was supported by the Medical Research Council of Canada (grant No. MT-3394). This manuscript is part XVII of papers having the general title *Biochemical Basis of Heart Function*. The β-adrenoceptor blocking agents used in this study were kindly provided by the Ayerst Laboratories and Poulenc Ltd, Montreal, Canada.

References

BASIL, B., JORDAN, R., LOVELESS, A.H. & MAXWELL, D.R. (1973). β-Adrenoceptor blocking properties and cardioselectivity of M & B 17803 A. *Br. J. Pharmac.*, **48**, 138–211.

BASIL, B., JORDAN, R., LOVELESS, A.H. & MAXWELL, D.R. (1974). A comparison of the experimental anti-arrhythmic properties of acebutolol (M & B 17,803), propranolol and practolol. *Br. J. Pharmac.*, **50**, 323–333.

COLEMAN, A.J. & LEARY, W.P. (1972). Cardiovascular effects of acebutolol (M & B 17803 A), in excercising man; A comparative study with practolol and propranolol. *Curr. Therap. Res.*, **14**, 673–678.

DHALLA, N.S., SULAKHE, P.V., FEDELESOVA, M. & YATES, J.C. (1974). Molecular abnormalities in cardiomyopathy. *Adv. Cardiol.*, **13**, 282–300.

DHALLA, N.S., YATES, J.C., WALZ, D.A., McDONALD, V.A. & OLSON, R.E. (1972). Correlation between changes in endogenous energy stores and myocardial function due to hypoxia in the isolated perfused rat heart. *Can. J. Physiol., Pharmac.*, **50**, 333–345.

DOHADWALLA, A.N., FREEDBERG, A.S. & VAUGHAN WILLIAMS, E.M. (1969). The relevance of beta-receptor blockade to ouabain-induced cardiac arrhythmias. *Br. J. Pharmac.*, **36**, 257–267.

HARIGAYA, S. & SCHWARTZ, A. (1969). Rate of calcium binding and uptake in normal animal and failing human cardiac muscle. Membrane vesicles (relaxing system) and mitochondria. *Circulation Res.*, **25**, 781–794.

HESS, M.L., BRIGGS, F.N., SHINEBOURNE, E. & HAMER, J. (1968). Effect of adrenergic blocking agents on the calcium pump of the fragmented cardiac sarcoplasmic reticulum. *Nature, Lond.*, **220**, 79–80.

KATZ, A.M., REPKE, D.I., TADA, M. & CORKEDALE, S. (1974). Propranolol-induced inhibition of cardiac microsomal calcium uptake, calcium binding, and epinephrine-stimulated adenylate cyclase. *Cardiovasc. Res.*, **8**, 541–549.

LAMEIJER, W. & VAN ZWIETAN, P.A. (1974). Comparison between the uptake of propranolol and its influence on the relative refractory period and on contractile force. *Arch. int. pharmacodyn.*, **210**, 306–320.

LOWRY, D.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

NAYLER, W.G. (1966). The effect of pronethalol and propranolol on lipid-facilitated transport of calcium ions. *J. Pharmac. exp. Ther.*, **153**, 479–484.

NOACK, E. & GREEFF, K. (1970). The influence of certain cardioactive drugs on the energy dependent uptake of calcium and adenine nucleotides into mitochondria. *Naunyn-Schmiedebergs Arch. Pharmak.*, **266**, 410–411.

NOACK, E. & GREEFF, K. (1971). The influence of some cardioactive drugs on the energy-dependent uptake of calcium, potassium and adenine nucleotides by mitochondria. *J. Molec. Cell Cardiol.*, **2**, 145–159.

PANG, D.C. & BRIGGS, F.N. (1973). Mechanism of propranolol inhibition of the cardiac sarcotubule-γ-$AT^{32}P$ reaction. *Biochem. Pharmac.*, **22**, 1301–1308.

SCALES, B. & McINTOSH, D.A.D. (1968). Effects of propranolol and its optical isomers on the radio calcium uptake and the ATPase of skeletal and cardiac sarcoplasmic reticulum fractions (SRF). *J. Pharmac. exp. Ther.*, **160**, 261–267.

SHINEBOURNE, E., WHITE, R. & HAMER, J. (1969). A quantitative distinction between the beta-receptor blocking and local anesthetic actions of anti-arrhythmic agents. *Circulation Res.*, **24**, 835–841.

SOLARO, R.J., GERTZ, E.W. & BRIGGS, F.N. (1972). Sucrose antagonism of drug and temperature effects on cardiac sarcoplasmic reticulum. *Biochim. Biophys. Acta.*, **255**, 751–761.

SULAKHE, P.V. & DHALLA, N.S. (1971). Excitation-contraction coupling in heart. VII. Calcium accumulation in subcellular particles in congestive heart failure. *J. clin. Invest.*, **50**, 1019–1027.

TAUSSKY, H.H. & SHORR, E. (1953). A microcolorimetric method for the determination of inorganic phosphorus. *J. biol. Chem.*, **202**, 675–685.

WHITE, R. & SHINEBOURNE, E. (1969). The interaction of sympathetic stimulation and blockade on the calcium uptake of the sarcoplasmic reticulum. *Cardiovasc. Res.*, **3**, 24.