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BIOCHEMICAL AND PHYSIOLOGICAL ADAPTATION TO CHRONIC PROPRANOLOL TREATMENT IN THE RAT

GORDON CRAMB,* NINA M. GRIFFITHS, JAMES F. AITON and NICK L. SIMMONS
Department of Physiology and Pharmacology, University of St. Andrews, Fife KY16 9TS, U.K.

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Abstract—The biochemical and physiological aspects of isoprenaline sensitivity in normotensive rats were examined during and after abrupt withdrawal of chronic propranolol treatment.

Serum propranolol concentrations in rats chronically treated for one month (0.125% propranolol in drinking water: 75–100 mg/kg/day) ranged from 7 to 23 ng/ml. At the height of the blockade, rats showed a decreased responsiveness *in vivo* to isoprenaline-induced increase in heart rate and fall in blood pressure; the ED_{50} values for isoprenaline being increased some 20- and 4-fold respectively. There was a 180% increase in β-receptor number in sarcolemmal membranes isolated from ventricular muscle of these animals, together with increased basal (290%), fluoride- (100%), forskolin- (80%) and isoprenaline-stimulated (125%) adenylate cyclase activity.

Twenty-four hours after propranolol withdrawal, serum propranolol concentrations were reduced by over 95%. At this time rats exhibited increased chronotropic and blood pressure responses to i.v. isoprenaline, indicated by the reduced ED_{50} values (2-fold and 12-fold respectively compared to controls). In addition, cardiac sarcolemmal β-receptor number and adenylate cyclase activities were still significantly elevated above those of controls; 35% increase in β-receptor number and increases of 96, 26, 13 and 37% in basal, fluoride-, forskolin- and isoprenaline-stimulated adenylate cyclase activities respectively.

Forty-eight hours after drug withdrawal serum propranolol concentrations were only just detectable at 0.5 ± 0.1 ng/ml. Although sarcolemmal β-receptor numbers were still elevated (23%) isoprenaline-stimulated adenylate cyclase activity had returned to control values. However, both the fluoride- and forskolin-stimulated enzyme activities were decreased below control values by 12 and 23% respectively, suggestive of a reduction in the catalytic capacity of the adenylate cyclase complex. In parallel with the reduction in β-receptor number and adenylate cyclase activity, the chronotropic response to i.v. isoprenaline had also returned to control values. In contrast, the blood pressure response to i.v. isoprenaline was still elevated in these animals indicated by the 5-fold reduction in the ED_{50} value compared with control animals.

The phenomenon of adrenergic receptor desensitization resulting from prolonged agonist application is now well accepted [1]. However considerable controversy still remains regarding the effects of chronic antagonist treatment on receptor number. Increases in β-receptor number as a result of chronic propranolol treatment have been reported in crude membrane preparations isolated from many tissues including rat cerebral cortex [2], cardiac muscle [3, 4] and lung [4] as well as circulating rat [4] and human [5] lymphocytes. It has been suggested that this general increase in β-receptor density as a result of chronic propranolol treatment may be associated with the elevated sensitivity to catecholamines found *in vivo* on propranolol withdrawal [6]. Other workers, however, have failed to find an increased catecholamine sensitivity *in vivo* [7, 8] or an increased receptor density in cardiac muscle *in vitro* [9, 10], during or after the abrupt withdrawal of the drug. The effect of chronic propranolol treatment on cardiac β-receptor number therefore remains controversial.

To date there has been no study of the effect of chronic β-blockade on β-receptor number and adenylate cyclase activity in a purified cardiac sarcolemmal preparation. It is important to note that it is only changes in the number of sarcolemmal β-receptors capable of coupling with the adenylate cyclase system and not alterations in the turnover of some intracellular receptor pool, which will be responsible for the catecholamine sensitivity of the myocardium. Accordingly we have examined β-receptor density and adenylate cyclase activity in rat cardiac sarcolemmal membranes isolated during or after withdrawal from propranolol treatment. These measurements *in vitro* were correlated with changes in sensitivity to isoprenaline assessed *in vivo* following identical conditions of chronic propranolol treatment.

MATERIALS AND METHODS

Biochemicals and reagents. Biochemicals and enzymes were purchased from Sigma, Poole, Dorset. ^{125}I-Cyanopindolol (specific activity > 2000 Ci/mmole) and ^{3}H-cyclic AMP (specific activity 40 Ci/mmole) were obtained from Amersham International, Bucks. Forskolin was purchased from Calbiochem, Bishops Stortford, Herts, and ±propranolol was a gift from ICI Pharmaceuticals, Macclesfield, Cheshire. General chemicals were of analytical grade and obtained from BDH, Poole, Dorset.

Animals. Male Wistar rats bred in the University

* To whom correspondence should be addressed.

of St. Andrews animal house were used during the study. All animals were allowed free access to food and water throughout the experimental period. Since propranolol is usually administered orally in man we decided to include the drug in the drinking water which removed the need for excessive handling of the animals and eliminated the possibility of stress induced by the surgical implantation and removal of infusion devices. Rats aged 40 days, weighing 150–190 g, were maintained for one month with 0.02% saccharin (control) or 0.02% saccharin + 0.125% propranolol (propranolol-treated) in their drinking water. In a random sample of animals the propranolol was removed from the drinking water either 24 or 48 hr before use in experiments (24 hr-withdrawn and 48 hr-withdrawn).

Blood propranolol concentrations. The carotid arteries were severed and blood collected by exsanguination. The concentration of propranolol and its active metabolites in rat serum samples was estimated by a radioreceptor assay [11] using the competitive displacement of the labeled antagonist (±) ^{125}I-cyanopindolol (^{125}I-CYP) from a rat lung membrane preparation. Membranes, prepared by the method of Barnett *et al.* [12] were incubated for 90 min at 30° in the presence of a series of standard propranolol concentrations or serum samples (diluted 1:2 with 50 mM Tris, 5 mM $MgCl_2$, 0.5% BSA pH 7.4) taken from treated and withdrawn rats. Standard propranolol solutions were prepared in a 1:2 dilution of control serum. Bound ^{125}I-CYP was separated from free by rapid filtration through glass-fibre filters (Whatman GF/F) under vacuum, followed by two 10 ml washes with ice-cold 10 mM Tris, 5 mM $MgCl_2$ pH 7.4. Radioactivity was determined using a Packard gamma scintillation counter.

Determination of heart rate and blood pressure. Animals were anaesthetised with urethane (1.3 g/kg, i.p.) and the jugular vein cannulated for injection of isoprenaline. The carotid artery was cannulated and blood pressure measured using a Statham pressure transducer connected to a Linseis pen recorder. ECG leads were connected to each limb and recordings made from Lead II and the heart rate determined from the ECG recording. Both heart rate and blood pressure responses to isoprenaline (0.1 ml bolus of 0.05–250 μM; 0.004–21 μg/kg) were recorded simultaneously.

Sarcolemmal isolation. Animals were killed by cervical dislocation before removal of hearts for sarcolemmal membrane preparation and the sampling of blood for the estimation of serum propranolol concentration. Sarcolemmal membranes were prepared from cardiac ventricular muscle by the method of Cramb and Dow [13]. In brief, sliced ventricles from six rats were incubated for 30 min at 37° in an isotonic buffer containing 5 μM $CaCl_2$ and 0.8 mg/ml collagenase. With all further processes carried out at 4°, the digested tissue was homogenized in a hypotonic $NaHCO_3$ buffer containing 0.5 mM dithiothreitol (DTT) and 1 mM phenylmethylsulphonyl fluoride (PMSF). Contractile proteins were extracted using a high ionic strength KCl/Na pyrophosphate buffer followed by final purification of sarcolemmal membranes by discontinuous sucrose gradient centrifugation. Sarcolemmal membrane fractions were collected at the 0–30% (Fraction A) and 30–35% (Fraction B) sucrose interfaces and stored at −70° until used. The extent of membrane purification was assessed by the increase in the specific activity of the sarcolemmal enzyme marker, potassium-stimulated *p*-nitrophenylphosphatase [13]. Activities were determined by the method of Bers [14] and expressed as μmoles *p*-nitrophenol produced per hr per mg membrane protein. To account for variations in sarcolemmal membrane purification, enzyme activities and binding data are expressed per unit potassium-stimulated *p*-nitrophenylphosphatase activity. Expressing the data in terms of other sarcolemmal marker enzymes, e.g. 5'Nucleotidase [13], gave similar results (data not shown). Adenylate cyclase activities in all membranes fractions were determined 5 days after isolation. Sarcolemmal marker enzyme activities indicated at least an eight-fold purification in both membrane fractions over the initial homogenate [13].

β-Receptor number. Sarcolemmal β-receptor number was determined by the specific binding and competitive displacement of (^{125}I-CYP) by (±)propranolol. Incubations were carried out for 3 hr at 30° in an assay medium comprising 50 mM Tris, 5 mM $MgCl_2$, 60 pM ^{125}I-CYP, 0.1% BSA, 40–60 μg/ml sarcolemmal protein (Fraction B), 0.005–1.0 μM propranolol, pH 7.4. Bound radioactivity was separated from free by rapid filtration on glass fibre filters as described above. Sarcolemmal β-receptor affinity and density were determined by Scatchard analysis [15]. Dissociation constants for propranolol binding were determined from the IC_{50} values calculated for the displacement of ^{125}I-CYP from the sarcolemma according to the equation,

$$K_I = \frac{IC_{50}}{1 + (H_T/K_D)}$$

where IC_{50} = propranolol concentration which half-maximally displaces ^{125}I-CYP from the sarcolemma, K_I = dissociation constant for propranolol, H_T = concentration of ^{125}I-CYP in the incubations and K_D = dissociation constant for ^{125}I-CYP [16].

Adenylate cyclase activity. Adenylate cyclase activity was determined by the method of Luzio *et al.* [17]. Assays were initiated by the addition of sarcolemmal membranes (Fraction A) to an incubation medium resulting in a final composition of 32 mM Tris/β-glycerophosphate, 0.5 mM ATP, 2.5 mM $MgSO_4$, 0.5 mM $MnSO_4$, 0.5 mM DTT, 6 mM theophylline, 2.5 mM phosphoenolpyruvate, 10 units/ml pyruvate kinase and 60–80 μg/ml membrane protein, pH 7.4. Incubations were carried out at 30° for 10 min before terminating by boiling. Assay tubes were centrifuged at 9000 *g* for 1 min (Beckman Microfuge) and supernatants sampled for cAMP by the method of Brown *et al.* [18]. Radioactivity was determined using a triton/toluene based scintillation cocktail. Isoprenaline, fluoride or forskolin were added to the incubations as required.

General. Protein was determined by the method of Lowry *et al.* [19] using bovine serum albumin as standard. All experimental results are means ± S.E.M. or means ± S.D. as indicated. Data were analysed using a non-paired Student's *t*-test with a probability of ≤ 0.05 being taken as significant.

RESULTS

Blood propranolol concentrations. Propranolol (0.125%) administered in the drinking water resulted in ingestion of 75–100 mg/kg/day (cf. man 1–5 mg/kg/day). At the height of the blockade, serum propranolol (plus active metabolites) concentrations were 15.4 ± 3.8 ng/ml (Table 1). Similar administration protocols have been shown to produce an effective β-blockade in the rat as judged by a decreased chronotropic response to isoprenaline [20]. The variability in blood propranolol concentrations (7–23 ng/ml) presumably reflects the fluctuating blood levels associated with oral dosing coupled with extensive first pass metabolism and rapid plasma clearance (approx. 63 ml/kg/min in the rat; [21]). These propranolol concentrations are three to eight times the K_D value obtained for propranolol binding to sarcolemmal β-receptors (see Table 2). Twenty-four hours after propranolol withdrawal, serum levels were reduced by 95% to 0.81 ± 0.11 ng/ml. After 48 hr serum propranolol concentrations were still detectable at 0.54 ± 0.07 ng/ml.

Heart rate and blood pressure. Following this oral treatment protocol and at these serum propranolol concentrations, propranolol-treated rats exhibited a reduction in basal heart rate and blood pressure (Table 1). Figures 1 and 2 show heart rate and blood pressure responses to an i.v. bolus injection of isoprenaline. In both cases the propranolol-treated animals showed a shift of the dose response curve to the right with significant increases in the ED_{50} values for isoprenaline (Table 1). These results reflect an extensive β-blockade by the circulating propranolol.

With regard to the 24 hr-withdrawn group of rats, both heart rate and blood pressure response curves were shifted to the left, indicative of an increased sensitivity to isoprenaline. There was also a significant reduction in the ED_{50} compared with controls and also the propranolol-treated animals (Table 1).

In the 48 hr-withdrawn rats, although the dose–response curve for isoprenaline-induced increase in heart rate was shifted to the left at lower isoprenaline concentrations, the ED_{50} value was not significantly different from controls (Table 1). However the blood pressure response to isoprenaline in these animals was still elevated as indicated by the markedly reduced ED_{50} value compared with controls (Table 1). Therefore, following this administration protocol these results show that a transient supersensitivity is associated with abrupt withdrawal from chronic propranolol treatment. In order to elucidate the mechanisms responsible for the enhanced sensitivity to isoprenaline, changes in the efficacy of the cardiac sarcolemmal β-receptor/adenylate cyclase complex were investigated.

Receptor number and affinity. Scatchard plots of binding data are shown in Fig. 3. Binding and dissociation constants were determined by a computer-fitted linear regression and are shown in Table 2. Compared to control membranes, receptor numbers were increased by 180% in membranes isolated from propranolol-treated rats. Similarly, small but significant increases in receptor number were still evident 24 hr (35%) and 48 hr (23%) after propranolol

Table 1. Measurements of serum propranolol, heart rat and blood pressure

Group	Serum propranolol concentration (ng/ml)	Basal heart rate (beats/min)	Basal mean arterial blood pressure (mm Hg)	ED_{50}-isoprenaline-induced increase in heart rate (ng/kg)	ED_{50}–isoprenaline-induced fall in blood pressure (ng/kg)
Control		296 ± 9	77 ± 1	81.8 ± 21	167 ± 30
Propranolol-treated	15.4 ± 3.8	**237 ± 7	**60 ± 5	**1799 ± 360	*748 ± 165
24 hr-Withdrawn	0.8 ± 0.1	290 ± 11	71 ± 5	*42.7 ± 8.7	**13.6 ± 2.9
48 hr-Withdrawn	0.5 ± 0.1	301 ± 11	76 ± 5	74.6 ± 17	**35.7 ± 11

Serum propranolol concentrations were determined by competitive displacement of ^{125}I-CYP from a rat lung membrane preparation (Materials and Methods) and the results are the mean ± S.D. for 11 animals.

Basal heart rate was determined from the ECG recording and blood pressure was measured from the carotid artery. The ED_{50} values for isoprenaline-induced increase in heart rate or fall in blood pressure were determined from cumulative doses of i.v. isoprenaline. All results are presented as the mean ± S.E.M. for six animals.

* $P < 0.05$.

** $P < 0.01$. Significantly different from control values.

Table 2. Dissociation and maximum binding constants for ±propranolol displacement of ^{125}I-CYP from sarcolemmal membranes isolated from control, propranolol-treated, 24 hr- and 48 hr-withdrawn rats

Group	Dissociation constant (K_D)	Maximum binding constant (B_{max})
Control	8.19 ± 0.32	8.42 ± 0.10
Propranolol-treated	8.39 ± 0.44	23.72 ± 0.42
24 hr-Withdrawn	8.30 ± 0.71	11.33 ± 0.41
48 hr-Withdrawn	9.07 ± 1.11	10.34 ± 0.47

Binding constants were calculated by a computer-fitted linear regression of Scatchard analysis of the data (see Fig. 3). Dissociation constants were calculated from the IC_{50} values (see Materials and Methods). Maximum binding is expressed as pmoles propranolol bound per unit K^+-stimulated *p*-nitrophenyl phosphatase. Results are given as means ± S.E.

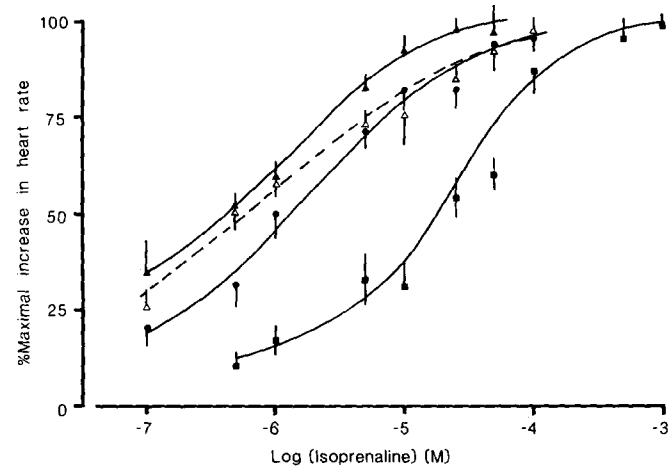

Fig. 1. Mean cumulative dose–response curves to i.v. isoprenaline (0.1 ml bolus) for control (●—●), propranolol-treated (■—■), 24 hr-withdrawn (▲—▲) and 48 hr-withdrawn (△—△) rats. Ordinate: % maximal increase in heart rate. Abscissa: isoprenaline concentration (M). All points are the mean ± S.E.M. for six animals.

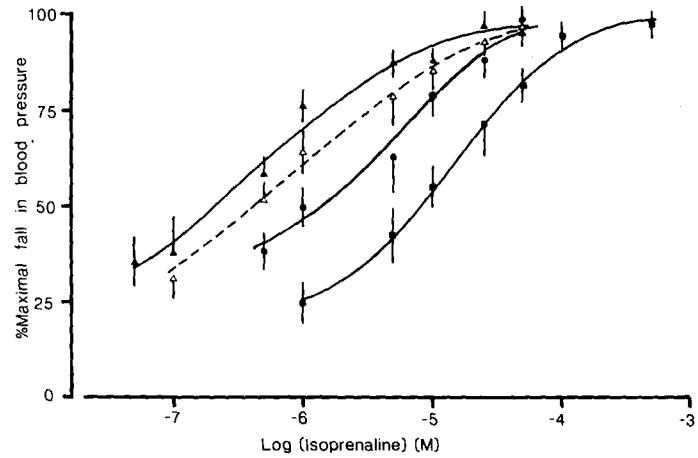

Fig. 2. Mean cumulative dose–response curves to i.v. isoprenaline (0.1 ml bolus) for control (●—●), propranolol-treated (■—■), 24 hr-withdrawn (▲—▲) and 48 hr-withdrawn (△—△) rats. Ordinate: % maximal fall in blood pressure. Abscissa: isoprenaline concentration (M). All points are the mean ± S.E.M. for six animals.

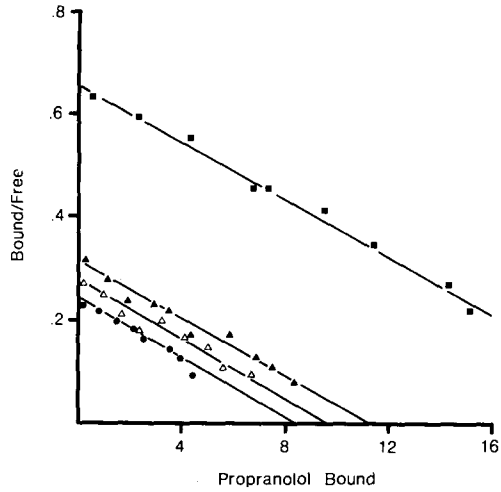

Fig. 3. Scatchard plots of propranolol displacement of ^{125}I-CYP (60 pM) binding to sarcolemmal membranes (fraction B) isolated from control (●——●), propranolol-treated (■——■), 24 hr-withdrawn (▲——▲) and 48 hr-withdrawn (△——△) rats. Ordinate: pmoles propranolol bound per unit K^+-stimulated *p*-nitrophenylphosphatase/free propranolol concentration (nM). Abscissa: pmole propranolol bound per unit K^+-stimulated *p*-nitrophenylphosphatase. Each point is the mean of six individual observations using the same membrane preparation. Maximum binding and IC_{50} constants were calculated by a computer-fitted linear regression programme (see Table 2).

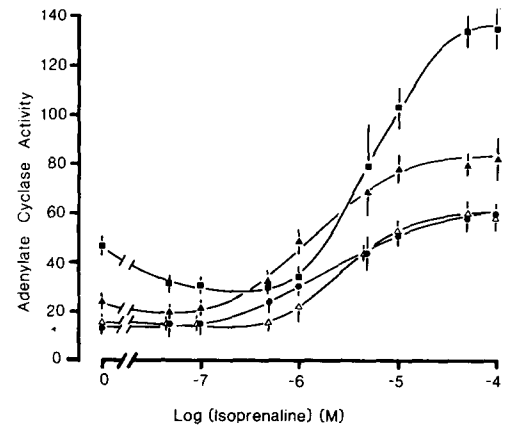

Fig. 4. Dose–response curves for isoprenaline stimulation of adenylate cyclase activity in sarcolemmal membranes (fraction A) isolated from control (●——●), propranolol-treated (■——■), 24 hr-withdrawn (▲——▲) and 48 hr-withdrawn (△——△) rats. Ordinate: pmole cAMP produced/min per unit K^+-stimulated *p*-nitrophenylphosphatase. Abscissa: isoprenaline concentration (M). Results are the mean ± S.D. of six measurements using the same membrane preparation. Maximum activities are shown in Table 3.

withdrawal. No significant change in receptor affinity for propranolol was found in membranes isolated from propranolol-treated or withdrawn rats.

Adenylate cyclase activity. Isoprenaline-stimulated adenylate cyclase activities in membranes isolated from control, propranolol-treated and withdrawn groups are presented in Fig. 4. Basal adenylate cyclase activity was elevated some 290% in propranolol-treated rats and 96% in the 24 hr-withdrawn group. Maximum isoprenaline-stimulated activity was increased 125% in propranolol-treated rats and 35% in the 24 hr-withdrawn group. Basal and isoprenaline-stimulated activities in membranes isolated from 48 hr-withdrawn rats were not significantly different from controls. Half-maximum activation of adenylate cyclase activity by isoprenaline was also increased in propranolol-treated rats (5.5 μM) as compared with control (2.5 μM) and the two withdrawn groups (2.0 μM, 24 hr and 3.0 μM, 48 hr). Addition of low concentrations of isoprenaline (<1 μM) to membranes obtained from propranolol-treated rats produced a decrease in enzyme activity.

In order to assess whether simultaneous changes in the density or responsiveness of the catalytic and regulatory components of the adenylate cyclase system [22, 23] accompanied the changes in β-receptor number, maximum fluoride- and forskolin-stimulated activities were determined. Fluoride- and forskolin-stimulated adenylate cyclase activities are presented in Table 3, together with the results for isoprenaline-stimulated activity. Maximum fluoride- and forskolin-stimulated activities were increased by 100 and 80% respectively at the height of the propranolol blockade. These parameters were still found to be elevated 24 hr after withdrawal of propranolol but had returned to below that of the controls at 48 hr.

Table 3. Basal and maximally-stimulated sarcolemmal adenylate cyclase activities in membranes isolated from control, propranolol-treated, 24- and 48 hr-withdrawn rats

Group	Basal	Isoprenaline-stimulated	Fluoride-stimulated	Forskolin-stimulated
Control	12.1 ± 2.4	59.1 ± 2.2	95.3 ± 3.5	249.5 ± 11.3
Propranolol-treated	**47.7 ± 3.3	**133.7 ± 7.7	**192.9 ± 10.9	**453.6 ± 36.4
24 hr-Withdrawn	**23.7 ± 1.4	**81.0 ± 7.9	**120.4 ± 6.0	*280.7 ± 18.2
48 hr-Withdrawn	*16.5 ± 2.2	59.9 ± 4.1	**83.4 ± 4.2	**192.4 ± 21.2

Maximal activities were determined in the presence of 100 μM isoprenaline, 25 mM fluoride or 100 μM forskolin. Results given are means ± S.D. for six measurements and are expressed as pmoles cAMP produced per min per unit K^+-stimulated *p*-nitrophenyl phosphatase.

* $P < 0.05$.

** $P < 0.01$. Significantly different from control values.

DISCUSSION

It is clear from the evidence *in vivo* that chronic oral propranolol treatment induced significant reductions in both basal heart rate and blood pressure. This was accompanied by parallel shifts of the dose–response curves for heart rate and blood pressure responses to isoprenaline, indicative of a competitive antagonism by the circulating propranolol. In addition to these changes found *in vivo* cardiac sarcolemmal membranes isolated from propranolol-treated rats exhibited large increases in β-receptor number (180%) and isoprenaline-stimulated adenylate cyclase activity (125%). These results agree well with those of Glaubiger and Lefkowitz [3] who reported a doubling in β-receptor number following chronic propranolol treatment of rats. Therefore at the height of the blockade when blood propranolol concentrations were over three-fold higher than the K_D calculated for receptor binding there is a substantial increase in the density of cardiac β-receptors capable of coupling with the adenylate cyclase system.

The increased isoprenaline-stimulated adenylate cyclase activity in membranes isolated from propranolol-treated rats can be explained by the paralleled increase in β-receptor number. However, the mechanisms responsible for the induction of large increases basal activity remains to be elucidated. Similarly, increased β-receptor number along with elevated basal and isoprenaline-stimulated adenylate cyclase activities have also been reported in rat cerebral cortex following 6-hydroxydopamine treatment [24]. Although the physiological significance of basal adenylate cyclase activity is unclear, these experiments indicate that the unstimulated enzyme rate is related to the intrinsic responsiveness of the system to β-agonists. Since both fluoride and forskolin-stimulated adenylate cyclase activities were also elevated, the increased basal activity may be related to the increased density of catalytic and regulatory components of the adenylate cyclase system in the sarcolemmal membrane. The increased receptor number may also be a significant factor in the regulation of basal activity.

Basal adenylate cyclase activity in membranes isolated from propranolol-treated rats was significantly reduced by low isoprenaline concentrations. The reason for this finding is unknown. No propranolol could be detected in the acid-extracts of these membrane preparations, indicating that the endogenous propranolol concentration was less than 0.3 ng/ml. The absence of any change in the K_D for propranolol binding to membranes isolated from propranolol-treated rats also indicates that no endogenous propranolol was associated with the sarcolemma after isolation. Therefore the inhibition of basal activity and the shift in the isoprenaline dose–response curve is possibly a function of the adenylate cyclase complex and is not due to residual propranolol.

Following propranolol withdrawal from the drinking water both basal heart rate and blood pressure values had returned to those of control rats within 24 hr, indicative of a diminished β-blockade. Serum propranolol concentrations also fell rapidly and were 5 and 3% of those found for propranolol-treated rats after 24 and 48 hr respectively. In parallel with the decline in serum propranolol and the diminished β-blockade there was a reduction in both sarcolemmal β-receptor number and hormone-sensitive adenylate cyclase activity. Sarcolemmal β-receptor number, however, was still greater than in controls both 24 and 48 hr after propranolol withdrawal. Aarons and Molinoff [4] have reported similar increases in β-receptor number in ventricle, lung and lymphocyte preparations isolated from rats 18 hr after propranolol withdrawal. Although this group reported that more than 90% of the circulating propranolol was eliminated within 18 hr of withdrawal, the blood propranolol concentrations were still significantly elevated (8 ng/ml; 24 nM). Such concentrations would result in a blockade comparable to that found in the propranolol-treated rats of the present study. However, the reported increase in receptor number 18 hr after propranolol withdrawal was not as extensive. No information was given on β-receptor number at the height of the blockade, nor when blood propranolol concentrations fell to zero. It is possible that at high propranolol doses the relative rate of reduction in receptor number to control values may exceed that of propranolol clearance from the blood.

Various clinical studies concerning the effects of abrupt withdrawal from propranolol treatment have yielded somewhat conflicting results. However, if the heightened sympathetic activity reported by some workers [6, 25–28] is a result of a general increase in tissue β-receptor density [28] then the rate of propranolol clearance must exceed the rate of reduction in β-receptor number to normal. Although basal heart rate and blood pressure were not elevated 24 hr after propranolol withdrawal, possibly as a result of a decrease in the sympathetic tone [29], both these parameters showed an increased sensitivity to i.v. isoprenaline. This supersensitivity to isoprenaline *in vivo* may be associated with the increased cardiac sarcolemmal β-receptor density and adenylate cyclase activity. Although β-receptors remain elevated 48 hr after propranolol withdrawal, isoprenaline-stimulated adenylate cyclase activity and chronotropic responses have returned to control values. Thus the changes in the chronotropic response to i.v. isoprenaline correlate well with changes in cardiac sarcolemmal adenylate cyclase activity *in vitro*.

Since sarcolemmal β-receptor density at 48 hr after propranolol withdrawal is still greater than in controls, the decrease in isoprenaline-stimulated adenylate cyclase activity may be accounted for by the decrease in responsiveness of both the catalytic and guanine nucleotide regulatory components as measured by decreased forskolin- and fluoride-activation respectively. Therefore an increase in β-receptor number does not necessarily result in an increase in the cAMP response to catecholamines. Similar findings with regard to the relationship between concurrent changes in β-receptor number, cAMP production and tissue adrenergic responsiveness have also been reported [30–32]. The sensitivity of a tissue to β-agonists will depend on receptor number and affinity together with the efficiency of coupling and activities of the other components of the adenylate

cyclase system. It is therefore suggested that for future studies it would be advantageous to measure not only β-receptor number but also hormone-sensitive adenylate cyclase activity.

If an increase in the number of cardiac β-receptors is responsible for the enhanced sympathetic activity associated with propranolol withdrawal, our experiments using the rat as a model system indicate that the supersensitivity should be at a maximum within 24 hr of withdrawal. In normal human subjects [28] and in patients treated for hypertension [33] supersensitivity has been reported within 24–48 hr of propranolol withdrawal, when blood propranolol concentrations would be negligible [34]. Likewise in some clinical studies in patients suffering from severe angina, an increased sympathetic sensitivity has been reported within 48 hr of propranolol withdrawal [27].

Although it is difficult to extrapolate the results obtained in rat to observations made in man, it is possible that the increased sympathetic activity is a result of the rapid removal of circulating propranolol, so effecting a transient increase in the density of functionally-operative, hormone-sensitive adenylate cyclase units within the sarcolemmal membrane. However, the occurrence of cardiac events appear to exhibit a bimodal distribution and can extend for up to 14 days after propranolol withdrawal [6, 27]. Assuming similar β-receptor turnover rates in man and rat it is difficult to relate these findings to those of the present study, with regard to the rate of decline in cardiac β-receptor density and adenylate cyclase activity. Since the blood pressure response to i.v. isoprenaline was still elevated 48 hr after propranolol withdrawal it is conceivable that significant changes in catecholamine sensitivity of other tissues may occur following prolonged β-blockade. As propranolol is a non-selective β_1, β_2-antagonist, those tissues where β_2-receptors predominate may not respond identically to those tissues containing mainly β_1-receptors on propranolol withdrawal. This is especially true of tissues which accumulate propranolol [21, 35, 36] and may account for the disparate changes in the sensitivities of heart rate and blood pressure to i.v. isoprenaline following propranolol withdrawal. Botting and Crook [20] demonstrated that propranolol induced an increase in catecholamine sensitivity but atenolol, a selective β_1-antagonist, failed to do so. This again implicates the involvement of tissues with β_2-subtype receptors in the "propranolol withdrawal syndrome".

It is suggested that the increased catecholamine sensitivity found both in normal subjects and patients for up to two days after propranolol withdrawal may be related to an increased myocardial β-receptor density. However, it is unlikely that the "propranolol withdrawal syndrome" seen in patients up to 14 days after propranolol withdrawal [6, 27] may still be related to increased cardiac β-receptor number. These later episodes may be associated with patient clinical status or may be a consequence of an imbalance in catecholamine sensitivity between various tissue types stemming from differential rates of recovery from β-blockade.

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