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ADAPTATIONAL RESPONSES TO PROLONGED β-ADRENOCEPTOR BLOCKADE IN ADULT RABBITS

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1 A method for measuring propranolol concentrations in plasma has been modified to permit estimations to be made on small volumes. The method has been used to correlate blockade of heart rate increases in response to intravenous isoprenaline, in both young and adult rabbits, with plasma concentrations of propranolol after subcutaneous injections of 2 or 4 mg/kg. It has been found that the relation between β-adrenoceptor blockade and plasma concentrations is the same in rabbits as that determined by previous workers in man.

2 Adult Dutch dwarf rabbits were treated for 9 weeks twice daily subcutaneously with 2 mg/kg propranolol, or 10 mg/kg practolol, or with saline.

3 The treatment caused no change in heart weight in relation to body weight or in the water content of the hearts, in contrast to effects previously observed in young rabbits.

4 As in the young rabbits, treatment did cause a prolongation of action potential duration, as measured with intracellular electrodes in hearts of animals killed 24 h after the last dose of drug.

5 In another series of experiments, with a similar regime of treatment for 6 weeks, a significant reduction of diastolic blood pressure was observed in the propranolol group.

6 During the course of treatment and at the end, there was no change in the heart-rate increases observed in response to intravenous isoprenaline administered not less than 15 h after the previous dose of β-blockers. Thus no functional alteration in sensitivity to β-adrenoceptor stimulation was apparent.

Introduction

In a previous paper it was shown that treatment of young rabbits for several weeks with β-adrenoceptor blocking drugs, at doses comparable to those used clinically, had a number of secondary effects which could be distinguished from the acute actions of the drugs (Vaughan Williams, Raine, Cabrera & Whyte, 1975). In acute experiments, β-blockers either have no effect on action potential duration, or shorten it (Papp & Vaughan Williams, 1969). However, after several weeks of treatment, action potential duration was greatly prolonged in cardiac muscle taken from animals killed 24 h after the last dose of drug, at a time when there was no residual block of the chronotropic or inotropic responses to isoprenaline. Prolonged treatment of young rabbits with β-blockers also caused a reduction of heart weight in relation to body weight, and induced some morphological changes, notably an increase in the relative volume of vascular elements and interfibrillar fluid (Vaughan Williams, Tasgal & Raine, 1977). These effects on heart weight and ultrastructure have recently been confirmed in an independent study (Florio, Nayler, Slade, Vaughan Williams & Yepez, 1980). However, the latter experiments, were carried out on rabbits which were initially nearly double the weight (mean 1640 g) of those used in the initial study (mean 930 g), and it was noted that the effects, though qualitatively similar, were quantitatively less marked. It seemed likely, therefore, that the age of the animals at the start of treatment might be a factor influencing the magnitude of the adaptational response to prolonged β-blockade. In this paper the effects of prolonged β-blockade on blood pressure, cardiac electrophysiology, heart growth and water content, and on body growth have been studied in adult rabbits. The effects of acute responses to isoprenaline and to injections of β-blockers have also been studied at intervals during the progress of the treatment, and at its end.

The results indicate that a clear distinction can be made between the effects of prolonged β-blockade on heart growth and on cardiac action potential duration.

The clinical pharmacology of propranolol in man has been extensively studied (reviewed by Nies & Shand, 1975) but since our experiments were performed in rabbits, it was important to establish (a) whether the rate of disappearance of propranolol from rabbit plasma was similar to that observed in

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man and (b) whether the relation between plasma concentration and functional β-blockade was the same in rabbits as in man. The standard propranolol assay (Shand, Nuckolls & Oates, 1970) requires aliquots of plasma of 4 ml, too great a volume to allow sequential measurements *in vivo* in small animals. In the first part of this paper a modification of the standard assay is described which enables accurate measurement of low concentrations of propranolol in small volumes of plasma. The relationship of the plasma propranolol concentration to the antagonism of the chronotropic response to isoprenaline was measured in rabbits, in order that a comparison might be made with that already established in man (Coltart & Shand, 1970). Propranolol levels in the rabbits have been estimated after both acute and chronic administration. The plasma concentration of propranolol was correlated with functional blockade after injections of 2 mg/kg subcutaneously, as used in our original papers, and after 4 mg/kg subcutaneously, the dose employed in a subsequent study. It is now possible to compare directly the drug levels achieved in the animal investigations with those observed in clinical therapy.

Methods

Measurement of β-blockade

Conscious rabbits were trained to sit quietly in open restraining boxes daily for increasing lengths of time. Electrocardiograms were recorded with subcutaneous fine needle electrodes. After several days' habituation, it was apparent that the animals would tolerate remaining in the boxes for many hours without distress, as judged by the stability of heart rate recordings. ECG recordings were made with a Devices AC1 preamplifier and DC5 recorder. Heart rate was read directly from a Devices ratemeter. Blood was withdrawn, and injections of isoprenaline introduced, through a tapered polythene cannula which was inserted into the marginal ear vein under procaine anaesthesia. The cannula was withdrawn at the end of each experiment. The peak increases in heart rate actually observed ranged from 5 to 100 beats/min. Log dose-response curves were drawn by eye and the doses of isoprenaline required to produce increases of 20, 40 and 70 beats/min were estimated by interpolation. The peak chronotropic response to isoprenaline occurred within 30 s of injection, and both heart rate and blood pressure returned to baseline levels within 2 to 3 min. At the end of each set of measurements, the first and last doses were repeated. Since the responses to these were similar to those first obtained, it was evident that no tachyphylaxis had been induced by the small doses of isoprenaline used in these experiments. The time required for each complete set of measurements was 12 to 18 min, the usual dose range required being 0.05 to 0.2 μg/kg. Propranolol 4 mg/kg was then injected subcutaneously and the doses of isoprenaline required to produce a chronotropic response of 20 to 70 beats/min were determined at various intervals after the propranolol injection. Blood (1 ml) was withdrawn into heparinized tubes immediately before determining each dose-response curve, centrifuged, and the plasma frozen for later measurement of propranolol concentration.

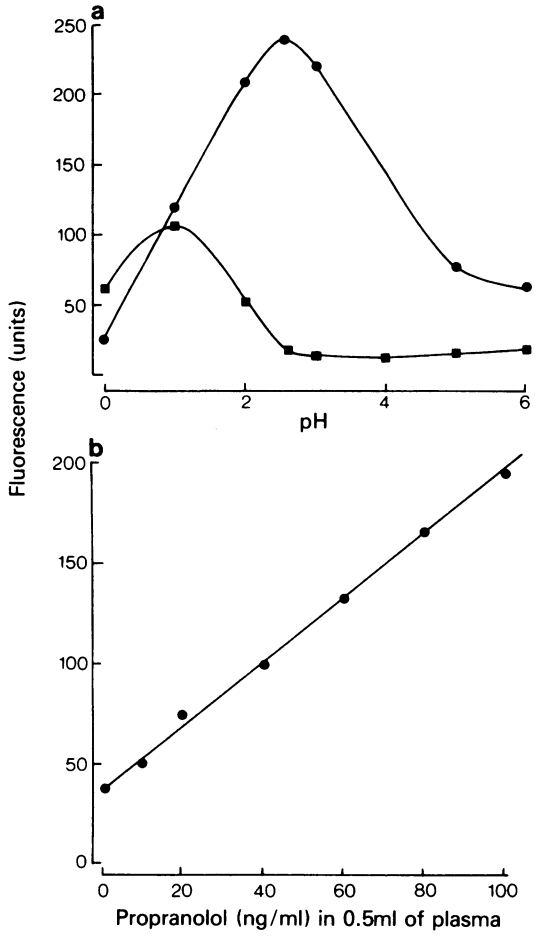

Figure 1 (a) Effect of pH on fluorescence of blank rabbit plasma (■) and of propranolol 100 ng/ml (●). (b) Standard fluorescence curve for propranolol obtained in the modified assay.

Measurement of propranolol concentration

The method of Shand, *et al.* (1970) was used. Since this method requires 8 to 10 ml of blood, a modification was made to permit measurements on smaller

volumes. As propranolol fluorescence is pH-dependent (Potter, 1967), the fluorescence of propranolol and of blank rabbit plasma were compared at different hydrogen ion concentrations (Figure 1a). In the present study, propranolol fluorescence was maximal at pH 2.6, and was decreased by half at pH 1.0, whereas the blank plasma had a peak fluorescence at pH 1.0 and the fluorescence was reduced at higher pH. Therefore the final extraction was made into 2.5 mM HCl, not 0.1 N HCl as in the method of Shand *et al.* (1970).

Rabbit plasma (0.5 ml) or homogenate of ventricular muscle was diluted with 1.5 ml H_2O and 0.5 ml N NaOH, and extracted into 12 ml heptane, containing 1.5% isoamyl alcohol, in a Vortex mixer. After centrifugation at 1000 rev/min for 5 min, 10 ml of the organic phase was extracted into 1.5 ml of 2.5 mM HCl, and the fluorescence measured in an Aminco-Bowman spectrophotometer (maximum excitation 300 mμ; maximum emission 358 mμ, uncorrected). Propranolol concentration was estimated by comparison with standards containing 25, 50 and 100 ng/ml propranolol added to plasma and carried through the assay. It was found possible to measure concentrations of propranolol of 10 ng/ml in 0.5 ml aliquots (Figure 1b).

Heart weight, and electrophysiological measurements

Cardiac muscle has such an avidity for oxygen that *in vitro* experiments must be performed on thin muscles if under-oxygenation of the deeper fibres is to be avoided. For this reason, for the experiments on adult rabbits, Dutch dwarf rabbits were chosen, since these weigh only about 2 kg when fully grown.

For the study of the effects on heart weight, three groups of rabbits, approximately 7 months old, were injected twice daily subcutaneously with saline 1 ml/kg (mean initial weight 1955 ± 32 g, $n = 13$), or with propranolol 2 mg/kg (init. wt. 1897 ± 31 g, $n = 10$), or with practolol (init. wt. 1859 ± 40 g, $n = 9$), for nine weeks. Not less than 24 h after the last dose, the animals were injected with heparin intravenously, were stunned, and their hearts were removed. The atria were separated from the ventricles, and the atria were set up for micro-electrode recording, as previously described (Vaughan Williams *et al.*, 1975). The right ventricular wall was dissected from the left (the septum being included with the left ventricle), and both ventricles were blotted and weighed separately. They were then dried to constant weight at 100°C.

In the propranolol group, after five weeks of treatment, blood was withdrawn at 0.5, 3.5 and 15 h after a standard subcutaneous dose of 2 mg/kg, and the plasma concentration of propranolol was estimated.

In a second series of animals, with a similar regime of dosage, but treated for 6 weeks instead of 9, measurements of blood pressure were made before, during and at the end of treatment, the records being taken not less than 15 h after dosage (i.e. when plasma concentrations were below blocking levels as described later). The right ear was anaesthetized with procaine at the base, a longitudinal skin incision about 1 cm long was made over the central artery, and a silk suture was passed beneath it. A small cut was made in the artery and a fine saline-filled polyethylene cannula introduced and tied in, the artery being compressed temporarily with a clamp. The blood pressure was recorded with a Consolidated Electrodynamics 4-327-L-221 transducer, and displayed on a Devices recorder (DC5). The blood pressure became stable after 15 min, and was recorded for 60 to 90 min. A further injection of procaine was given, the cannula was removed, the artery tied, and the skin sutured. Dose-response curves for isoprenaline were determined.

The mean initial weights of the second series of rabbits were: saline-treated controls, 1839 ± 46 g ($n = 7$); propranolol group, 1795 ± 23 g ($n = 6$); practolol group 1877 ± 58 g ($n = 7$). Electrophysiological studies were carried out in both series of animals.

Propranolol hydrochloride was supplied by I.C.I., isoprenaline sulphate by Burroughs Wellcome, and all other drugs and reagents were obtained from B.D.H. Results have been expressed as means ± s.e. mean, and the significance of differences between groups evaluated by Student's *t* test (2-tailed), and differences within groups by the paired *t* test.

The animals were kept in separate cages and fed R.14 maintenance pellets (Labsure Animal Foods) and water *ad libitum*. They were weighed thrice weekly and on the day they were killed.

Results

β-Adrenoceptor blockade and blood levels of propranolol

Chronotropic responses to intravenous injections of isoprenaline were measured in seven young rabbits (New Zealand White approximately 8 weeks old) at various times after a subcutaneous injection of 4 mg/kg of propranolol, and the mean results are presented in Figure 2a; 15 min after a 4 mg/kg injection of propranolol the dose-ratio for isoprenaline was 92.5, declining to 40 at 1 h, 3.5 at 4 h and 2.1 at 7 h as the plasma concentration of propranolol fell. Despite the wide range of dose-ratio, the log dose-response curves were parallel throughout, implying that the antagonism was competitive at all times. Blood was withdrawn after 0.25, 1, 4 and 7 h. At

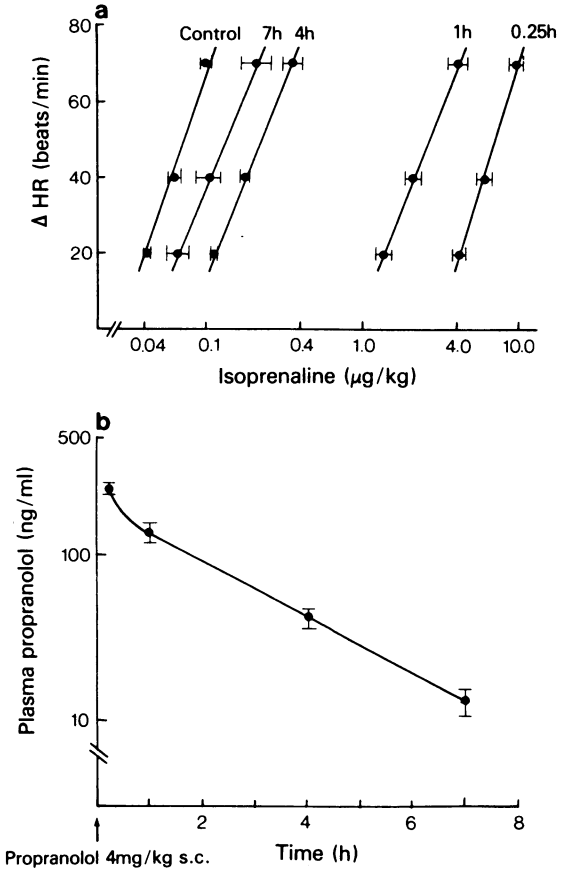

Figure 2 (a) Decay of blockade of chronotropic responses to intravenous isoprenaline in a conscious rabbit after 4 mg/kg propranolol s.c. Ordinate scale: changes in heart rate (beats/min). Abscissa scale: dose of isoprenaline in μg/kg (log scale). Dose-response curves obtained before, and starting 0.25, 1, 4 and 7 h after the injection. Points give means (n = 7); horizontal lines show s.e. means. (b) Decay of plasma concentration of propranolol after an injection of 4 mg/kg propranolol s.c. Ordinate scale: mean concentration of propranolol in ng/ml; vertical lines show s.e. mean. Abscissa scale: time in h after the injection (n = 7).

15 min the mean propranolol concentration was 250 ± 16 ng/ml, declining to 13 ± 2.4 ng/ml at 7 h (Figure 2b). Excluding the 15 min reading, when it may be assumed that the distributive phase was incomplete, the decay curve fitted a single exponential with a half-time of 1.8 h, which may be compared with the half-time of 2.3 h observed by Shand *et al.* (1970) after 10 mg propranolol intravenously in man.

For the reasons given above, Dutch dwarf rabbits were chosen for the study of the effect of prolonged propranolol treatment in adult rabbits, and it was important to determine whether plasma levels of propranolol achieved in this strain were comparable to those observed in the young N.Z. rabbits. In an earlier paper (Vaughan Williams *et al.*, 1975), it was found that after a subcutaneous 2 mg/kg dose of propranolol, blockade had declined to a low level after about 5 to 6 h and this correlates well with the rate of decline of plasma level and a similar low degree of blockade reported here in N.Z. Whites at 7 h after 4 mg/kg propranolol subcutaneously. When the Dutch dwarf rabbits had been treated for 5 weeks with twice daily subcutaneous doses of 2 mg/kg propranolol, blood was withdrawn 12 h after the last dose of propranolol, and the mean plasma level was found to be 4 ng/ml i.e. at the limit of sensitivity of the method; 3 h later the next dose of propranolol (2 mg/kg s.c.) was given and the mean plasma level after 30 min was 188 ± 9 ng/ml (n = 5), and 200 min later it had fallen to 46 ± 8 ng/ml (which gives a half-life of 1.4 h). This evidence suggests that there was no great difference between the two strains, either in the plasma levels achieved or in the duration of blockade.

In man the minimally effective propranolol concentration in reducing exercise tachycardia is 10 ng/ml (Coltart & Shand, 1970). Taking this as an end-point, interpolation from the above data showed the duration of action of a 4 mg/kg dose to be 7.75 h, and that of a 2 mg/kg dose to be 5.5 h. This compares with a duration of effective blockade of 5.52 ± 0.24 h after a 2 mg/kg dose, determined by isoprenaline challenge in young rabbits (Vaughan Williams *et al.*, 1975). The parallel log dose-response curves obtained in N.Z. whites after a single dose of propranolol (Figure 2a) suggested that it was acting as a competitive antagonist.

In our studies of long-term β-blockade in rabbits the animals were killed 24 h after the last dose of drug. It was confirmed (Vaughan Williams *et al.*, 1975) that no residual β-blockade persisted *in vitro* in cardiac muscle taken from these animals, on challenge with isoprenaline, and from the data presented above, it was apparent that the plasma concentration would be below blocking levels. However, it was possible, in spite of the absence of functional blockade, that some drug might have remained in the cardiac muscle. Homogenates were, therefore, made of ventricles taken from rabbits treated for 24 days with 4 mg/kg propranolol twice daily subcutaneously, and excised 24 h after the last dose. The mean fluorescence in them was found to be 30.8 ± 2.9 units (n = 6), which may be compared with a mean fluorescence of 28.0 ± 2.7 units found in homogenates of ventricles taken from saline-treated litter mates (n = 7). Thus the residual propranolol remaining in the tissue after 24 h was below the accurately measurable level.

Body growth: first series

In the studies previously reported (Vaughan Williams *et al.*, 1975) on growth, heart weight etc. there were several series of experiments, in which mongrel rabbits and New Zealand Whites were used, with essentially similar results, so that there was reason to believe that the strain employed was of minor importance. All the rabbits were very young (5 to 6 weeks), and gained about 30 g daily and doubled their weight during a 6 weeks' treatment period. The Dutch dwarf rabbits used here were about 7 months old and almost fully grown. During the 9 weeks treatment they increased their weight by only 19% (controls), 20.5% (propranolol) and 8.5% (practolol). The slightly lower growth rate of the practolol-treated animals was significantly different from the controls ($P < 0.05$), and may be compared with similar significant reductions of growth in the previous studies (Vaughan Williams *et al.*, 1975, Figure 2 and Florio *et al.*, 1980, Figure 1).

Heart weight

The evidence given above indicated that in both N.Z. whites and Dutch dwarfs, twice daily subcutaneous injections of 2 mg/kg propranolol would have achieved substantial functional β-blockade for about half of each day. Previous evidence (Vaughan Williams *et al.*, 1975) showed that 10 mg/kg of practolol in N.Z. whites would have provided blockade for about three quarters of each day. As will be seen, the adaptational responses to prolonged treatment with both drugs in the Dutch dwarf rabbits were very similar.

The mean ventricular and atrial wet and dry weights of the hearts taken from the three groups of animals are presented in Table 1. The mean ventricular weight in the practolol groups was again significantly less than that of the controls, but when allowance was made for the slightly lower body weight in this group, there was no change in ventricular weight expressed as a percentage of body weight. Thus in these adult animals, in clear contrast to the effects previously observed in younger rabbits, prolonged treatment with β-blockers had no effect on the heart weight in relation to body weight. There was also no change in the water content of the treated hearts in comparison with the controls.

Although left ventricular wet and dry weights were similar in all groups (Table 1), the right ventricular weight of the propranolol-treated animals was greater

Table 1 Effects of 9 weeks' treatment with propranolol and practolol on heart weight in Dutch dwarf adult rabbits

(A) *Ventricular wet weight*

Treatment	(*n*)	*Ventricular wet wt. (g)*	*Wet wt. as % body wt.*	*L.V. wet wt. (g)*	*L.V. wet wt. (% body wt.)*	*R.V. wet wt. (g)*	*R.V. wet wt. (% body wt.)*
Saline 1 ml/kg b.d.	(13)	4.11 ± 0.13	0.172 ± 0.004	3.22 ± 0.11	0.135 ± 0.003	0.80 ± 0.04	0.034 ± 0.001
Propranolol 2 mg/kg b.d.	(10)	3.93 ± 0.09	0.173 ± 0.004	3.05 ± 0.07	0.134 ± 0.003	0.81 ± 0.03	0.036 ± 0.001
Practolol 10 mg/kg b.d.	(9)	3.52 ± 0.11**	0.168 ± 0.004	2.79 ± 0.09*	0.134 ± 0.004	0.68 ± 0.02*	0.033 ± 0.001

(B) *Ventricular dry weight*

		Ventricular dry wt. (g)	*Dry wt. as % body wt.*	*L.V. dry wt. (g)*	*L.V. dry wt. (% body wt.)*	*R.V. dry wt. (g)*	*R.V. dry wt. (% body wt.)*
Saline	(13)	0.93 ± 0.04	0.039 ± 0.001	0.73 ± 0.03	0.031 ± 0.001	0.20 ± 0.01	0.009 ± 0.001
Propranolol	(10)	0.92 ± 0.03	0.040 ± 0.001	0.71 ± 0.02	0.031 ± 0.001	0.21 ± 0.01	0.009 ± 0.001
Practolol	(9)	0.81 ± 0.02*	0.039 ± 0.001	0.64 ± 0.02*	0.030 ± 0.001	0.17 ± 0.01*	0.008 ± 0.001

(C) *Atrial weight*

		Atrial wet wt. (g)	*Wet wt. as % body wt.*	*Atrial dry wt. (g)*	*Dry wt. as % body wt. × 10²*
Saline	(13)	0.524 ± 0.026	0.022 ± 0.001	0.109 ± 0.009	0.463 ± 0.034
Propranolol	(10)	0.466 ± 0.023	0.020 ± 0.001	0.100 ± 0.007	0.435 ± 0.025
Practolol	(9)	0.415 ± 0.018**	0.020 ± 0.001	0.084 ± 0.005*	0.400 ± 0.020

L.V. = left ventricle; R.V. = right ventricle; b.d. = twice daily.
*$P < 0.02$; **$P < 0.005$.

than for controls, and in the practolol group was less than that of the controls. The ratio of right ventricular to left ventricular weight was thus significantly different in the two treated groups (0.265 ± 0.006, propranolol; 0.244 ± 0.005, practolol; $P < 0.02$). The reason for this difference is unclear, but could be related to the smaller extent of blockade by practolol of vascular and/or bronchial β_2-receptors in the lungs.

Electrophysiology

In our previous studies of the effects of prolonged β-blockade, the drug-treated groups were litter mates of the saline-treated animals, and individual intracellular records from the rabbits in each group were pooled as a single group of records for each drug. The drug-treated adult rabbits in the present series of experiments were not litter mates of the saline-treated animals. Accordingly, 10 to 15 records were obtained from each rabbit, and the means of these measurements were taken as single results, so that there was only one set of measurements per animal. The measurements made in the 6 week series were very similar to those of the 9 week series, and so the results of the two series have been taken together. Representative records are presented in Figure 3 and demonstrate a striking extension of the plateau of the action potential, similar to that previously observed in younger rabbits. The mean results are given in Table 2 (*n* refers to the total number of rabbits from both 6 and 9 week series). Prolonged treatment of adult rabbits had no effect on resting or action potentials, or on the maximum rate of depolarization, but significantly prolonged the plateau of the action potential, especially the time from the peak to repolarization to the zero line and the effect on this parameter was actually greater than that previously seen in the younger rabbits.

Not only the repolarization, but the depolarization phase was also significantly affected by the prolonged treatment. In atrial muscle depolarization has 3 distinct phases; (A) a slow depolarization 'foot', as the capacity of the cell in which the electrode is situated discharges into the approaching action potential in neighbouring fibres; (B) a very rapid linear phase of depolarization, as fast inward current flows; (C) a further slower phase of depolarization. There is some variation from cell to cell in the relative duration and magnitude of these phases (Vaughan Williams, 1958). In any given record it is not difficult to identify the end of the fast linear phase, and to measure the magnitude of the further depolarization which occurs (Figure 3, y) and the time from the end of the fast phase to the peak of the action potential (x). In the saline-treated controls the mean value of x was 1.61 ± 0.19 ms, but in the propranolol group it was 2.51 ± 0.28 ms ($P < 0.001$) and in the practolol group

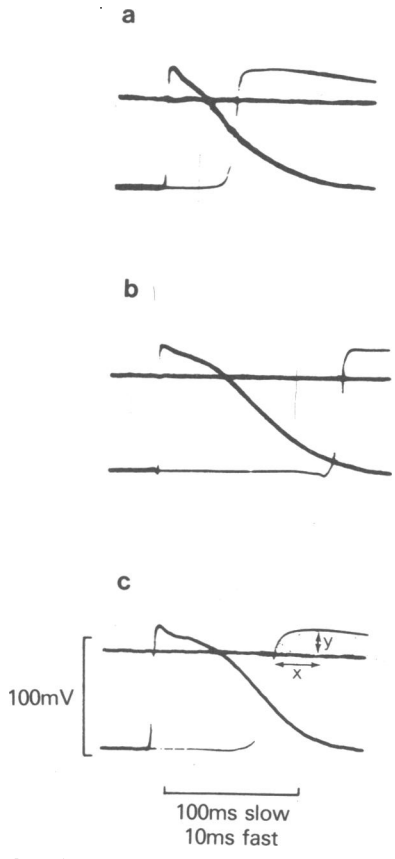

Figure 3 Effect of prolonged β-adrenoceptor blockade on action potential duration. The rabbit was killed 24 h after the last dose of drug. (a) Intracellular potential record from saline-treated animal. (b and c) Records from animals treated for 9 weeks with propranolol (b) and practolol (c), and killed 24 h after the last doses of drug. x = time (ms) from the transition between the end of the very fast linear phase of depolarization and the peak height of the action potential. y = additional depolarization (mV) after the end of the fast linear phase. Prolonged β-blockade significantly increased both x and y.

it was 4.17 ± 0.71 ms ($P < 0.001$). Likewise the magnitude of depolarization after the fast linear phase was 7.2 ± 1.1 mV (controls), but 12.2 ± 1.7 ($P < 0.025$) and 11.6 ± 1.1 ($P < 0.02$) in the propranolol and practolol groups respectively. These changes may be related to some changes in the velocity constants αm and βm, and αh and βh, which determine the time of opening and closing of sodium channels. But whatever the detailed explanation, it is clear that prolonged β-blockade had induced a significant alteration in the mechanism by which the fibre depolarized.

Contractions As previously found in younger rabbits, prolonged β-blockade had no negative inotropic effect. The mean magnitudes of the contractions of the isolated atria, measured in the same conditions as described previously (Vaughan Williams *et al.*, 1975) were 0.57 ± 0.11 g ($n = 13$) in the controls; 0.56 ± 0.09 g ($n = 9$) in the propranolol group; and 0.69 ± 0.11 g ($n = 10$) in the practical group.

It is emphasized that all these records were obtained from the hearts of animals killed at least 24 h after the last injection of β-blocker, when no drug would be present in the tissue, so that the changes observed represent a secondary adaptation to prolonged β-blockade. In acute *in vitro* experiments in previously untreated animals propranolol and practolol either do not affect action potential duration (APD) or shorten it by a few ms (Papp & Vaughan Williams, 1969). It was, therefore, of interest to determine whether, in these adult animals in which APD had already been lengthened as an adaptation to prolonged β-blockade, acute exposure to β-blockers *in vitro* would still cause shortening of APD.

In several animals from each group, after control records had been taken, the solution was changed to one containing propranolol 0.2 or 0.4 μg/ml, or practolol 1.0 or 2.0 μg/ml. Atria from the propranolol-treated animals ($n = 8$) were exposed to the propranolol solutions, and others from the practolol-treated animals ($n = 8$) to the practolol solutions. Atria from saline-treated animals were exposed to either propranolol ($n = 4$) or practolol ($n = 4$). The effects of these acute exposures to the drugs are presented in Table 3, depicted as differences from the measurements first made in the drug-free solutions. It is apparent that both propranolol and practolol still had their normal small effect in shortening APD in all three groups. The 'class 1' (local anaesthetic) effect of propranolol (Vaughan Williams, 1974) on fast inward current is also apparent as a small reduction in the maximum rate of depolarization (MRD). Thus prolonged β-blockade, though it induced a prolongation of APD, did not prevent the usual shortening effect on APD by acute blockade being superimposed on the 'basic' longer APD. This observation is obviously relevant to studies in man of the effect of β-blockers on the Q–T interval if the patients had been administered the drug a short time before the ECG records were taken.

Effect of prolonged β-blockade on sensitivity to isoprenaline

In the previous experiments on young rabbits, treated with β-blockers for 6 weeks and killed 24 h after the last dose, the atria studied *in vitro* exhibited no difference in chronotropic or inotropic responses to isoprenaline, in comparison with saline-treated control litter mates. It was of interest, therefore, to determine whether any change in sensitivity to isoprenaline could be observed *in vivo* in adult rabbits during treatment with β-blockers for a similar period.

Increases in heart-rate, measured by ECG in conscious adult rabbits, were produced in response to intravenous isoprenaline before treatment, and after 3 days and 6 weeks of treatment. The records were made not less than 15 h after the previous injection of drug, so that negligible amounts of β-blocker would be present in the plasma and in the heart. The results for the saline-treated, propranolol-treated, and practolol-treated animals are presented in Figure 4, and indicate that treatment did not alter heart-rate responses to isoprenaline.

Table 2 Effect of 6 and 9 weeks' treatment with propranolol and practolol on atrial intracellular potentials in adult rabbits

Treatment twice daily (no. of animals	*Resting potential (mV)*	*Action potential (mV)*	*Maximum rate of depolarization (V/s)*	*Zero volts (ms)*	*Action potential duration to 50% repolarization (APD_{50}) (ms)*	*Action potential duration to 90% repolarization (APD_{90}) (ms)*
Saline 1 ml/kg (17)	80.1 ± 1.2	98.7 ± 1.3	89.4 ± 4.6	30.6 ± 2.7	59.1 ± 2.9	108.5 ± 2.3
Propranolol 2 mg/kg (15)	78.6 ± 1.4	98.6 ± 1.4	83.7 ± 5.0	38.7 ± 2.3*	64.0 ± 2.1	115.1 ± 3.3
Practolol 10 mg/kg (13)	78.1 ± 1.2	100.2 ± 1.7	85.3 ± 6.2	44.0 ± 3.3**	67.8 ± 3.0*	110.3 ± 2.8

*$P < 0.05$; **$P < 0.005$.

Table 3 Changes in parameters of intracellularly recorded atrial action potentials of chronically-treated adult rabbits following acute addition of propranolol or practolol

Treatment group and drug added (μg/ml)		*Total no. of fibres*	*Resting potential (mV)*	*Action potential (mV)*	*Maximum rate of depolarization (V/s)*	*Action potential duration to*		
						Zero volts (ms)	*50% (ms)*	*90% (ms)*
Controls								
Propranolol	(0.2)	25	−2.5 ± 1.6	+1.0 ± 0.6	−9.3 ± 8.9	−0.2 ± 2.7	−5.9 ± 2.9	−2.3 ± 2.1
Propranolol	(0.4)	18	−0.4 ± 1.3	−0.4 ± 1.4	−14.9 ± 6.5*	−5.1 ± 3.5	−8.1 ± 5.2	+0.8 ± 1.8
Practolol	(1.0)	15	0.0 ± 4.4	+1.1 ± 1.4	+0.3 ± 6.1	−5.7 ± 4.3	−8.1 ± 1.7***	−3.9 ± 2.2
Practolol	(2.0)	21	+3.3 ± 2.0	+1.4 ± 1.1	+8.7 ± 9.0	−7.1 ± 3.9	−9.6 ± 1.0***	−6.2 ± 1.7***
Propranolol-treated								
Propranolol	(0.2)	48	+2.2 ± 1.8	+0.9 ± 0.9	−6.1 ± 7.3	−5.7 ± 2.1**	−8.1 ± 6.2	+2.6 ± 1.2
Propranolol	(0.4)	36	+0.2 ± 3.3	−1.2 ± 3.3	−7.9 ± 11.6	−5.6 ± 5.5	−2.5 ± 1.9	−0.6 ± 2.8
Practolol-treated								
Practolol	(1.0)	48	+1.3 ± 1.5	+0.4 ± 0.9	−2.7 ± 4.4	−8.3 ± 3.9*	−6.1 ± 2.2**	−1.1 ± 2.2
Practolol	(2.0)	43	+0.8 ± 1.6	+0.4 ± 1.1	−1.2 ± 4.3	−4.1 ± 2.3	−4.5 ± 2.6	−3.7 ± 3.7

The values represent the means ±s.e. of the differences in measurements made after acute exposure to the drugs from measurements previously made in drug-free solution.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

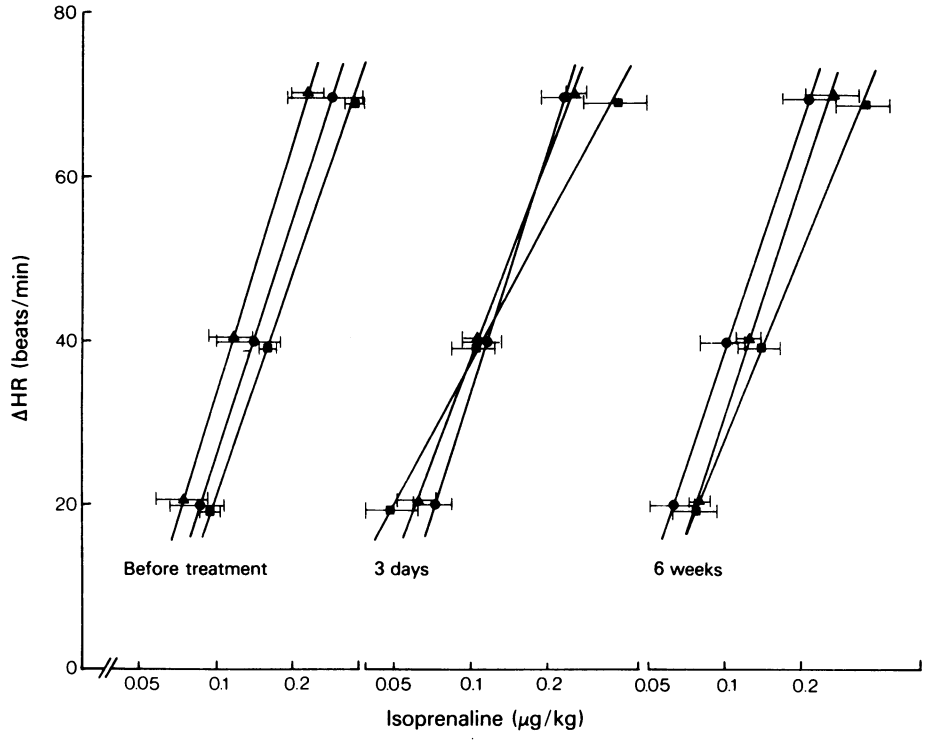

Figure 4 The effect of prolonged β-blockade on sensitivity to the chronotropic action of isoprenaline. Heart rate increases in response to intravenous injections of isoprenaline were measured not less than 15 h after the preceding dose of drug (or saline). Ordinate scale: increase in heart rate. Abscissa scale: dose of isoprenaline (μg/kg) on log scale. Prolonged treatment with propranolol (▲) or practolol (■) caused no change in sensitivity to isoprenaline; (●) = Control. Horizontal bars indicate s.e. mean.

Effects of prolonged β-blockade on the efficacy of acute blockade of the chronotropic responses to isoprenaline

When the measurements described in the preceding section had been completed, 10 mg/kg practolol was injected subcutaneously into the practolol-treated animals, and 2 mg/kg propranolol into the propranolol-treated animsls. The dose-response curves were then repeated, and exhibited the expected parallel shift to the right (Figure 5). After the acute injection of practolol, the dose-ratio (13) observed at 6 weeks was slightly (but not significantly, $P = 0.20$) different from that obtained after 3 days (8). In the propranolol-treated animals, however, the dose-ratio (31) at 6 weeks was significantly ($P < 0.05$) smaller than that obtained at 3 days (71).

Effects of prolonged β-blockade on blood pressure

Before the dose-response curves for isoprenaline on heart rate described in the preceding two sections were obtained, blood pressure was recorded for 1.5 to 2 h. In addition, blood pressure was also recorded in these rabbits (second series) after 3 weeks of treatment, the measurements being made not less than 15 h after the previous injection of drug or saline. The results are presented in Figure 6.

Blood pressure was the same in all groups before treatment. In the saline-treated animals the mean systolic and diastolic blood pressures rose by 11 ($P < 0.01$) and 3 mmHg respectively during the 6 weeks. In the practolol group, the systolic pressure also rose by 11 mmHg ($P < 0.02$), but the diastolic pressure fell by 2 mm (NS from saline-treated controls). In the propranolol group the systolic pressure rose by only 4 mmHg (NS from initial value) and the diastolic pressure fell by 7 mmHg ($P < 0.01$). In this group the systolic pressure was significantly different from that observed in the controls both at 3 weeks ($P < 0.025$) and 6 weeks and the diastolic pressure was significantly different at 6 weeks ($P < 0.05$).

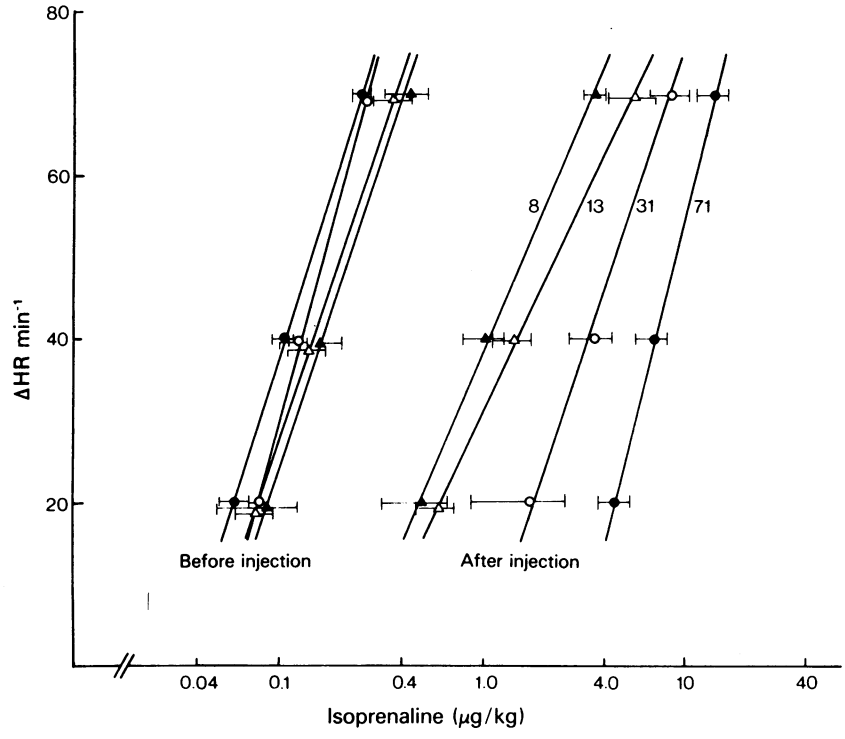

Figure 5 Effect of prolonged β-blockade on sensitivity to the acute β-blocking action of propranolol and practolol. Adult rabbits had been treated for 3 days (closed symbols) or for six weeks (open symbols) with twice daily subcutaneous injections of either 2 mg/kg of propranolol (circles) or of 10 mg/kg of practolol (triangles). Heart rate increases in response to intravenous injections of isoprenaline were measured not less than 15 h after the preceding injection of drug (left) and the measurements were then repeated 10 to 15 min after an acute subcutaneous injection of 10 mg/kg of practolol into the rabbits previously treated with practolol, or of 2 mg/kg of propranolol into those previously treated with propranolol. Ordinate scale: increase in heart rate. Abscissa scale: dose of isoprenaline (log scale). The numerals indicate the isoprenaline dose-ratios. Sensitivity to the acute blocking action of practolol was not significantly affected by 6 weeks pretreatment, but 6 weeks pretreatment with propranolol caused a significant reduction in sensitivity to the acute blocking action of propranolol. Horizontal bars indicate s.e. mean.

Discussion

The discovery that prolonged β-blockade in rabbits induced secondary adaptational changes in the electrophysiology and ultrastructure of cardiac muscle raised a number of questions, to some of which the work described above has provided answers. In young rabbits, of mean initial weight less than 1 kg and growing at about 30 g per day, twice daily doses of β-blockers in the clinical range, administered for 6 weeks, caused a reduction of heart weight in relation to body weight. The drug-treated hearts also contained more water than those of saline-treated litter mates. It has been found that in mature Dutch dwarf rabbits, treated with comparable doses of propranolol or practolol for 9 weeks, there was no change in heart weight relative to body weight, and the treated hearts had a water content similar to that of the controls.

β-Blockers are used to treat patients with hypertrophic cardiomyopathy (HOCM). Therapy is especially effective in infants and the obstruction may not recur on cessation of treatment (Shand, Sell & Oates, 1971). Östman-Smith (1976) found that the ventricular hypertrophy induced in rats by making them swim for several hours per day does not occur in rats treated with guanethidine (which causes degeneration of sympathetic nerve-endings). Further, ventricular hypertrophy in hypertensive rats was reversed by α-methyldopa, whereas a greater reduction of blood pressure by the vasodilator hydralazine did not cause any regression of the hypertrophy (Sen, Tarazi, Khairallah & Bumpus, 1974). It is possible, therefore, that in cardiac muscle not yet fully differentiated, sympathetic drive may be a necessary stimulus for hypertrophy to develop. Furthermore, the results of Sen *et al.* suggest that for the hypertrophy to regress, the sympathetic

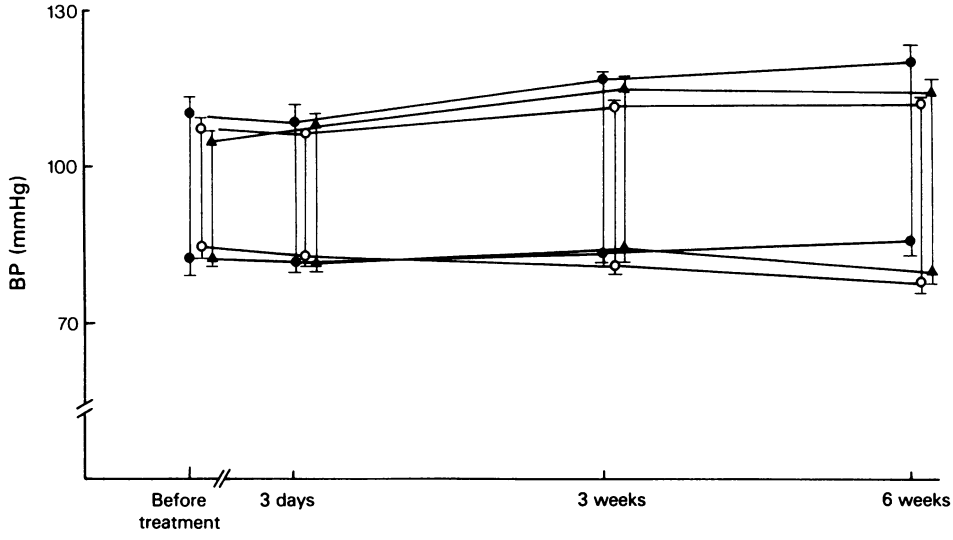

Figure 6 Effect of prolonged β-blockade on blood pressure in adult rabbits. Blood pressure was measured in conscious animals not less than 15 h after the preceding injection of drug (or saline). (●): saline-treated animals; (○): propranolol-treated; (▲): practolol-treated. During the 6 week period both diastolic and systolic pressures rose slightly in the saline-treated rabbits. In the treated animals the systolic pressure rose less, and the diastolic pressure fell slightly.

drive must be abolished or reduced, diminution of after-load alone being insufficient. This view is supported by our own finding that in adult rabbits prolonged β-blockade with a dosage regime adequate to induce secondary electrophysiological changes caused no change in heart weight, even though blood pressure was reduced. We were careful to employ doses which produced a degree of blockade not in excess of that achieved in man. It is possible, of course, that higher doses, or a longer period of treatment, might have induced secondary changes in heart weight in adult animals also. The point of importance here is that the electrophysiological changes were differentiated from the effects on heart weight in adult animals at the dosage level actually used.

In contrast to the absence of effect on heart weight, the electrophysiological adaptation to prolonged β-blockade, notably the extension of the plateau phase of the action potential, was even more marked in the adult rabbits than that previously observed in young rabbits. There was also a significant change in the phase of *de*polarization. Intracellular potentials were recorded in hearts taken from animals killed 24 h after the last dose of drug, so that no β-blocker remained in the muscle. In acute experiments on previously untreated animals, exposure to propranolol or practolol caused a shortening of APD by a few ms (Papp & Vaughan Williams, 1969). It was found that after prolonged β-blockade a similar shortening by a few ms from the more prolonged APD still occurred on acute exposure to propranolol or practolol.

The chronotropic responses to intravenous isoprenaline, recorded *in vivo* in conscious adult rabbits were unaffected by prolonged β-blockade, and this result is in accord with our previous evidence in young rabbits. Glaubiger & Lefkowitz (1977) found that chronic propranolol treatment in rats increases the number of cardiac β-adrenoceptors. If so, our results imply that this increase is not great enough to result in any pharmacologically demonstrable supersensitivity, either *in vivo* or *in vitro*. On the other hand, the blockade of the chronotropic response to isoprenaline produced *acutely* by an injection of propranolol was significantly less (dose-ratio = 31) after 6 weeks of propranolol treatment, than that produced by the same blocking dose after only 3 days of treatment (dose-ratio = 71). Sensitivity to the acute blocking action of practolol, however, was not reduced by 6 weeks treatment with practolol. The reason for this difference is not apparent, because no change in sensitivity to isoprenaline itself as observed *in vitro* and *in vivo*, had been induced by either drug. Nevertheless, it is of interest that in man the haemodynamic response to acute administration of propranolol is greatly diminished after long-term propranolol therapy (Brundin, Edhag & Lundman, 1976).

Both systolic and diastolic pressures rose slightly in the saline-treated adults during 6 weeks. In the propranolol-treated group, in contrast, there was a small but significant fall in mean diastolic pressure after 6 weeks. Farmer & Levy (1968) treated hypertensive dogs with oral propranolol 5 mg/kg daily for 4

weeks, and then with 5 mg/kg twice daily for a further 4 weeks, but no significant reduction in blood pressure was observed. However, it is not known for what proportion of each day such oral dosing would have maintained effective β-blockade. The dosage regime employed in the present experiments would have provided effective blockade for about half of each day, and it is of interest that a significant reduction of blood pressure in these normotensive rabbits was observed.

The results presented in this paper indicate that secondary adaptational changes to prolonged β-blockade occur in adult animals as well as in very young rabbits, and may, therefore, be of relevance to the clinical use of β-blockers. Patients treated for several weeks with β-blockers exhibited a prolongation of Q–T interval (Raine & Pickering, 1977). In matched control (untreated) patients, Q–T shortened from 0.38 to 0.27 during exercise (and QT_c from 0.42 to 0.40) but in patients on long-term β-blockade Q–T shortened from 0.40 to only 0.33 on exercise (and QT_c *lengthened* from 0.41 to 0.42). However, the heart-rates of the treated patients were lower than at the start of treatment, and it is uncertain whether the customary correction $QT(s)/\sqrt{RR(s)}$ (Bazett, 1920) is appropriate in these circumstances. Bertil Olsson has shown (personal communication) that right ventricular monophasic action potentials become longer in patients on long term β-blockade. A uniform prolongation of APD, by prolonging absolute refractory period, could have a 'class 3' anti-arrhythmic effect (Vaughan Williams, 1974), and the induction of a prolonged APD by long-term β-blockade may partly explain the disparity between the benefit of acute and long-term treatment with β-blockers after myocardial infarction (Raine & Vaughan Williams, 1978).

The single dose pharmacokinetic experiments which are described in this paper were undertaken to determine the plasma levels to be expected during subsequent prolonged dosing experiments, to ensure that doses were not being given which would lead to plasma levels in excess of those observed in man. The plasma levels were also measured after 5 weeks of dosing to ensure that similar levels were still being achieved during prolonged treatment in the adult animals used in this study. Coltart & Shand (1970) found, in acute experiments in man, that effective β-blockade was exerted at plasma propranolol concentrations in the range 10 to 100 ng/ml. More than 80% block of exercise tachycardia, and an isoprenaline dose-ratio of 30, was observed at a propranolol concentration of 100 ng/ml. The evidence of the present paper indicates that the relation between propranolol concentration and β-blockade in rabbits is the same as in man.

The parallel displacement to the right of the log dose-response curves to isoprenaline after propranolol (Figure 2a) confirm that throughout its duration of action, propranolol behaved as a competitive β-adrenoceptor antagonist. A similar competitive antagonism to isoprenaline after intravenous administration of propranolol has been demonstrated in man (McDevitt & Shand, 1975). However, there is no such simple relationship between propranolol concentration and degree of β-blockade after oral administration in man. This discrepancy may arise because after oral but not parenteral administration significant amounts of 4-hydroxypropranolol are formed by hepatic metabolism (Paterson, Conolly, Dollery, Hayes & Cooper, 1970). This metabolite is equipotent to propranolol (Fitzgerald & O'Donnell, 1971) but is not detected by the standard propranolol assay. Nevertheless it is unlikely that such metabolites are involved in the long term effects of β-blockade described here and in our previous papers. 4-Hydroxypropranolol has a shorter half life than propranolol (Paterson *et al.*, 1970) and in addition, propranolol has always been given by parenteral injection in our studies.

Adult Dutch dwarf rabbits, which had been treated for 5 weeks with propranolol 2 mg/kg twice daily were injected, 15 h after the previous dose, with 2 mg/kg propranolol subcutaneously and the plasma concentration of propranolol was estimated at intervals thereafter; 200 min after propranolol administration, mean plasma concentration was 46 ng/ml, which is comparable with the concentration of 55 ng/ml observed in young N.Z.W. rabbits, not previously treated, after a subcutaneous injection of 4 mg/kg. The half-life for propranolol in the chronically-treated adult animals was 1.4 h. Although this is a little shorter than the half-life in untreated N.Z. rabbits (1.8 h), the dosage in the Dutch dwarf rabbits was still sufficient to induce an adaptational prolongation of action potential duration. The comparable plasma levels in the two groups also indicate that there was no significant accumulation of propranolol during chronic treatment. During chronic oral administration in man there was an increase in the steady-state plasma levels of propranolol (Evans & Shand, 1973a, b), which was attributed to reduced hepatic extraction due to saturation of binding sites in the liver. However, there are no studies on plasma concentration during chronic parenteral administration in man.

It is clear from the above measurements of plasma levels, and from previous studies of the duration of functional blockade, that in the previously reported investigations on young New Zealand rabbits and in that on Dutch dwarf rabbits described here, twice daily subcutaneous injections of 2 mg/kg propranolol would have provided only intermittent β-blockade for up to half of each day. The short half-life of propranolol is due to its rapid hepatic metabolism (Shand,

Evans & Nies, 1971) but other β-blockers with longer plasma half-lives than propranolol would give a longer duration of blockade. Metoprolol for example has a half-life of 3.8 h in man (Bengtsson, Johnsson & Regårdh, 1975) while that of practolol is 10 h (Fitzgerald & Scales, 1968). In young rabbits practolol 10 mg/kg subcutaneously effectively blocked responses to isoprenaline for 9.25 h (Vaughan Williams *et al.*, 1975). Nevertheless, for propranolol at least it is clear that no significant concentration of drug remains in the body 24 h after a dose of 2 mg/kg subcutaneously. This is confirmed both by the time course of decline in plasma concentration (Figure 2b) and more directly by the absence of propranolol in ventricular muscle homogenates. Faulkner, Hopkins, Boerth, Young, Jellet, Nies, Bender & Shand (1973) and Coltart, Cayen, Stinson, Goldman, Davies & Harrison (1975) have also shown in man that no propranolol remains in the body 24 to 48 h after drug withdrawal.

To conclude, the first section of this paper has served to show that the relation between plasma propranolol concentration and cardiac blockade is the same in rabbits as in man, and demonstrates that the doses used in previous studies on young rabbits (Vaughan Williams *et al.*, 1975; 1977) and in the present investigation on adult rabbits do not give rise to plasma concentrations in excess of those used for the treatment of angina pectoris and hypertension in man.

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