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EFFECT OF PROLONGED β-ADRENOCEPTOR BLOCKADE ON HEART WEIGHT AND ULTRASTRUCTURE IN YOUNG RABBITS

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1 Young rabbits were treated for 30 days or 6 weeks with propranolol or oxprenolol to provide dose-levels similar to those used clinically.

2 Relative to litter matched controls the treated rabbits exhibited a reduced rate of growth.

3 Morphometric studies showed that prolonged β-adrenoceptor blockade had no effect on the myofibrillar volume as a proportion of cell volume.

4 Prolonged β-blockade increased the proportion of the extracellular space occupied by vascular tissue.

Introduction

β-Adrenoceptor blocking drugs have been increasingly used during the past 15 years for the treatment of hypertension, of certain cardiac arrhythmias and of angina pectoris, yet their mode of action, especially in hypertension, is not fully understood. Several hypotheses have been advanced (cf Vaughan Williams, 1977 for review). Recently it has become clear that the effects of prolonged treatment with β-blocking agents differ markedly from their acute actions. For example, acutely, propranolol either does not alter, or may even shorten, the duration of the cardiac action potential (APD) (Morales-Aguilerá & Vaughan Williams, 1965), but after a few weeks' treatment with doses of β-blockers in the clinical range, APD is greatly lengthened in both atrium and ventricle (Vaughan Williams, Raine, Cabrera & Whyte, 1975). The Q-T interval of the ECG is prolonged in both animals and man (Raine & Pickering, 1977) and the prolongation persists for many days after cessation of treatment. Six weeks' treatment of very young rabbits with propranolol (2 mg/kg) or practolol (10 mg/kg) twice daily caused a small reduction in the rate of body growth, but a much more pronounced reduction in the rate of growth of the heart (Vaughan Williams *et al.*, 1975). Furthermore, the treated hearts contained significantly more water (2.73 ± 0.09 g H_2O/g dry wt.) than hearts of control saline-treated littermates (2.36 ± 0.12 g H_2O/g dry wt.). Thus it was clear that prolonged interference with the sympathetic control of the heart led to profound and persistent secondary adaptive changes in the heart itself.

A number of investigations, physiological, biochemical and morphological, were undertaken to elucidate the nature of this adaptation. There was no evidence of any negative inotropic effect of prolonged β-blockade, nor was there any reduction in the inotropic or chronotropic responses to isoprenaline (Vaughan Williams *et al.*, 1975). In view of the significant increase in the water content of the treated hearts, it seemed probable that there would have been some detectable associated morphological changes, yet no abnormality of any cardiac organelles has been reported after treatment with much higher doses of β-blockers for very prolonged periods. However, a recent morphometric study, revealed that 6 weeks' treatment of young rabbits with clinical doses of propranolol or practolol caused a highly significant increase in extracellular vascular and connective tissue elements accompanied by a reduction of the organelle-free extracellular space (Vaughan Williams, Tasgal & Raine, 1977). This was of interest, because it implied that there would be a shorter pathway for the diffusion of oxygen from capillary to cell wall, which could be of significance in angina pectoris. However, the myofibrillar volume expressed as a proportion of intracellular volume, was identical in the treated and control hearts, which accorded with the absence of any negative inotropic effects of the prolonged treatment.

The present investigation has carried these studies a stage further and has been addressed in particular to the following question. The previous morphometric studies were done on very young animals (mean weight, 700 g at the start of treatment), and it was of interest to determine whether similar changes would be observed if treatment were started on larger rabbits.

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Methods

Effect of long-term β-adrenoceptor blockade on the hearts of young rabbits

Two sets of experiments were performed with the young rabbits. In the first series (Table 1) 50 to 56-day-old rabbits (male New Zealand Whites) were used and injections of propranolol or oxprenolol were continued for 30 days. In the second series, older rabbits were used (67 to 70 days, mean initial weight 1639 ± 31 g controls; and 1630 ± 28 g for propranolol-treated).

In the first series the individual groups contained litter mates from the litters of 14 does, and in the second series, from 9 does. The litters of rabbits were assigned to the individual groups so that every rabbit had at least one litter mate in each of the other groups. The control rabbits ($n = 45$) received 1 ml/kg of 0.9% w/v NaCl solution (saline) subcutaneously, twice daily. The others were given 1 ml/kg of saline containing 2.0 mg/kg of either propranolol ($n = 42$) or oxprenolol ($n = 15$) subcutaneously, twice daily for 30 or 42 days as shown in Table 1. This protocol was similar to that described by Vaughan Williams *et al.* (1975). The rabbits were fed *ad lib* SG IV standard diet and water and were weighed daily.

No drugs were given on the last day of the experiment. Each rabbit was stunned by a blow on the head. Its heart was rapidly removed, and rinsed with an ice-cold, modified Krebs-Henseleit buffer (1932) containing, (mM): NaCl 115.0, $NaHCO_3$ 25.0, KCl 4.0, KH_2PO_4 0.9, $MgSO_4$ 1.1, $CaCl_2$ 1.5 and glucose, 11.0, and equilibrated with 95% O_2 and 5% CO_2.

The hearts were blotted and weighed, and (1) perfusion-fixed for microscopy (Nayler, Grau & Slade, 1976) as described below; or (2) dried at 100°C to constant weight; or (3) perfused by the Langendorff technique as previously described (Nayler, Grau & Yepez, 1977) contractions being measured by a Grass FTO3 force displacement transducer attached to the ventricular apex, and displayed on a Devices recorder at 1 mm/s paper speed. The hearts were perfused at a pressure of 60 to 80 mmHg, maintained with a Watson Marlow roller pump. Some of the hearts (Table 1) were allowed to beat spontaneously; others were paced at 140/min as previously described (Nayler *et al.*, 1977).

Fixation and counting techniques

To determine whether prolonged β-adrenoceptor blockade alters the fine ultrastructure of heart muscle, hearts from control ($n = 6$) and propranolol-treated ($n = 7$, series 2) rabbits were perfusion-fixed by retrograde perfusion, with 4% glutaraldehyde prepared in 0.2 mol/litre sodium cacodylate buffer, pH 7.3. After 10 min perfusion, multiple ($n = 6$) 1 mm^3 biopsies of left ventricular wall and papillary muscle were taken from each heart and immersed for 2 h in 4% buffered glutaraldehyde, followed by two 10 min rinses in 0.1 mol/litre sodium cacodylate buffer, pH 7.3. After 2 h post-fixation in 1% osmium tetroxide prepared in 0.1 mol/litre sodium cacodylate buffer, pH 7.3, the specimens were dehydrated, rinsed in propylene oxide and embedded (Nayler *et al.*, 1976). Silver sections were cut on an LKB ultratome III, counterstained with lead citrate and examined in an AEI 801 electron-microscope. At the same time some of the perfusion-fixed material was prepared for light microscopy.

Prints of 100 transverse and 100 longitudinal sections were prepared from control and treated hearts, at a final magnification of 8,000. A 1.5 cm grid was superimposed on each print, and the organelle underlying each cross on the grid was identified by eye, the counting being assisted by computer as previously described (Vaughan Williams *et al.*, 1977); 54,000 points were counted per treatment.

Reagents and statistical analysis

Analytical reagent grade chemicals were used for the Krebs-Henseleit buffer solution, which were freshly prepared daily. Racemate propranolol was obtained from ICI, and oxprenolol from CIBA.

Results have been expressed as mean ± s.e. of n experiments and the statistical significance of differences calculated by Student's t test, or the (2×2) X^2 test for the morphometric data.

Results

Effect of β-adrenoceptor blockade on growth

The gain in weight of the three groups of rabbits is plotted in Figure 1. Both treated groups in the first series of experiments grew less rapidly than the controls, the difference becoming statistically significant from the 15th day onwards in the propranolol group, and from the 25th day onwards in the oxprenolol group. Over the whole 30 day period, mean growth rates were: controls, 53.2 ± 1.7 g/day; oxprenolol, 45.3 ± 1.6; propranolol, 44.2 ± 1.6 g/day. The statistical significance of both these differences was $P < 0.02$. In the second series of experiments, in which the animals were already older at the start of treatment, a statistically significant reduction in growth rate did not become apparent until the 33rd day of treatment onwards (Figure 1b). The mean rates of growth over the whole 42 day period were: controls 39.9 ± 1.8 g/day; propranolol 30.6 ± 1.2 g/day ($P < 0.001$). These effects of β-receptor blockade on growth are

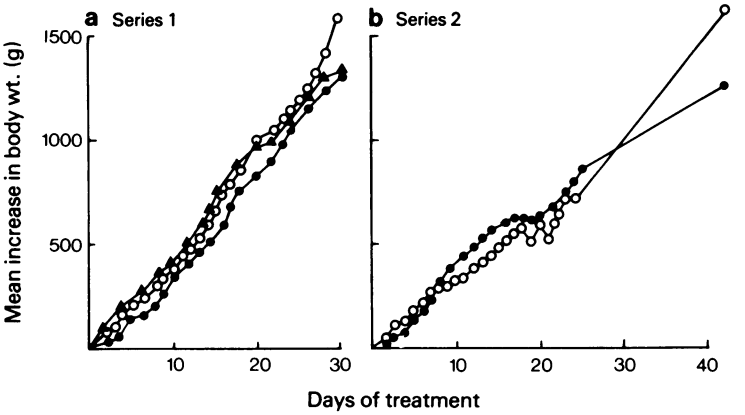

Figure 1 Growth of rabbits treated with β-adrenoceptor antagonists compared with that of litter mate controls. (a). Rabbits were 50 to 56 days old initially. Controls (1 ml/kg saline twice daily, ○); mean initial weight 894 g (n = 26); propranolol (2 mg/kg s.c. twice daily, ●) mean initial weight 867 g (n = 21); oxprenolol (2 mg/kg s.c. twice, daily, ▲) mean initial weight 863 g (n = 15). (b) Rabbits were 67 to 70 days old initially. Controls: mean initial weight 1452 g (n = 19); propranolol: mean initial weight 1303 g (n = 21). Dosage as in (a) Note that not all of these rabbits were used for the experiments described in Table 1; those that were used were selected on a random basis.

very similar to those previously described (Vaughan Williams *et al.*, 1975).

Heart weight/body weight

Table 1 shows that for both series of experiments, prolonged β-adrenoceptor blockade resulted in a significant ($P < 0.05$) reduction in the heart (wet) weight/body weight ratio. However, the reduction in the dry weights of the treated hearts was much more significant and when the water content of these hearts was determined and expressed in terms of g H_2O/g dry weight we found, as Vaughan Williams *et al.* described (1975), a significant increase in the water content (Table 1) of the hearts. This increase was most marked ($P < 0.001$) in the series 2 rabbits, i.e. in those that had been treated for 42 instead of only 30 days.

In vitro *spontaneous heart rate and peak systolic tension*

We found, as did Vaughan Williams *et al.* (1975), that the *in vitro* spontaneous heart rates of the preparations from the treated animals were significantly faster than the controls. The peak systolic tension of these spontaneously beating preparations was lower in the treated hearts than in controls. When the frequencies were equalised by pacing, however, (bottom line, Table 1), there was no evidence of any negative inotropic effect induced by the prolonged β-blockade, again in agreement with the previous evidence.

Ultrastructure

The electron and light micrographs shown in Figure 2 are representative of the fields seen for control and long-term (42 days) propranolol-treated hearts. The myofilaments remained in array and the fibres of normal dimensions. The cell membranes remained intact and the cytosol contained abundant glycogen. The morphometric results have been summarised in Table 2. In Table 2A, the counts have been expressed as percentages of the total area counted. In Table 2B the counts have been presented as percentages of the total count for the compartment, intracellular or extracellular, in which each organelle was contained. It is immediately apparent from Table 2A that the relative volume of the intracellular compartment was 60% in the saline-treated controls, but only 56.46% in the treated animals. From Table 2B it is apparent that the connective tissue and vascular compartments had expanded relatively at the expense of the organelle-free interfibrillar fluid and of the T-system. These changes are similar to those observed previously in the younger rabbits, and are discussed in detail below.

Discussion

It was previously reported that 6 weeks' treatment of young rabbits with doses of practolol or propranolol within the clinical range and producing plasma levels comparable with those observed in man, caused

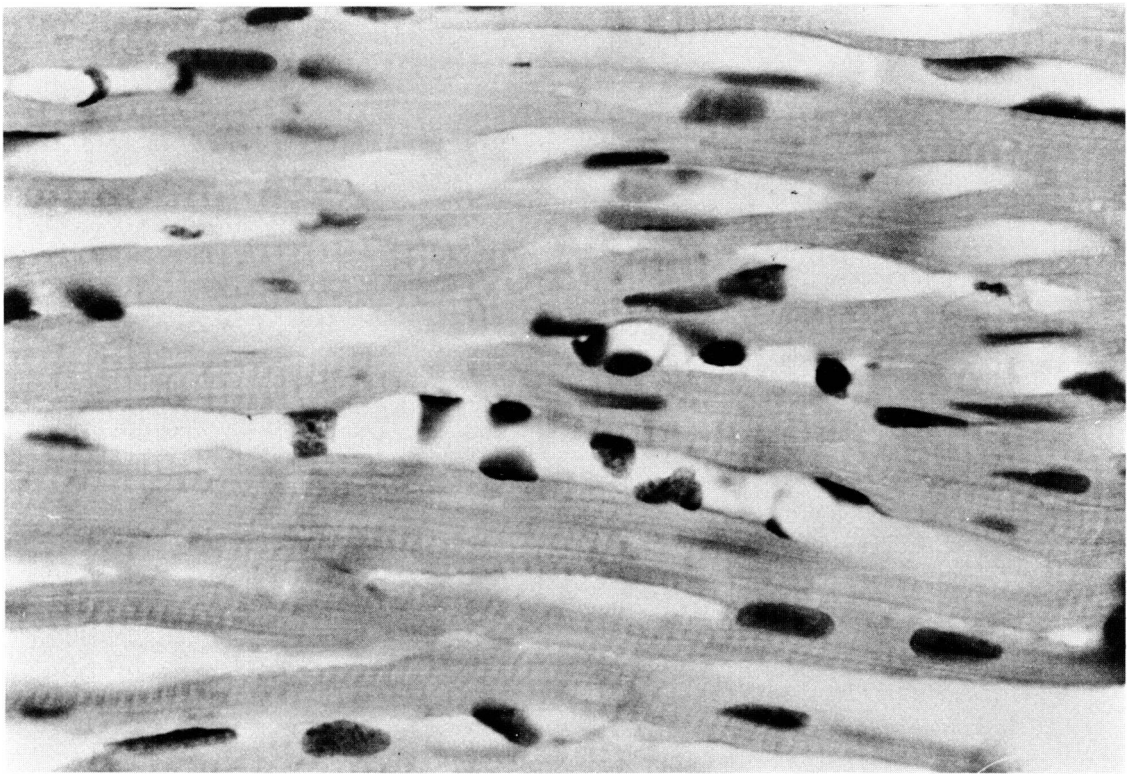

Figure 2(a) Magnification × 725.

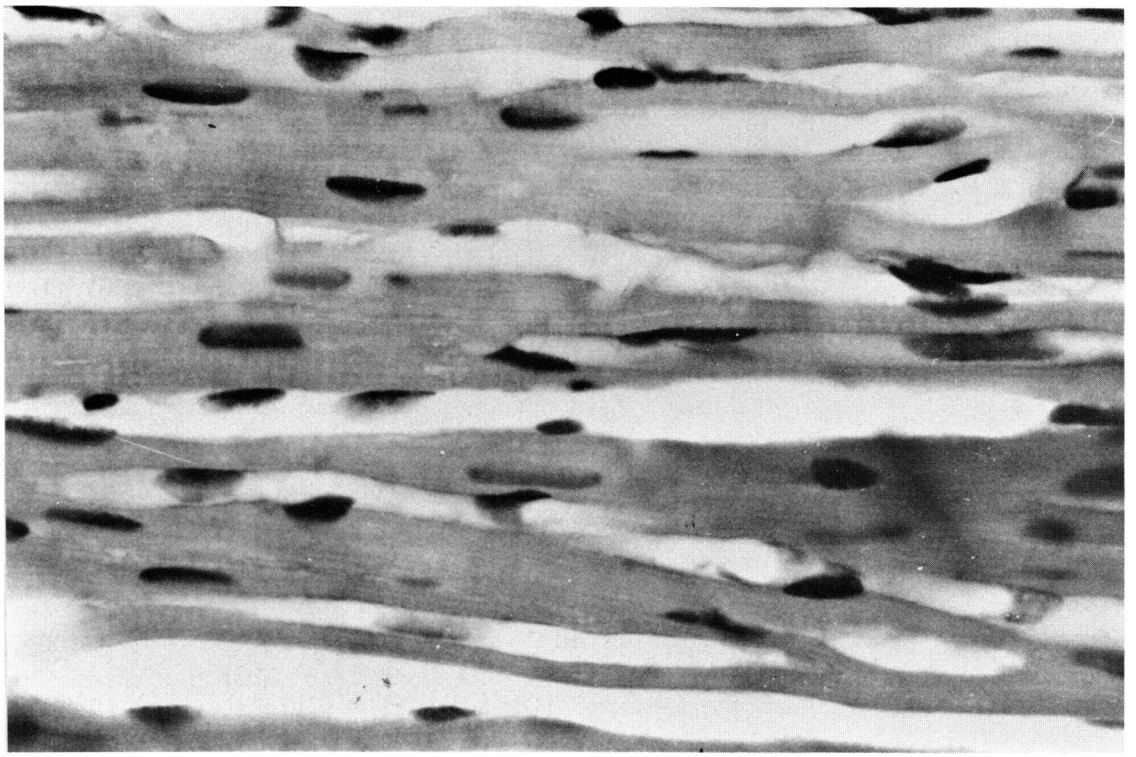

Figure 2(b) Magnification × 700.

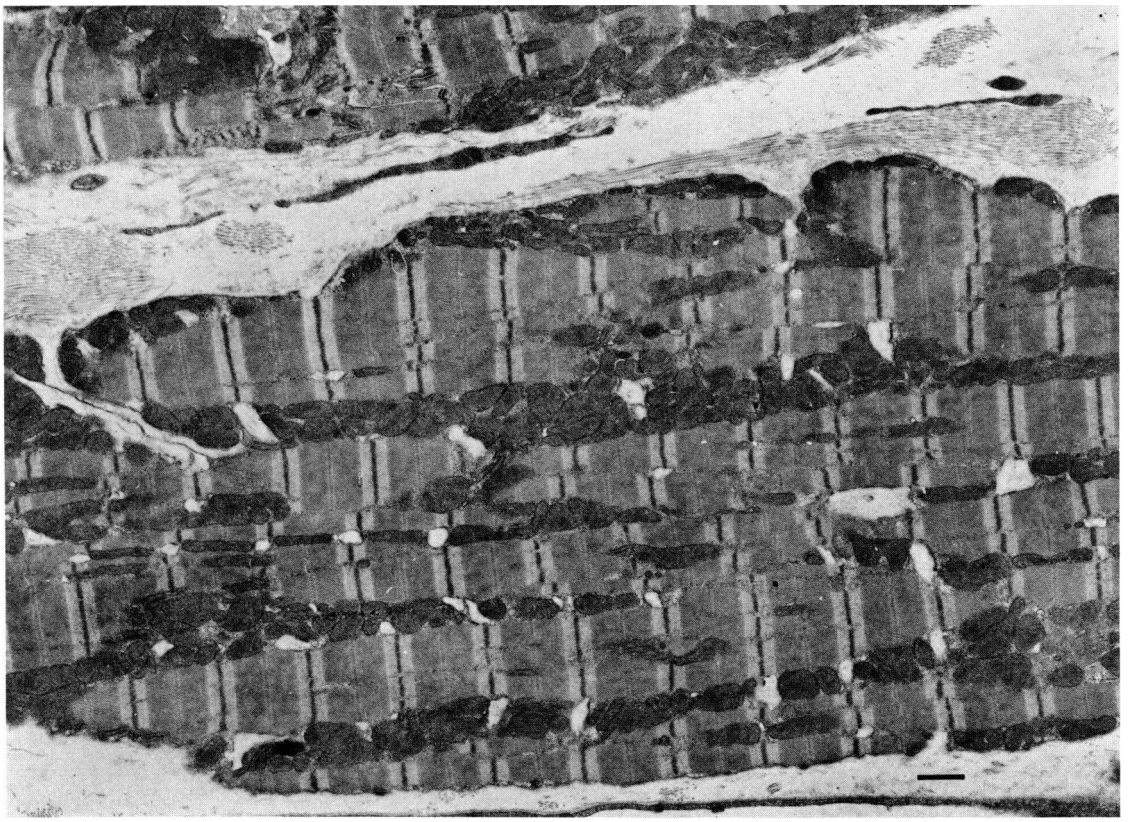

Figure 2(c) Magnification × 590.

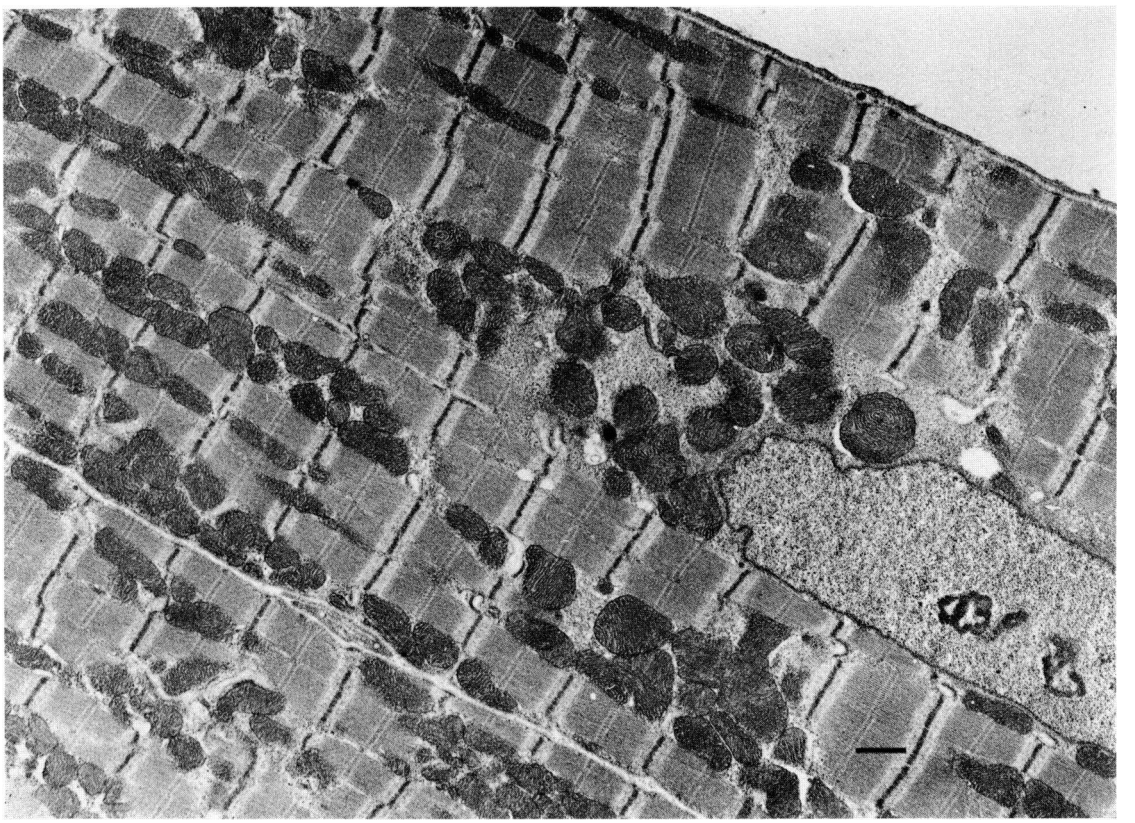

Figure 2(d) Magnification × 6080.

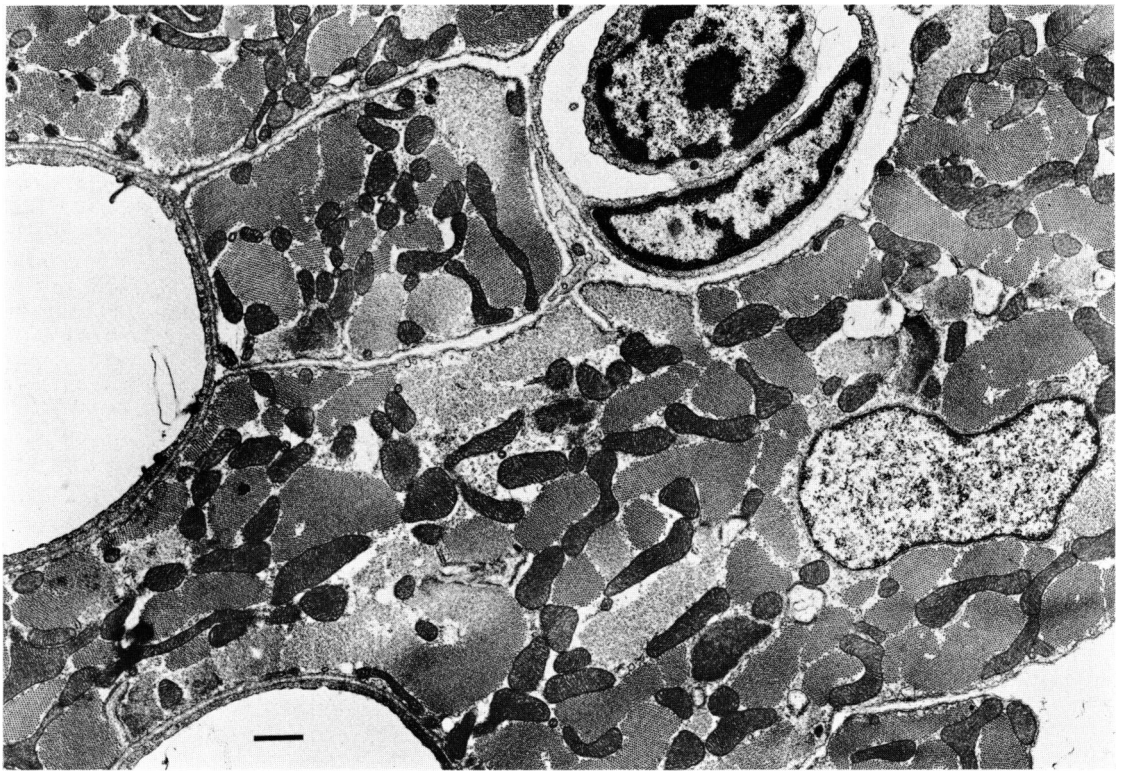

Figure 2(e) Magnification ×6240.

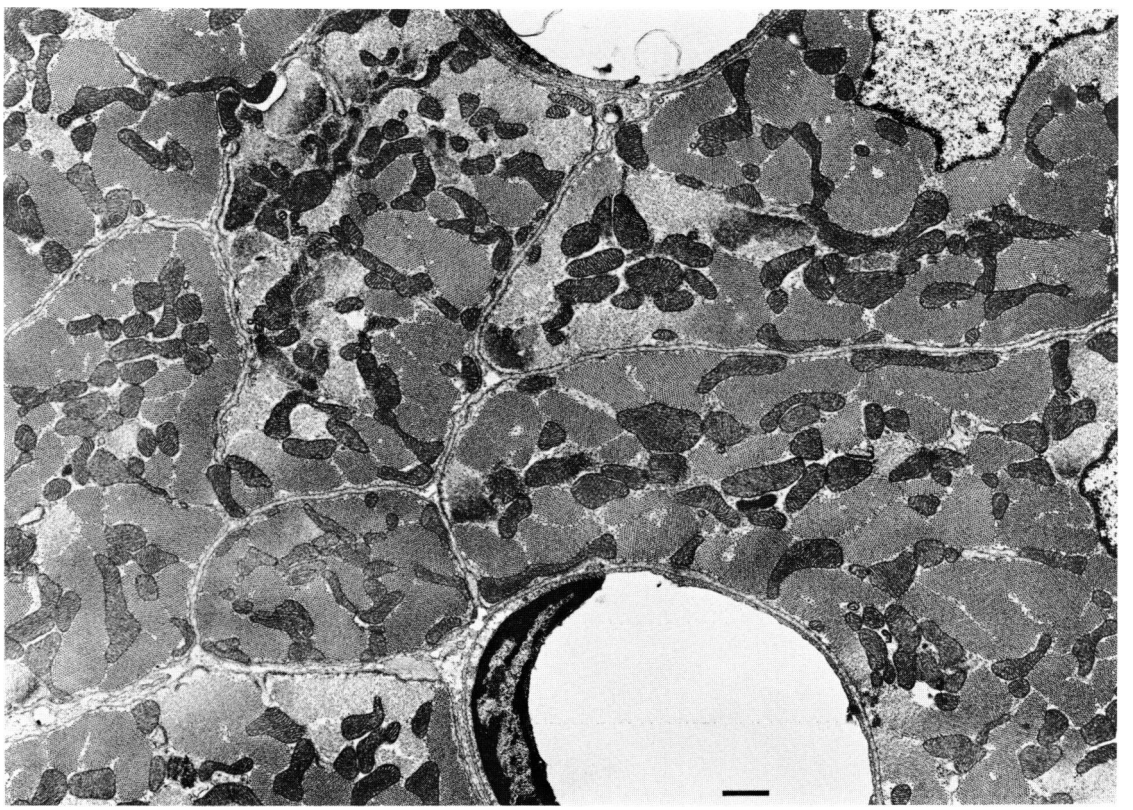

Figure 2(f) Magnification ×6240.

Figure 2 (a and b) Light micrographs of longitudinal sections of left ventricular free wall of hearts from (a) saline-treated control and (b) propranolol-treated rabbits. (c to f) Electronmicrographs of longitudinal (c, d) and transverse (e, f) sections from left ventricular free wall of hearts from saline-treated control (c and e) and propranolol-treated (d and f)-rabbits. Marker bar, 1 micron.

secondary effects which were distinct from the acute effects of β-blockade, and which persisted when the drug had been eliminated from the body (Vaughan Williams *et al.*, 1975; 1977). The main findings were bradycardia *in vivo*, tachycardia *in vitro*, a prolongation of QT in the *in vivo* ECG and of the duration of intracellularly recorded atrial and ventricular potentials *in vitro*, a reduction in dry weight of the hearts of the treated animals in relation to body weight, and an increase in the water content of the treated hearts. Also, the treated animals grew more slowly during treatment than their control litter mates, the difference becoming statistically significant from the fourth week onwards.

In the present investigation most of the earlier experiments of Vaughan Williams *et al.* (1975; 1977) (except for electrophysiological measurements) have been repeated and additional investigations have been made. It was confirmed that subcutaneous injection of young rabbits with 2 mg/kg twice daily of propranolol or oxprenolol significantly reduced the rate of growth. The dry weights of the hearts of the treated animals, killed 24 h after the last dose of drug, were smaller than those of the control litter-mates and the water contents were higher. It was confirmed that the frequency of spontaneously beating, treated hearts *in vitro*, was significantly higher than that of controls, and that when the heart rates were equalised by pacing, there was no evidence of any negative inotropic effect induced by the prolonged β-blockade. In confirmation of previous results it was also found that, *in vivo*, prolonged β-blockade induced a significant bradycardia.

Even in the absence of any autonomic influences, the sinus node has a 'basal' rate, which is modified in the long-term by factors such as 'training' and thyroid state. Deviations from this basal rate are produced in the short term by the accelerator of the sympathetic or the brake of the vagus. Prolonged β-blockade reduces background sympathetic tone (Raine & Chubb, 1977) which may explain the lowered heart rate *in vivo*. However, as an adaptation to this lower sympathetic tone, the basal rate may go up a little after prolonged β-blockade, which would explain the faster sinus rate observed *in vitro* (i.e. in total absence of any central autonomic control).

Table 1 Effect of long-term β-adrenoceptor blockade on heart/body weight ratio, heart water content, spontaneous heart rate and contractile force

	Series 1			*Series 2*	
	Control	*Propranolol*	*Oxprenolol*	*Control*	*Propranolol*
Days of treatment	30	30	30	42	42
n	10	5	5	6	7
Age at start of expt. (days)	50 to 56	50 to 56	50 to 56	65 to 70	65 to 70
Body weight (g)	913 ± 18	919 ± 24	890 ± 22	1639 ± 31	1630 ± 28
Increase in body weight (g)	1595 ± 56	1325 ± 31	1360 ± 28	1676 ± 65	1228 ± 36
		$P < 0.002$	$P < 0.02$		$P < 0.001$
Heart (mg)	2.62 ± 0.08	2.49 ± 0.04	2.40 ± 0.09	2.99 ± 0.09	2.75 ± 0.06
Body (g)		$P < 0.05$	$P < 0.05$		$P < 0.05$
Water content of heart (W − D)/D	3.06 ± 0.06	3.21 ± 0.02	3.31 ± 0.08	2.63 ± 0.14	4.32 ± 0.02
		$P < 0.02$			$P < 0.001$
In vitro					
n	4	4	4	4	4
Spontaneous heart rate (beats/min)	126 ± 3	136 ± 2	142 ± 4	130 ± 7	140 ± 3
Peak systolic tension (g)					
(a) Spontaneous	15.2 ± 2.3	10.7 ± 1.8	10.3 ± 0.6	14.6 ± 0.7	10.7 ± 0.6
		$P < 0.01$	$P < 0.01$		$P < 0.01$
(b) Paced (140/min)	13.6 ± 1.0	14.7 ± 1.2	14.2 ± 0.9	16.6 ± 0.8	16.3 ± 0.5
		NS	NS		NS

Results are mean ± s.e. of *n* experiments. Tests of significance relate to the significance of the difference between the results obtained for the control (saline-treated) rabbits and those obtained for rabbits that had been treated with 2.0 mg/kg propranolol or oxprenolol, twice daily, for either 30 or 42 days. The drugs were given subcutaneously. W = wet wt.; D = dry wt. NS = not significant ($P > 0.05$).

Ultrastructure

The initial weights of the rabbits used here were twice as great as in the series described previously (Vaughan Williams *et al.*, 1977). There were also several differences between the techniques employed. The tissue was perfusion-fixed (instead of superfusion), and the magnification was 8000, instead of 3500. However, 200 pictures of 27 × 23 cm were counted, so that the total area sampled was 194,000 μm^2 per treatment (in comparison with 164,000 μm^2 previously). The higher magnification used made it possible to distinguish the T-system from other 'organelle-free' sarcoplasm. In spite of these differences the counts from the two independent studies are strikingly similar, and are commented upon in detail below.

Extracellular space The treated hearts in both the previous and present studies contained more water than the controls. Both of the morphometric studies have indicated that this expansion was located mainly in the connective tissue elements (+16.82% here, and +38.4% in the previous study) and vascular compartment (+3.34% ($P_{\bullet} < 0.002$) here and +43.8% previously) whereas the organelle-free intercellular compartment was actually reduced (−6.79% here, and −19.85% in the previous study). Thus the view that prolonged β-blockade leads to a reduction in the pathway for diffusion from capillary to cell-membrane has been confirmed, the changes being more marked in the younger animals.

Intracellular compartment In the previous study the myofibrillar counts, expressed as % of intracellular volume, were identical (difference = −0.18%; $P = 0.91$) and the present investigation gave the same result, (difference = −0.41%; $P = 0.57$). The physiological evidence from both studies showed that isolated cardiac muscle from treated animals, driven at the same frequency as muscle from saline-treated controls, contracted with no less force. Thus it would appear that prolonged β-blockade, at least in normal animals, causes no reduction of myofibrillar substance nor of contractile force.

Mitochondria and sarcoplasm In the previous study the mitochondrial volume relative to the total intracellular volume was reduced by −4.44%, and the 'sar-

Table 2 (A) Organelle counts expressed as a percentage of the total sample volume

		Control	*Propranolol*	*Difference*	X^2	*P*
Intracellular						
Myofibrils		33.53	31.43	−2.1	54.2	1.8×10^{-11}
Mitochondria		19.05	18.67	−0.38	2.6	0.11
Sarcoplasm		4.82	4.06	−0.76	36.3	1.7×10^{-7}
Nucleus		0.76	0.79	+0.03	0.15	0.7
T-system		1.81	1.51	−0.30	14.9	0.01
	Total	59.97	56.46	−3.51		
Extracellular						
Connective tissue		4.25	5.40	+1.15	78.2	9.1×10^{-17}
Vasculature		17.00	19.12	+2.12	81.5	1.8×10^{-17}
Interfibrillar fluid		18.54	18.81	+0.27	1.2	0.27
Artifacts		0.15	0.13	−0.02	0.32	0.57
	Total	39.94	43.46	+3.52		

(B) Organelles as % of intracellular or extracellular totals

Intracellular	*Control*	*Propranolol*	*Difference*	X^2	*P*	Δ%
Myofibrils	55.90	55.67	−0.23	0.32	0.57	−0.41
Mitochondria	31.70	33.07	+1.37	11.7	0.06	+4.09
Sarcoplasm	8.03	7.19	−0.84	15.2	0.009	−10.46
Nucleus	1.28	1.39	+0.11	1.5	0.22	+3.95
T-system	3.02	2.67	−1.81	6.6	0.01	−11.59
Extracellular						
Connective tissue	10.64	12.43	+1.79	37.0	1.2×10^{-7}	+16.82
Vasculature	42.57	43.99	+1.42	9.6	0.002	+3.34
Interfibrillar fluid	46.42	43.27	−3.15	47.0	7.0×10^{-10}	−6.79
Artifacts	0.37	0.31	−0.06	1.2	0.27	−16.2

coplasm' was increased by a corresponding amount (+4.53%) both changes being highly significant. However, in the present study, the mitochondrial volume was slightly increased, but the change was not statistically significant. Possibly the difference between the present and former results is related to the different fixative procedures that were used, perfusion fixation being used in the present studies and immersion fixation in the earlier study (Vaughan Williams *et al.*, 1977). Alternatively it may be that in the earlier experiments, due to the younger age of the animals or for some other reason, the β-blockade had restricted or delayed the conversion of some intercellular sarcoplasmic elements into formed mitochondria. The observation could be of interest in a study of the control of mitochondrial synthesis, and an investigation of the effects of prolonged β-blockade in young rabbits on mitochondrial enzymes is in progress.

In the present study the relative volume of the T-system (not distinguished from 'sarcoplasm' in the previous series) was slightly but significantly reduced. Since the fluid within the T-tubules is, strictly speaking, 'extracellular', this result is in agreement with the observation, in both studies, of a reduction in the organelle-free extracellular space.

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