Adaptation to Prolonged $\beta$-Blockade of Rabbit Atrial, Purkinje, and Ventricular Potentials, and of Papillary Muscle Contraction

Time-Course of Development of and Recovery from Adaptation

A.E.G. Raine and E.M. Vaughan Williams

SUMMARY Groups of littermate rabbits were treated for various periods up to 6 weeks with twice daily subcutaneous injections of saline, $\beta$-propranolol, DL-propranolol, or metoprolol, the latter two at doses equivalent to those used in clinical therapy. Investigations were made at a sufficient time (20–24 hours after the most recent dose) to ensure that the drugs would have been eliminated from the body, so that any observed changes would represent an adaptation to treatment, not effects due to the presence of the drugs. The ECG was recorded in vivo at regular intervals during treatment. After several days, Q-T, was prolonged by the $\beta$-blockers, reaching a peak effect at about 3 weeks from the start of treatment, and returned to control values at 3 weeks after cessation of treatment. Action potential duration, measured in vitro by intracellular recording, was also prolonged uniformly in atria and ventricles over a similar time-course, unrelated to cardiac frequency, but shortened in distal Purkinje cells. Peak tension was not altered in propranolol-adapted papillary muscles, but the relationship of rate of rise of tension to peak tension was steeper. It is concluded that these effects represent a myocardial adaptation to prolonged $\beta$-blockade.


DESPITE the widespread clinical use of $\beta$-adrenergic antagonists, many questions about their mode of action remain unanswered. It is often difficult to correlate their therapeutic action with acute $\beta$-receptor blockade, and this has led to suggestions that factors other than $\beta$-adrenergic antagonism may be important (Koch-Weser, 1975). An alternative possibility is that part of the beneficial effects of $\beta$-blockade may be slow in onset, developing after the first week or two of treatment. It is well known, for example, that the fall in vascular resistance which may accompany and partly account for the hypotensive effect of propranolol is delayed. Investigations in animals of the cellular effects of prolonged $\beta$-blockade were begun several years ago, and it was shown that treatment of young rabbits for several weeks with $\beta$-adrenoceptor blocking drugs at doses comparable with those used clinically had a number of secondary effects which could be distinguished from the acute actions of the drugs (Vaughan Williams et al., 1975; Raine and Vaughan Williams, 1980). Atrial transmembrane action potential duration was greatly prolonged, a surprising finding since, in acute experiments, $\beta$-blockers either have no effect on action potential duration or shorten it, in both atrial (Papp and Vaughan Williams, 1969) and ventricular muscle (Davis and Temte, 1968). Chronic treatment of young rabbits with $\beta$-blockers also caused a reduction of heart weight in relation to body weight and produced some morphological changes, notably an increase in the relative volume of vascular elements and interfibrillar fluid (Vaughan Williams et al., 1977).

In the light of this evidence that prolonged $\beta$-blockade might produce adaptational changes within cardiac muscle, we have now studied changes in action potential duration (APD) and in contractility during chronic treatment in atrial, ventricular and Purkinje tissue. The time course of development of these changes and of their regression after propranolol withdrawal has been investigated, and has been found to parallel the time course of reduction in biochemical indices of sympathetic activity during long-term $\beta$-blockade, which we have also previously reported (Raine and Chubb, 1977).

Methods

Littermate rabbits of either sex weighing 1.5–2.0 kg were injected twice daily subcutaneously with equal volumes of DL-propranolol, 4 mg/kg ($n = 10$; $\beta$-propranolol, 4 mg/kg ($n = 4$); metoprolol, 6 mg/
kg (n = 6); or saline, 1 ml/kg (n = 6), for a total of 24 days. To monitor electrophysiological changes in vivo, electrocardiograms were recorded by limb leads (fine needle electrodes) with a Devices AC1 preamplifier and DC5 pen recorder at a paper speed of 100 mm/sec. All electrocardiograms were recorded 20 hours after the previous dose of drug, by which time negligible amounts would remain in the body, so that any observed changes could be attributed to a secondary adaptation to the prolonged blockade and not to the blockade itself. Recordings were made before treatment began, and on days 5, 9, 15, and 22 of treatment. RR, PR, QRS, and QT intervals were measured and QTc derived from the equation, QTc = QT(sec)/√RR (sec).

Twenty-four hours after the final dose, the animals were injected with 100 units of heparin iv, stunned, and their hearts were removed rapidly. The superior cervical ganglia were excised for separate biochemical analysis of tyrosine hydroxylase and dopamine-β-hydroxylase activity, as an index of sympathetic function (Thoenen, 1972). The atria were suspended horizontally in modified Locke’s solution gassed with 95% O2 and 5% CO2 at 32°C intervals were measured and QTc derived from the equation, QTc = QTc/sec = √RR (sec).

Measurement of Contractions

Papillary muscles were suspended horizontally in the tissue bath as described above. The base of the muscle was fixed and the free end connected to an RCA 5734 transducer mounted on a millimeter vernier. Developed tension (T) and dT/dt were displayed on a Tektronix D13 oscilloscope, and length-tension curves obtained by increasing the length in steps by adjustment of the vernier to Lmax, the length at which developed tension was maximal. Time-to-peak tension was also measured.

Results

Electrophysiological Effects of Chronic Treatment with DL-Propranolol, D-Propranolol, and Metoprolol

Groups of 4–6 rabbits were dosed for 24 days as described. Twenty-four hours after the final injection, lack of residual β-blocking effect was confirmed functionally by the absence of any reduction in the chronotropic response to isoproterenol measured by ECG recording in vivo. In previous experiments it had been shown that, at this time, samples of plasma and of ventricular tissue contained no measurable concentration of propranolol (Raine and Vaughan Williams, 1980). Although there was no remaining acute drug effect, there were marked changes in the transmembrane action potentials recorded in vitro from cardiac muscle after chronic treatment. Similar effects on the action potential were seen in both atrial and ventricular muscle and these are illustrated in Figure 1. In each experiment records were obtained from 7–10 different areas and the results for each treatment group were pooled and analyzed statistically (Table 1). The notable feature is that 24 days' treatment with both propranolol (which is nonselective) and metoprolol (which is selective for β1-receptors) caused a consistent (P < 0.001) prolongation of action potential duration, in both atria (+23%) and ventricles...
VENTRICLE ATRIUM

FIGURE 1 Intracellular potentials from the right atrium and right ventricular papillary muscle of rabbits killed 24 hours after prolonged treatment with saline, racemic propranolol, dextro-propranolol, or metoprolol. In each panel the horizontal trace gives the zero potential, the second trace gives the intracellular record, and the bottom trace the differentiated potential (dV/dt). In the atrial preparations contractions also were recorded.

Effect of Frequency of Stimulation on APD

Cardiac action potential duration is frequency-dependent (Hoffman and Suckling, 1964), and drugs which produce bradycardia would also prolong APD. To measure the extent to which changes in heart rate would influence repolarization, papillary muscle cells were impaled and the stimulus cycle length was decreased from 2000 to 200 msec during a continuous impalement. As shown in Figure 2, action potential duration shortened only at cycle lengths less than 500 msec from both control and treated animals. However, the 50 msec prolongation of APD, induced as an adaptation to prolonged β-blockade, was maintained at all frequencies.

Changes in APD in the Ventricular Conducting System after Chronic β-Blockade

Action potentials were recorded at intervals along the length of the right ventricular conducting system dissected from hearts of four rabbits treated with propranolol 2 mg/kg b.d. sc, for 6 weeks, and six controls given saline. The His bundle was stimulated at a frequency of 1 Hz. The resting potential and action potential amplitudes were not altered by propranolol treatment, but there were striking effects on APD. Normally, APD in the conducting tissue lengthens progressively from the bundle of His as far as the most distal false tendons, then shortens abruptly by 100 msec or more as ventricular muscle is reached (Myerburg et al., 1970). The controls showed this pattern (Fig. 3). In contrast, in the propranolol-treated rabbits, action potential duration was much more uniform (+25%). In contrast, cardiac action potential duration of animals treated with dextropropranolol was similar to that of controls. Resting membrane potential and action potential amplitude were unchanged in all the groups, and there were negligible differences in the maximum rate of depolarization (MRD).

D-propranolol, which has the same local anesthetic activity as dl-propranolol, but only 1/10 the β-blocking potency, did not alter action potential duration. The cardioselective drug metoprolol, and the non-selective propranolol, caused highly significant prolongations (P < 0.001) in APD_{at}, APD_{va}, and APD_{wm}.

TABLE 1 Repolarization Times (msec) from Peak to 20%, 50%, and 90%

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Atrium</th>
<th>Ventricle</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. of fibers</td>
<td>No. of fibers</td>
</tr>
<tr>
<td></td>
<td>APD_{20}</td>
<td>APD_{50}</td>
</tr>
<tr>
<td>Saline 1 ml/kg b.d.</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>d-Propranolol 4 mg/kg b.d.</td>
<td>4</td>
<td>42</td>
</tr>
<tr>
<td>dL-Propranolol 4 mg/kg b.d.</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>Metoprolol 6 mg/kg b.d.</td>
<td>6</td>
<td>50</td>
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Control
Propranolol
Metoprolol

APD
Cycle length (msec)

FIGURE 2
Frequency-dependence of action potential duration. Ordinate: APD90, msec. Abscissa: stimulation cycle length, msec. Intracellular records made on papillary muscles taken from animals treated for 24 days, and killed 24 hours after the final dose. The controls were saline-treated littermates of the rabbits given racemic propranolol, 4 mg/kg; or metoprolol, 6 mg/kg (sc b.d.).

throughout the conducting system; the APD of the ventricular free wall was 50 msec longer than in the controls [as already demonstrated in the isolated papillary muscle (Table 1)]. Similarly, at the proximal end of the conducting system, APD was also longer in the bundle of His taken from treated rabbits, than in the controls. In Purkinje cells in the central region, however, APD was shorter than in the controls.

Time Course of Adaptation to Prolonged β-Blockade

A prolongation of ventricular APD, provided it is uniform and unaccompanied by changes of conduction pathway, should be detectable as a prolongation of Q-T interval in the electrocardiogram. ECG records were made on a number of rabbits, which were then separated into four groups on the basis of Q-Tc calculation, so that the mean Q-Tc was approximately the same in each group. They were injected (sc) twice daily for 24 days with saline, 1ml/kg (n = 6); D-propranolol, 4 mg/kg (4); DL-propranolol, 4 mg/kg (10); or metoprolol, 6 mg/kg (6). ECG records were made on the 5th, 9th, 15th, and 22nd day after the start of treatment, 20 hours after the previous injection. There was no significant change in the P-R and R-R intervals, confirming the absence of acute blockade at the time of measurement, or of any adaptational bradycardia. QRS width was also unaltered, but in the groups receiving propranolol and metoprolol, Q-T interval was lengthened significantly (P < 0.05) on the 9th, but not on the 5th day, and remained at the prolonged level (Fig. 4) on the 15th and 22nd day (P < 0.005). A slight prolongation of Q-T on the 22nd day in the group on D-propranolol was not statistically significant.

Correlation between Q-T, APD, and Refractory Period

To establish whether the time-course of the adaptation to β-blockade observed in vivo as a prolongation of Q-T was paralleled by the prolongation of APD observed in vitro, four litters of rabbits were divided into groups and injected twice daily s.c. with 4 mg/kg DL-propranolol, and the animals were killed after 3, 6, 12, and 24 days of treatment, one rabbit from each of the litters being in each of the groups. The remaining six littermates were killed on day 0, to serve as controls. The ECG was recorded before the rabbits were killed, and the hearts were then removed for measurement in vitro of atrial and ventricular intracellular potentials and refractory period.

As depicted in Figure 5, the ventricular APD90 was actually reduced after 3 days (P < 0.01), but increased progressively thereafter, being 40 msec longer than controls after 24 days (P < 0.001). The Q-T changes paralleled those of ventricular APD. The effective refractory period was not significantly different after 3 days from that of control littermates killed at the start of the experiments, but increased progressively thereafter (+36 msec at 24 days, P < 0.001). Adaptation of APD in the atria was more rapid than in the ventricles, the prolongation being highly significant after 6 days of treatment, and lengthening by only a further few milliseconds thereafter.

Regression of Q-T Lengthening after Cessation of β-Blockade

Adaptive changes were defined as effects induced by treatment, and persisting after elimination from
the plasma and tissues of the drugs used (Raine and Vaughan Williams, 1980). To answer the question, therefore, for how long such changes would persist after cessation of therapy, a group of four rabbits was treated with propranolol, 4 mg/kg, for 28 days, and at this time the Q-Tc was prolonged by 0.041 above the value observed in saline-treated littermate controls (n = 2). Treatment was stopped, and it was found that 10 days later, the Q-Tc had not altered. Gradually, however, the Q-Tc shortened again, until, at 3 weeks after cessation of treatment, it was no longer significantly different from the controls (Fig. 6).

**Adaptive Changes in Contractions of Papillary Muscles after Prolonged β-Blockade**

Length-tension curves were obtained from isolated papillary muscles of approximately uniform width (1.5 mm) taken from rabbits treated with propranolol for 12 or 24 days, or with metoprolol for 24 days, or with saline for 24 days. The lengths...
obtained by stretching from the completely relaxed length have been expressed as a percentage of the range of lengths between that at which developed tension was maximal (Lmax), and that at which developed tension was just detectable (Lo). (% = 100 × (L - Lo)/(Lmax - Lo)). The peak tension developed at Lmax was similar (28–32 mg/mm) in all groups, and the complete length-tension curves were almost identical for the papillary muscles taken from saline-treated and drug-treated animals (Fig. 7). Thus there was no evidence of any negative inotropic effect after prolonged treatment, confirming our initial observations on atrial muscle; (Vaughan Williams et al., 1975); indeed, it was noted that treated muscles appeared to have a more rapid maximum rate of rise of tension (dT/dt max) than controls. This observation was examined in greater detail with isolated papillary muscles from 17 controls and 16 propranolol-treated animals. Peak tension at Lmax was similar in the two groups, but dT/dt max was greater and time-to-peak tension shorter in the treated group, although these differences did not attain statistical significance (Table 2). However, a significant linear correlation (P = 0.001) was demonstrated between peak tension and dT/dt max in each group (Fig. 8), and when the gradients of the regression lines were compared, that for the treated group was steeper (P = 0.01). This suggests that, in the propranolol-adapted papillary muscles, there was a more rapid rate of attainment of peak tension, particularly marked in the more strongly contracting preparations (Fig. 8).

Discussion

These studies have shown that in rabbits treated twice daily (sc) with doses of β-blockers equivalent to those used clinically (Raine and Vaughan Williams, 1980), action potential duration gradually increases as an adaptation to treatment during the first 3 weeks. The increase occurs uniformly in both atrial and ventricular muscle. In the ventricular conducting system, although APD is lengthened in the His bundle, it is shortened in preterminal Purkinje fibres, so that the usual disparity of APD in these regions is reduced. It may be concluded that the primary effect leading to these changes is repeated, though intermittent, blockade of cardiac β-adrenoceptors, since similar effects were produced both by propranolol, which blocks both β1 and β2 receptors, and by metoprolol which is selective for β1 receptors. APD was not prolonged by D-propranolol, which is 100 times less active than DL-propranolol as a β-blocker, but is equipotent with DL-propranolol in restricting fast inward current in cardiac muscle and as a local anaesthetic in nerve (Dohadwalla et al., 1969).

Prolonged treatment with β-blockers also caused a lengthening of Q-T interval in vivo, measured 20 hours after the previous injection of drug. The in vitro measurements of APD were made in animals

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Parameters of Contraction in Isolated Papillary Muscle</th>
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<tr>
<td>Treatment</td>
<td>No. of animals</td>
</tr>
<tr>
<td>Saline 1 ml/kg b.d.</td>
<td>17</td>
</tr>
<tr>
<td>DL- Propranolol 4 mg/kg b.d.</td>
<td>16</td>
</tr>
<tr>
<td>P value</td>
<td>0.818</td>
</tr>
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There was clearly no significant change in peak tension as a result of prolonged β-blockade, but there appeared to be a trend towards a shortening of time-to-peak, only just not significant.
controls (n=17). y=0.27x + 0.15. r=0.924
propranolol (n=16). y=0.40x + 0.04. r=0.963

Figure 8 Rate of rise of tension (dT/dt) as a function of peak tension at Lmax compared in separate papillary muscles from 16 propranolol-treated animals and 17 controls. Ordinate: maximum rate of rise of T g/sec x 0.4. Abscissa: peak tension, g x 10^-1. Treatment for 24 days with propranolol, 4 mg/kg, induced an adaptive increase in rate of rise of papillary muscle tension in comparison with saline-treated littermates. Linear regressions gave highly significantly slopes (P < 0.001) for both groups, and the slopes were significantly different from each other (P < 0.01).

Killed not less than 24 hours after the last dose of drug. The effects represented, therefore, an adaptation to the treatment, because negligible amounts of drug would have been present in the tissues at the time the observations were made (Raine and Vaughan Williams, 1980). The prolongation of Q-T and APD developed gradually after a period of a few days, becoming maximal at 3 weeks. Q-T remained prolonged for 10 days after cessation of treatment, and declined to control values at 3 weeks. In vitro APD was uniformly prolonged in comparison with controls over a wide range of stimulation frequencies and, in vivo, the prolonged Q-T was not associated with any adaptational bradycardia.

The prolongation of APD as an adaptational response to prolonged β-blockade contrasts with the effects of acute blockade, which either does not alter or shortens APD (Papp and Vaughan Williams, 1969; Davis and Temte, 1968). In the presence of propranolol, norepinephrine lengthens APD in guinea pig atria (Pappano, 1971) and in Purkinje fibers (Giott et al., 1973), presumably as a result of stimulation of α-adrenoceptors. The adaptational prolongation of APD observed in our experiments, however, was much greater than could have been accounted for by α-adrenergic stimulation (Rosen et al., 1977). Furthermore, it was shown previously (Raine and Chubb, 1977) that prolonged β-blockade reduced the activities of enzymes involved in norepinephrine synthesis, and the time-course of the development of that decrease has been shown in this study to parallel that of the adaptive changes in APD. In any complex control system, repeated interruption of the effector output at one point will also affect afferent input, and could lead to long-term readjustments, which might include a reduction of sympathetic efferent traffic (Lewis, 1976; Vaughan Williams, 1977).

The adaptational lengthening of APD after prolonged β-blockade involved primarily an increase in plateau height and duration, rather than of the "tail" of the action potential, and it may be associated with an increased rate of development of tension. One explanation for these changes might be an increased activation of slow inward current, since dT/dt is intimately associated with the release of intracellular calcium from the sarcoplasmic reticulum, which in turn is triggered by calcium entering the cell as slow inward current (Fabiato and Fabiato, 1977). It must be stressed that the relationship between activation of slow inward current, duration of the plateau, and activation of contraction is complex, and as yet poorly understood; increases in tension development may be associated with either lengthening or shortening of APD (Allen, 1977). Nevertheless, changes similar to those we report are produced by epinephrine, which increases plateau duration and contractile force, and shortens time to peak contraction (Beresiwicz and Reuter, 1977) and increases slow inward current (Vassort and Reuter, 1977). It may appear paradoxical to suggest that prolonged β-blockade produces effects similar to those of epinephrine. However, although voltage-dependent slow channels operate in the absence of adrenergic influence, slow inward current is normally augmented in vivo by β-receptor stimulation. One might speculate that prolonged β-blockade leads to a compensatory increase in the number of non-dependent channels relative to that of catecholamine-dependent channels, perhaps by inducing synthesis of new channels, which would
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account for the delay in onset of the adaptation. A similar delay is observed in the appearance of increased myocardial RNA content and protein synthesis in response to hypoxia or elevated work-load (Meerson, 1975). The biochemical basis for shortening of APD in hypoxia and its amelioration by glucose (McDonald and McLeod, 1973) is unknown, but it is of interest that APD in ischemia is correlated with intracellular glycogen content (Cowan and Vaughan Williams, 1980), and that myocardial glycogen content is greatly increased (+40%) by β-blockade (Smithen et al., 1975).

It is not clear why prolonged β-blockade caused shortening of APD in Purkinje cells, in contrast to ventricular muscle. Propranolol acutely shortens APD in Purkinje cells (Davis and Temte, 1968) but this acute effect could not explain our findings because no propranolol remained in the tissue at the time recordings were made. APD in Purkinje cells is inherently longer than in ventricular muscle, and is determined mainly by outward potassium currents, as slow inward current is of small amplitude (Noble, 1975). There are so many factors which could be involved in a change of APD that further experiments would be required to elucidate their individual contributions to the APD shortening in the preterminal Purkinje cells. The possibility that these cells are more susceptible to hypoxia could be considered; also, the activation of outward K+ current by a possibly increased inward Ca2+ current might be involved (Bassingthwaighte et al., 1976).

There are grounds for believing that the adaptational prolongation of APD reported here is not irrelevant to the use of β-blockers in humans. It has recently been shown that similar plasma propranolol concentrations block β-receptors to the same extent in rabbits and humans (McDevitt and Shand, 1975; Raine and Vaughan Williams, 1980) and long-term β-blockade causes a lengthening of human APD as measured with intracardiac suction electrodes (Edvardsson and Olsson, 1978). The Q-T is longer, especially in exercise, at a given rate than in pre-treatment controls (Vaughan Williams et al., 1980), and in comparison with matched patients not receiving β-blockers (Raine and Pickering, 1977). A prolongation of APD, provided that it is uniform and not associated with great heterogeneity within the myocardium such as occurs in the "long Q-T syndrome," should have an antiarrhythmic effect (Raine and Vaughan Williams, 1978; Vaughan Williams, 1980), as might also the increased uniformity of APD within the ventricular conducting system.

It has been noted that there is similarity between patients adapted to prolonged β-blockade (Brundin et al., 1976) and athletes at a high level of fitness, in that the usual hemodynamic effects of acute β-blockade are much attenuated. The implication is that in both groups excitation-contraction coupling currents are less dependent on adrenergically controlled slow channels. Abrupt cessation of β-blockade could add back the adrenergically controlled channels, to the now larger number of non-adrenergically dependent channels, and in patients with narrowed coronary arteries could provoke an excessive oxygen demand, i.e., the "rebound" phenomenon. Our own finding that the electrophysiological adaptation to prolonged β-blockade persists for 10 days, and that the Q-T interval returns to normal only after about 3 weeks, suggests that if β-blockade has to be discontinued in anginal patients, dosage should be reduced gradually during at least a similar period.

Acknowledgments

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