J. Physiol. (1984), **355**, 523–546
With 10 text-figures
Printed in Great Britain

EFFECTS OF SELECTIVE α_1-, α_2-, β_1- AND β_2-ADRENOCEPTOR STIMULATION ON POTENTIALS AND CONTRACTIONS IN THE RABBIT HEART

BY I. D. DUKES AND E. M. VAUGHAN WILLIAMS
From the University Department of Pharmacology, Oxford OX1 3QT

(*Received 19 March 1984*)

SUMMARY

1. Selective adrenoceptor agonists and antagonists have been used to analyse the effects of stimulation of individual types of adrenoceptor in various parts of the rabbit heart.

2. The selective α_1- and α_2-adrenoceptor agonists used were St 587 and BHT 933 respectively, and the antagonists were prazosin (α_1) and WY 25309 (α_2).

3. The selective β_1- and β_2-adrenoceptor antagonists were atenolol and ICI 118551, respectively. Pirbuterol was a highly selective β_2-adrenoceptor agonist. The non-selective agonists noradrenaline, adrenaline and isoprenaline were also employed with various combinations of antagonists.

4. Phenylephrine was found to stimulate β- as well as α-adrenoceptors. Rimiterol was a β-adrenoceptor agonist, partially selective for β_2-adrenoceptors.

5. In the sinus node β_1-, but not β_2-adrenoceptor stimulation increased the fast phase of depolarization ($\dot{V}_{max}$).

6. Both β_1- and β_2-adrenoceptor stimulation increased the slope of slow diastolic depolarization, accelerated repolarization and increased maximum diastolic potential.

7. After blockade of both β_1- and β_2-adrenoceptors α_1-adrenoceptor stimulation caused bradycardia, due exclusively to delayed repolarization. α_2-adrenoceptor stimulation had no effect.

8. In Purkinje cells and papillary muscle both β_1- and β_2-adrenoceptor stimulation accelerated repolarization. Stimulation of α_2-adrenoceptors had no effect.

9. β_1-, not β_2-adrenoceptor stimulation augmented peak contractions 3–5-fold, and greatly increased rate of development of tension. After β-blockade α_1-adrenoceptor stimulation moderately increased peak contractions (up to 47%), but increased time-to-peak and duration of contractions.

10. These patterns of adrenoceptor-mediated effects were unchanged in animals pre-treated with sufficient 6-hydroxydopamine to eliminate responses to sympathetic nerve stimulation.

11. The results would be consistent with β_1-, not β_2-adrenoceptor stimulation increasing inward calcium current, and with stimulation of α_1-adrenoceptors delaying its inactivation, rather than increasing its magnitude.

INTRODUCTION

In several previous studies of the effects of adrenergic stimulation of the heart (for example Hauswirth, Noble & Tsien, 1968; Brown, DiFrancesco & Noble, 1979), the adrenergic stimulant was adrenaline which activates all types of adrenoceptor. β-adrenoceptors were subdivided by Lands, Arnold, McAuliffe, Luduena & Brown (1967) into β_1-adrenoceptors mediating the cardiac effects of sympathomimetic drugs, and β_2-adrenoceptors mediating bronchodilator and vasodilator actions. On this basis, it was rational to employ 'cardioselective' drugs for the treatment of asthmatics who needed β-adrenoceptor blockade, but whether or not cardioselectivity is a desirable property in other respects (e.g. for the treatment of hypertension) is unproven. Prichard (1971) observed that in many patients it was impossible to block the tachycardia induced by isoprenaline or by the Valsalva manoeuvre or by exercise, with the β_1-adrenoceptor selective antagonist practolol, even when administered in very high doses, which suggested that the human heart may contain β_2-adrenoceptors. Recent studies with the non-selective ^{125}I-labelled cyanopindolol and various selective agonists and antagonists, (Brodde, Karad, Zerkowski, Rohm & Reidemeister, 1983; Heitz, Schwartz & Velly, 1983) indicated a mixed population of binding sites, the latter authors concluding 'that β_1- and β_2-adrenoceptors coexist in the left ventricle and left atrium of the human heart'. Binding studies do not establish the functional significance of the sites, but Wilson (1984) has reported a positive inotropic effect of β_2-adrenoceptor stimulation in excised human atrial appendage.

It is important to know how closely animal tissues used for research resemble their human counterparts. There is evidence that in rat hearts, β_1-adrenoceptors predominate (Bryan, Cole, O'Donnell & Wanstall, 1981), and that both β_1- and β_2-receptors coexist in cat hearts (Carlsson, Ablad, Branstrom & Carlsson, 1972), but conflicting conclusions have been reached about the existence of single or mixed populations in guinea-pigs (Johansson & Persson, 1983) and rabbits. Functional evidence was obtained for the mediation of increases in heart rate by β_2-adrenoceptors in conscious rabbits (Vaughan Williams, Raine, Cabrera & Whyte, 1975), and the presence of β_2-adrenoceptors has recently been demonstrated in rabbit atria by binding studies (Brodde, Leifert & Krehl, 1982). In contrast, Costin, O'Donnell & Wanstall (1983) maintained that rabbit atria contained β_1-adrenoceptors only.

Kass & Weigers (1982) reported that in calf Purkinje fibres, noradrenaline (which stimulates both α- and β-adrenoceptors) lengthened action potential duration (a.p.d.) at low concentrations, and shortened it at higher concentrations, but the possibility that different types of adrenoceptor might be involved was not investigated. Evidence for the existence and functional role of α-adrenoceptors in the heart was obtained many years ago (Govier, 1968; Giotti, Ledda & Mannaioni, 1973). More recently interest has been aroused in the possibility that stimulation of α-adrenoceptors may be involved in the genesis of cardiac arrhythmias in ischaemic and reperfused myocardium (Sheridan, Penkoske, Sobel & Corr, 1980).

Phenylephrine has often been employed as a selective α-adrenoceptor agonist, but it has been found to have, in guinea-pig atria, some action on β-receptors also (Reinhardt & Wagner, 1974), and we have found the same in rabbits (Dukes &

Vaughan Williams, 1984*a*). Methoxamine, another reputed α-adrenoceptor agonist, blocks β-receptors and is negatively inotropic (Blinks, 1964). Innemee, de Jonge, van Meel, Timmermans & van Zweiten (1981) described highly selective α_1- and α_2-adrenoceptor agonists St 587 and BHT 933 (Kobinger & Pichler, 1977), respectively. As for α-adrenoceptor antagonists, it is well known that prazosin is α_1-selective (Davey, 1980) and yohimbine α_2-selective (Langer, 1974). Yohimbine, however, has additional effects, including blockade of 5-hydroxytryptamine (5-HT) receptors. The compound WY 25309 is more selective than yohimbine for α_2-adrenoceptors, and lacks antagonist activity at 5-HT receptors (Lattimer, Rhodes, Ward, Waterfall & White, 1982; Pierce & Waterfall, 1982). We have, therefore, employed in addition to noradrenaline and phenylephrine, St 587 and BHT 933 as α_1- and α_2-adrenoceptor agonists, and prazosin and WY 25309 as selective α_1- and α_2-antagonists, respectively. Recently, some highly selective β_1- and β_2-adrenoceptor agonists and antagonists have also been made available, so that it has now become possible to undertake a systematic analysis of the effects mediated individually by the four types of adrenoceptor in different parts of the heart.

METHODS

New Zealand White rabbits of either sex weighing 800–1200 g were stunned and their hearts rapidly removed. For measurements of heart rate and contractions the atria were suspended in an isolated organ bath at 32 °C and contractions were recorded with a Dynamometer UPI transducer and displayed on a Devices chart recorder. For the experiments with micro-electrodes a portion of the right atrium containing the sino-atrial node was pinned to the Silastic base of an organ bath at 37 °C. Results were recorded on tape (Racal Store 4), and measured subsequently via analog-to-digital transient recorders by a computer program, as described elsewhere (Vaughan Williams, 1977; Dukes & Vaughan Williams, 1984*b*). Peaks and troughs of the digitalized wave forms were identified with a resolution of 0·4 mV and 0·25 ms.

Twelve more rabbits were pre-treated with an initial dose of 6-hydroxydopamine of 25 mg kg^{-1} I.P., followed by 50 mg kg^{-1} the next day and every 48 h thereafter. This regime has already been shown to abolish both the dilation of the pupil on stimulation of the cervical sympathetic and the bradycardic response to β-blockade (Vaughan Williams & Dukes, 1983) in anaesthetized rabbits.

The bath solution contained (mmol l^{-1}): NaCl, 125; KCl, 5·6; $NaHCO_3$, 25; Na_2HPO_4, 0·4; $MgCl_2$, 1·0; $CaCl_2$, 2·16; glucose, 11·0; and was equilibrated with 95% O_2 and 5% CO_2. Intracellular potentials were recorded from ventricular and Purkinje cells as previously described. The preparations were paced at 1·6 Hz.

Drugs were gifts from their manufacturers as follows: atenolol and ICI 118551 (β_1- and β_2-adrenoceptor selective antagonists respectively) from ICI; the selective β_2-adrenoceptor agonists, rimiterol and pirbuterol from Riker 3M and Pfizer, respectively; the α_1- and α_2-adrenoceptor agonists St 587 and BHT 933 respectively from Boehringer Ingelheim; the selective α_1-adrenoceptor antagonist prazosin from Pfizer, and the α_2-receptor antagonist WY 25309 from Wyeth. Other compounds were obtained commercially as follows: isoprenaline sulphate (Wellcome); L-noradrenaline, phenylephrine, and 6-hydroxydopamine (Sigma).

RESULTS

Sino-atrial node

Presence of β_2-adrenoceptors. Dose–response curves relating increases in heart rate to agonist concentration on a logarithmic scale for isoprenaline (non-selective β-agonist) and for the β_2-adrenoceptor agonists rimiterol (Cooke, Kerr, Willey,

Hoare, Grant & Crompton, 1974) and pirbuterol (Moore, Constantine & Barth, 1978) are shown in Fig. 1*A*, and for isoprenaline alone, and in the presence of the β_1-adrenoceptor selective antagonist atenolol, and the β_2-adrenoceptor selective antagonist ICI 118551 (Bilski, Halliday, Fitzgerald & Wale, 1983) in Fig. 1*B*. ICI 118551 shifted the isoprenaline dose–response curve to the right, implying that part

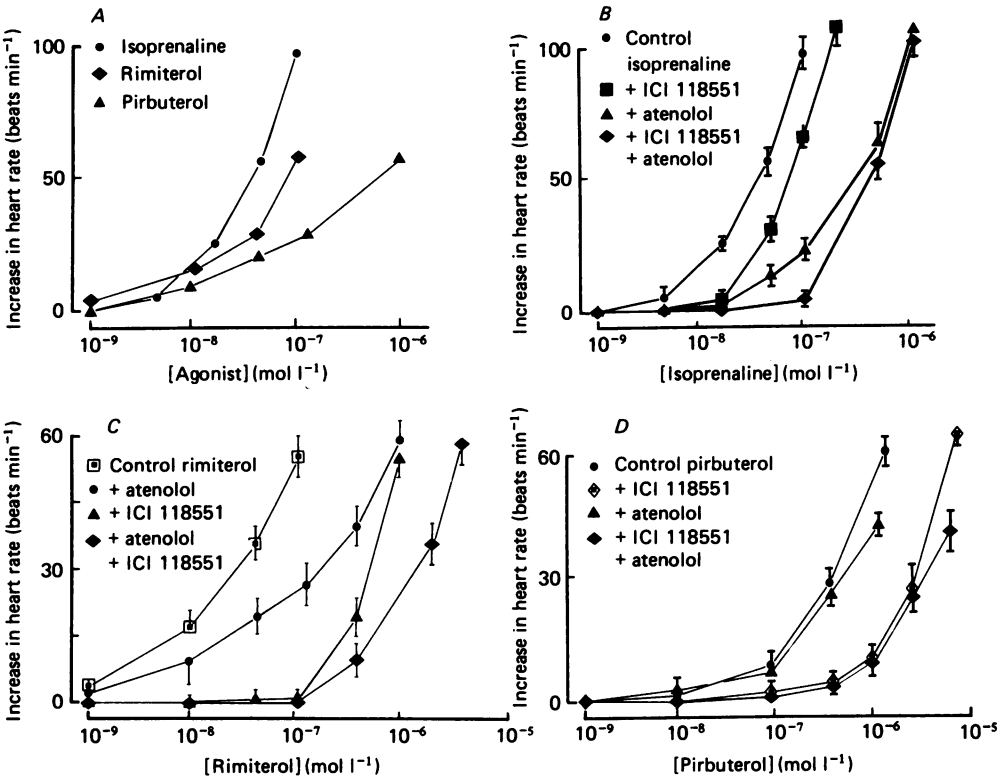

Fig. 1. Dose–response curves relating increases in heart rate and agonist concentration on a logarithmic scale. *A*, effect of isoprenaline (non-selective) and rimiterol and pirbuterol (β_2-adrenoreceptor selective). *B*, effect of increasing concentrations of isoprenaline, alone or in the presence of the selective β_1-adrenoceptor (atenolol, 10^{-6} mol l^{-1}) or β_2-adrenoceptor (ICI 118551, 10^{-6} mol l^{-1}) antagonists, or both. *C*, effect of increasing concentrations of rimiterol and *D*, pirbuterol, alone and in the presence of the selective β_1-adrenoceptor (atenolol, 10^{-6} mol l^{-1}) or β_2-adrenoceptor (ICI 118551, 10^{-6} mol l^{-1}) antagonists, or both.

of the response to isoprenaline alone was mediated by β_2-adrenoceptors, as is consistent with the responses to rimiterol and pirbuterol alone (Fig. 1*A*). The isoprenaline dose–response curve was not shifted to the right in a parallel fashion by atenolol alone but a combination of both β_1- and β_2-adrenoceptor blockade did so. This again would be consistent with the effects of isoprenaline at the lower concentrations in the presence of atenolol being due to the excitation of a number of unblocked β_2-adrenoceptors sufficient to mediate a submaximal effect.

The effects of rimiterol and pirbuterol, alone and in the presence of atenolol or ICI 118551 or both, are shown in Fig. 1*C* and *D* respectively. Selective β_1-adrenoceptor

blockade by atenolol had little effect on the dose–response curve for pirbuterol, and ICI 118551 caused a parallel shift to the right, indicating the high selectivity of pirbuterol for β_2-adrenoceptors. The Figure also demonstrates that β_2-adrenoceptors alone can mediate an increase in heart rate. In contrast, the non-parallel shift of the rimiterol dose–response curve by atenolol implies that rimiterol was only partially selective, stimulating both β_1- and β_2-adrenoceptors, like isoprenaline. Some selectivity for β_2-adrenoceptors, however, was indicated by the fact that ICI 118551 was more potent than atenolol with rimiterol as agonist (Fig. 1*C*), whereas the reverse was the case with isoprenaline as the agonist (Fig. 1*B*).

Differential electrophysiological effects of β_1- and β_2-adrenoceptor stimulation. The well known effects of adrenergic stimulation in the sino-atrial node of steepening the slope of the slow diastolic depolarization, increasing the maximum rate of depolarization ($\dot{V}_{max}$) of the action potential, and shortening the a.p.d. are illustrated in Fig. 2*A*. In the presence of atenolol the effect on $\dot{V}_{max}$ was abolished (Fig. 2*B*), but in spite of blockade of the β_1-adrenoceptors isoprenaline still caused a small increase in heart rate, and shortened the a.p.d. The effects of pirbuterol and rimiterol, illustrated in Fig. 2*C* and *E* respectively, confirm that selective β_2-adrenoceptor stimulation did not increase $\dot{V}_{max}$, but nevertheless augmented heart rate, partly by steepening the slope of diastolic depolarization, and partly by accelerating repolarization. Although the points at which the maximum diastolic potential was reached are not easy to detect from the superimposed records, the mean of measurements by computation from the digital recorders demonstrated a statistically significant acceleration of repolarization by β_2-adrenoceptor stimulation (Figs. 3*B* and 4*C*). Atenolol had no influence on the effects of pirbuterol (Fig. 2*D*) but ICI 118551 completely abolished them, as illustrated in Fig. 2*F*, in which the two superimposed action potentials are indistinguishable.

The effects of atenolol and ICI 118551 alone, and of a range of concentrations of the three agonists, isoprenaline, pirbuterol and rimiterol, both alone and in the presence of antagonists, have been measured on heart rate, peak action potential amplitude, maximum diastolic potential, take-off potential, a.p.d. and on $\dot{V}_{max}$ and rates of repolarization and diastolic depolarization. The important features extracted from this mass of information have been presented in Figs. 3 and 4.

It was apparent from Fig. 1*A* that pirbuterol, even at the highest concentration used, did not increase heart rate by much more than 50 beats min^{-1}. Comparison of Fig. 3*A* and *B* illustrates that at 10^{-8} and 10^{-7} mol l^{-1} concentrations of isoprenaline the shortening of repolarization time accounted for 80 % and 46 % respectively of the total reduction of peak-to-peak interval, and with pirbuterol, at concentrations of 10^{-7} and 10^{-6} mol l^{-1} acceleration of repolarization was responsible for 74 % and 68 % of the reductions in peak-to-peak intervals. After blockade of β_1-adrenoreceptors with atenolol, acceleration of repolarization now accounted for 60 % of the peak-to-peak interval reduction by isoprenaline at 10^{-8} mol l^{-1}, while at 10^{-7} mol l^{-1} the mean shortening of repolarization (−44·5 ms) actually exceeded the mean total shortening of the peak-to-peak interval (−40·4 ms). These findings indicate that both β_1- and β_2-adrenoceptor stimulation increase heart rate largely by accelerating repolarization as well as by a change in the slope of the slow diastolic depolarization. In addition, β_1- but not β_2-adrenoceptor stimulation, increases $\dot{V}_{max}$. As can be seen

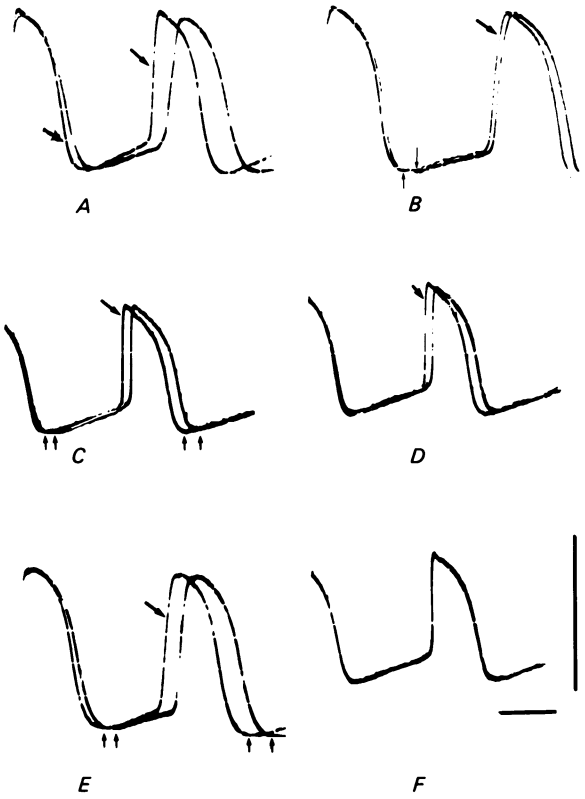

Fig. 2. Superimposed intracellularly recorded potentials from the sino-atrial node. *A*, *C* and *E*, effects respectively of isoprenaline (10^{-8} mol l^{-1}), rimiterol (10^{-7} mol l^{-1}) and pirbuterol (10^{-7} mol l^{-1}). The upper arrows indicate the tracings taken after exposure to the drugs, the other tracings being the pre-drug controls. The lower arrows are placed at the points of maximum diastolic potential, easily detected by computer but less easy to identify on the superimposed records. *B*, isoprenaline, in the presence of the β_1-adrenoceptor selective antagonist atenolol (10^{-6} mol l^{-1}), still caused some shortening of a.p.d. but no longer increased $\dot{V}_{max}$ of the action potential upstroke. *D* and *F*, effects of pirbuterol in the presence of atenolol and ICI 118551, respectively, at concentrations of 10^{-6} mol l^{-1}. In *F*, the control potential and that recorded in the presence of ICI 118551 and pirbuterol are superimposed. Thus the β_2-adrenoceptor selective compound, ICI 118551, abolished the effects of pirbuterol, but atenolol had no effect (*D*). Calibrations: vertical bar, 60 mV; horizontal bar, 100 ms.

from Fig. 1 *D* there was some minor stimulation of β_1-adrenoceptors by the highest concentrations of pirbuterol because atenolol, without effect at the lower concentrations of pirbuterol, had a small effect on responses at the highest concentrations. All the detailed results with pirbuterol in the presence of atenolol have not been presented, because the effects of atenolol were otherwise non-significant. The detailed results with rimiterol have likewise been omitted, since they resembled those obtained with pirbuterol.

The conclusion that β_1- but not β_2-adrenoceptor stimulation increased the currents responsible for the rapid phase of depolarization is reinforced by the evidence presented in Fig. 4 *A*, which indicates that the isoprenaline-induced increases of

overshoot potential were almost abolished by β_1-adrenoreceptor blockade, and that pirbuterol alone had little effect. Increases in $\dot{V}_{max}$ induced by isoprenaline were also abolished by β_1-adrenoceptor blockade, whereas pirbuterol alone had no significant effect on $\dot{V}_{max}$, except at the highest concentration, probably due to β_1-receptor stimulation because the effect was not abolished by ICI 118551 (Fig. 3*D*). The

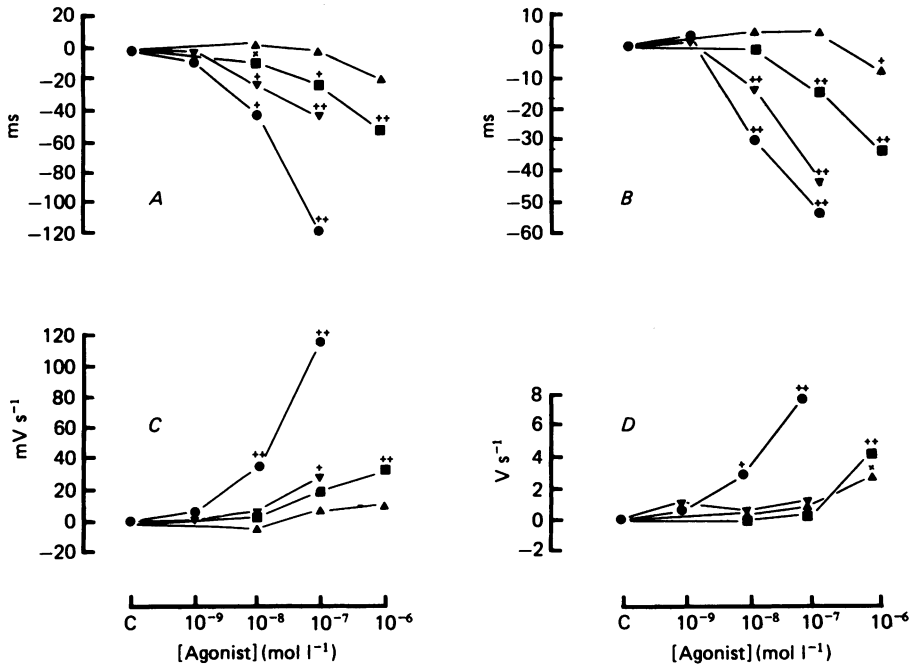

Fig. 3. Mean differences from controls of various electrophysiological measurements in the rabbit sino-atrial node. The significance of differences is indicated thus: ×, $P < 0{\cdot}05$; +, $P < 0{\cdot}01$; ++, $P < 0{\cdot}001$: bars showing S.E. of mean have been omitted for clarity. Ordinates: *A*, shortening of peak-to-peak interval between action potentials (ms). *B*, duration of repolarization from peak to maximum diastolic potential (ms). *C*, slope of the slow diastolic depolarization (mV s^{-1}). *D*, maximum rate of depolarization ($\dot{V}_{max}$) of action potential upstroke (V s^{-1}). All measurements returned to control values on wash-out of the agonists. ●, isoprenaline; ▼, isoprenaline in the presence of atenolol (10^{-6} mol l^{-1}); ■, pirbuterol; ▲, pirbuterol in the presence of ICI 118551 (10^{-6} mol l^{-1}). Abscissae: agonist concentrations on logarithmic scale (mol l^{-1}); C, pre-drug control.

β_2-selective antagonist ICI 118551 did not alter the effects of isoprenaline on overshoot potential or $\dot{V}_{max}$ (not shown). In contrast, both β_1- and β_2-adrenoceptor stimulation increased the slope of the *slow* diastolic depolarization (Fig. 3*C*) and accelerated repolarization (Fig. 4*C*). The latter effect may have been due to an increase in potassium permeability, because it was associated with a more negative maximum diastolic potential (Fig. 4*B*), as discussed later.

The take-off potential, measured as the point at which extrapolation forward of the slow diastolic depolarization intersected extrapolation backwards of the action potential upstroke, was made more negative by β_1-adrenoceptor stimulation only, because a dose-related effect of isoprenaline was abolished by atenolol (Fig. 4*D*) but

not by ICI 118551. Pirbuterol again had no significant effect, except at the highest concentration used.

Presence of α-adrenoceptors. Phenylephrine, at concentrations of 10^{-5} mol l^{-1} and above, induced a dose-related tachycardia in isolated rabbit atria, but, as can be seen from Fig. 5*A*, this was caused by stimulation of β-adrenoceptors because in the

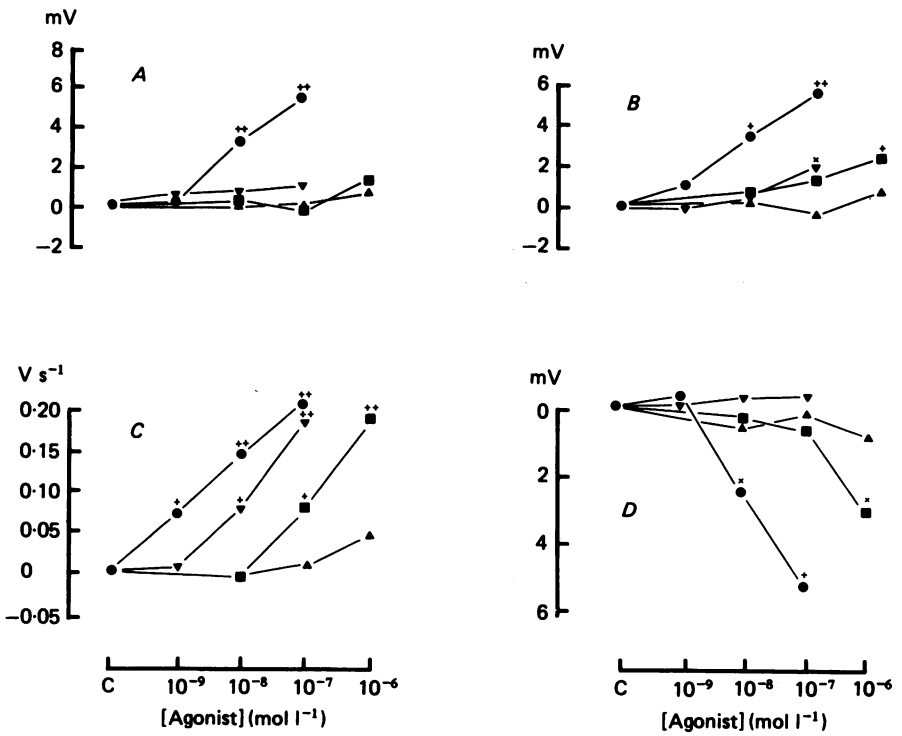

Fig. 4. Further differences from control of electrophysiological measurements in the sino-atrial node. Symbols as for Fig. 3. Ordinates: *A*, peak action potential amplitude (overshoot) (mV); *B*, maximum diastolic potential (mV); *C*, mean rate of repolarization (V s^{-1}); *D*, take-off potential of action potential upstroke (mV). Abscissae: as for Fig. 3.

presence of propranolol, which blocks both β_1- and β_2-adrenoceptors, phenylephrine induced a bradycardia at the much lower concentration of 2×10^{-7} mol l^{-1} and above. Evidence that the bradycardia was mediated by α_1-adrenoceptors is presented in Fig. 5*B*, showing that the α_1-adrenoceptor selective agonist St 587 caused falls in heart rate over a similar range of concentrations, but the α_2-selective BHT 933 was without effect. Thus, after elimination of other effects, all α_1-adrenoceptor agonists should cause a bradycardia, and evidence that noradrenaline slows heart rate after selective blockade of β_1- and β_2-adrenoceptors is presented in Fig. 5*C*. The Figure also shows, however, that although the maximal effect of noradrenaline was to reduce frequency by 25 beats min^{-1} and occurred at 10^{-5} mol l^{-1}, both St 587 and phenylephrine caused further bradycardia at higher concentrations, implying an effect additional to stimulation of α-adrenoceptors. Fig. 5*D* shows that after pre-treatment with the α_1-adrenoceptor selective antagonist prazosin (10^{-6} mol l^{-1})

none of the agonists (still in the presence of atenolol and ICI 118551) had any effect at concentrations up to 10^{-5} mol l^{-1}, but that at higher concentrations phenylephrine and St 587, but not noradrenaline, still caused bradycardia, confirming a non-adrenergic action, observed at very high concentrations only. The α_2-adrenoceptor antagonist WY 25309 had no effect on the bradycardia.

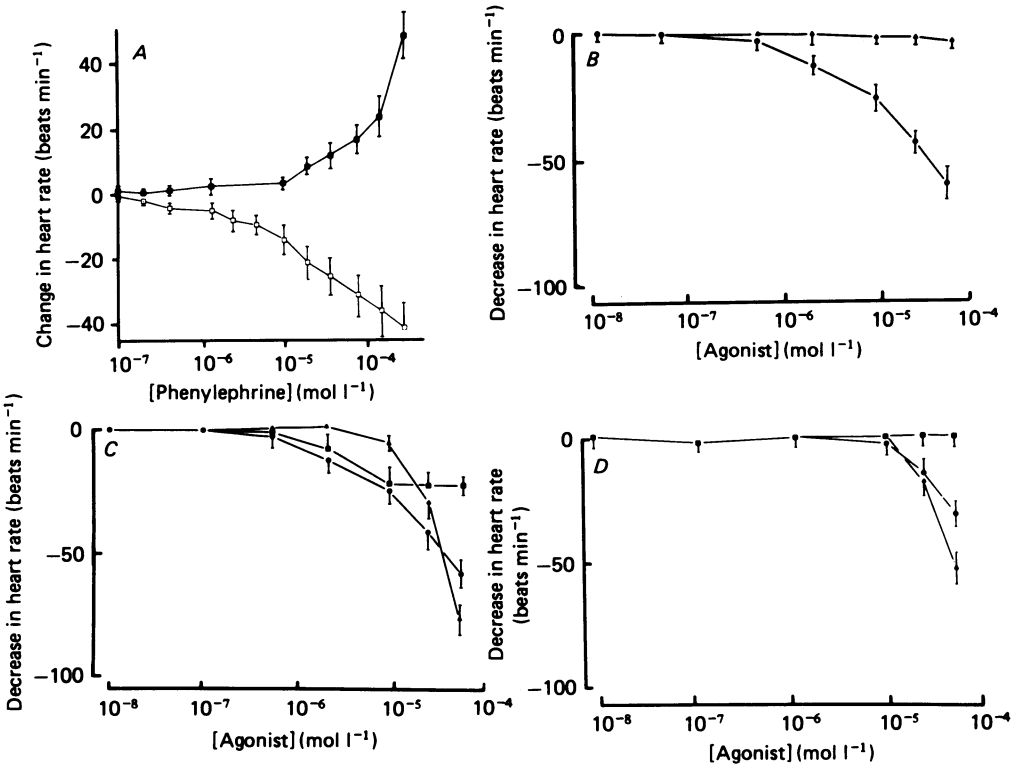

Fig. 5. Log dose–response curves for α-adrenoceptor agonists alone and in the presence of various antagonists. *A*, Phenylephrine increased heart rate at concentrations above 10^{-5} mol l^{-1} (■), but in the presence of propranolol (10^{-6} mol l^{-1}) (□) caused bradycardia at concentrations above 10^{-7} mol l^{-1}. Ordinate: change in heart rate (beats min^{-1}). Abscissa (in all panels): concentration of agonist on logarithmic scale. *B*, effects on heart rate of the selective α_1- and α_2-adrenoceptor agonists St 587 (●) and BHT 933 (▲) respectively. Ordinate (and for *C* and *D*): decrease in heart rate (beats min^{-1}). *C*, effect of St 587 (●) alone compared with those of phenylephrine (▲) and noradrenaline (■) in the presence of both the selective β_1- and β_2-adrenoceptor antagonists atenolol and ICI 118551 respectively, at concentrations of 10^{-6} mol l^{-1}. *D*, effects of St 587 (●) and of phenylephrine (▲) and noradrenaline (■) in the presence of the β-blockers, after exposure to prazosin (10^{-6} mol l^{-1}).

The electrophysiological changes responsible for the slowing of heart rate are illustrated in Fig. 6. Noradrenaline alone (Fig. 6*A*), like isoprenaline, accelerated heart rate by increasing the slope of slow diastolic depolarization and $\dot{V}_{max}$, and by shortening the a.p.d. After blockade of both types of β-adrenoceptor, noradrenaline had no effect on slow diastolic depolarization or on the upstroke of the action potential, but *delayed* repolarization (Fig. 6*B*). The α_1-adrenoceptor agonist St 587

alone had effects similar to those of noradrenaline after β-blockade (Fig. 6*C*). Analysis of the digital records showed that the bradycardia induced by St 587 was exclusively due to delay of repolarization, by a mean of 26 ms at 10^{-6} mol l^{-1}. Other parameters measured, maximum diastolic potential, peak potential, take-off potential, maximum rate of depolarization, and slope of slow diastolic depolarization were all unchanged.

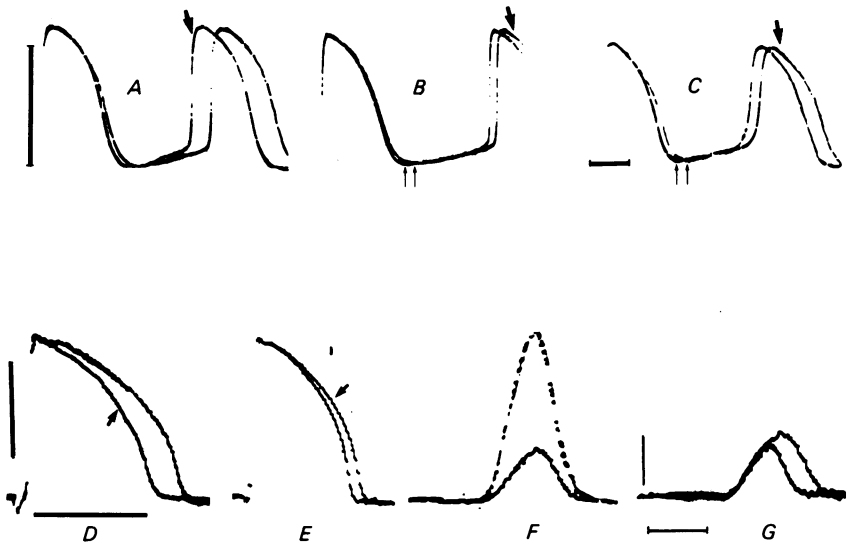

Fig. 6. *A*, *B* and *C*, intracellular potentials recorded from sino-atrial node. The upper arrows denote tracings taken in the presence of the agonists for comparison with the pre-drug control tracings. The lower arrows indicate the points at which the maximum diastolic potentials were reached. *A*, effect of noradrenaline (10^{-7} mol l^{-1}) alone, and in the presence (*B*) of atenolol and ICI 118551 at 10^{-6} mol l^{-1}. *C*, effect of St 587 (5×10^{-6} mol l^{-1}). Vertical and horizontal bars: 60 mV and 100 ms respectively. *D* and *E*, records from papillary muscle; effect of noradrenaline (10^{-7} mol l^{-1}) alone (*D*), and in the presence of the β-blockers (*E*). Vertical and horizontal bars: 60 mV and 100 ms. *F* and *G*, papillary muscle contractions before and after exposure to noradrenaline (10^{-7} mol l^{-1}) alone (*F*), and in the presence of atenolol and ICI 118551 at 10^{-6} mol l^{-1} (*G*). Vertical bar: 1 g; horizontal bar: 100 ms.

The prolongation of a.p.d. was abolished by prazosin at 10^{-6} mol l^{-1}, but the α_2-adrenoceptor selective antagonist WY 25309 had no effect. The α_2-adrenoceptor agonist BHT 933 alone had no effect on sinus node potentials even at the highest concentration used, 10^{-5} mol l^{-1}.

The effects of phenylephrine on sinus node potentials resembled those of noradrenaline. Phenylephrine alone increased the slopes of slow diastolic depolarization and action potential upstroke, and shortened the a.p.d., but lengthened the a.p.d. after combined β_1- and β_2-adrenoceptor blockade, the latter effect being abolished by prazosin, but not by WY 25309.

Elimination of presynaptic effects. The possibility had to be considered that the results might have been influenced by transmitter release from surviving autonomic nerves. For example, presynaptic β-adrenoceptors could have augmented release of noradrenaline (Langer, 1980). Atenolol at concentrations up to 10^{-5} mol l^{-1} had no

effect on the spontaneous frequency of isolated atria, from which it could be concluded that there was no significant 'background sympathetic tone'. Indeed, atenolol had no significant effect on any electrophysiological parameter, except for a small reduction in $\dot{V}_{max}$ at 10^{-5} mol l^{-1}, and therefore the detailed results have not been presented. ICI 118551, likewise had no significant effect at 5×10^{-7} mol l^{-1}, but at 10^{-6} and 4×10^{-6} mol l^{-1} overshoot potential and $\dot{V}_{max}$ were reduced in a concentration-related manner, and repolarization was delayed by 7 and 17 ms respectively. The reduction in $\dot{V}_{max}$ must have been a direct effect of the drug and not due to blockade of a background β_1-adrenoceptor stimulation, because if the latter had been present $\dot{V}_{max}$ would have been depressed by atenolol also.

The above conclusion that presynaptic influences were unlikely to be important was indirect, from the absence of the effect of an antagonist. Dose–response curves for isoprenaline and rimiterol in atria taken from chemically sympathectomized rabbits were compared with control curves and were not significantly different, providing direct evidence that the agonists used were not causing release of presynaptic transmitters in amounts sufficient to affect the results. The bradycardia induced by St 587 and by noradrenaline in the presence of atenolol and ICI 118551, was of equal magnitude in the controls and in animals pre-treated with 6-hydroxydopamine, and the dose–response curves were superimposable.

Papillary muscles

Effects of β-adrenoceptor stimulation

Resting potential. Neither of the β-receptor antagonists, atenolol or ICI 118551, had any significant effect on resting potential. The selective β_2-adrenoceptor agonist pirbuterol, and the less selective rimiterol, made the resting potential slightly more negative at the highest concentrations. The non-selective agonists adrenaline and isoprenaline were a little more potent in hyperpolarizing the resting potential, and although the maximum effect was small (2 mV only), it was statistically significant. The phenomenon is described in more detail below (Table 2).

Repolarization. The effects on the a.p.d. from its peak to 90% repolarization ($a.p.d._{90}$) of increasing concentrations of the four agonists, alone and in the presence of antagonists, are shown in Fig. 7, and representative tracings of the effects of isoprenaline and adrenaline in Fig. 8. All four agonists shortened the a.p.d., isoprenaline being the most potent. (The effects on the a.p.d. from its peak to 20% and 50% repolarization ($a.p.d._{20}$ and $a.p.d._{50}$ respectively) were similar to those on $a.p.d._{90}$, so have not been presented.) It is apparent that the a.p.d.-shortening effects of isoprenaline were only partially blocked by the β_1-adrenoceptor antagonist atenolol, implying that β_2-adrenoceptors also shortened the a.p.d. (Fig. 7*A*). This is confirmed by the fact that the highly selective β_2-adrenoceptor agonist pirbuterol, shortened $a.p.d._{50}$ and $a.p.d._{90}$ in a dose-dependent manner, and that this effect was unaffected by atenolol, but abolished by the β_2-adrenoceptor antagonist ICI 118551 (Fig. 7*D*).

Effects on depolarization. In contrast to the similarity of the effects of β_1- and β_2-adrenoceptor stimulation on repolarization, their effects on depolarization differed. In Table 1 the mean effects of increasing concentrations of the four agonists are presented on the maximum rate of depolarization ($\dot{V}_{max}$) and overshoot potential, the

magnitudes of which in papillary muscle are determined mainly by fast inward (sodium) current. The three drugs with β_1-adrenoceptor agonist activity increased both overshoot and $\dot{V}_{max}$, but in the presence of atenolol the effects on the latter were abolished, and on the former attenuated, implying that they were due to excitation of β_1-adrenoceptors. In confirmation, the highly selective β_2-adrenoceptor agonist

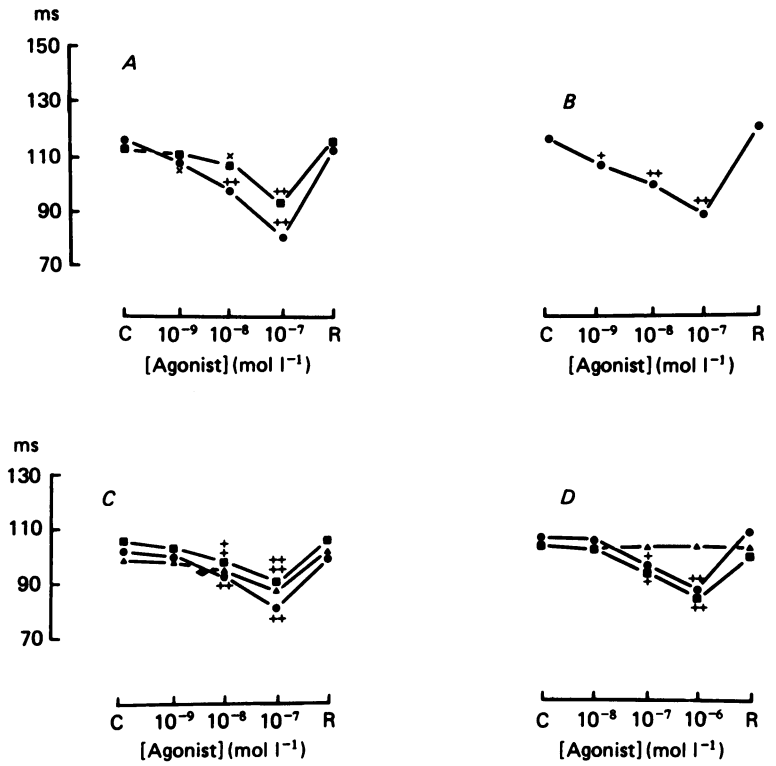

Fig. 7. Effects of *A*, isoprenaline and *B*, adrenaline (non-selective agonists), *C*, rimiterol (partially selective β_2-adrenoceptor agonist), and *D*, pirbuterol (highly selective β_2-agonist) on a.p.d. in papillary muscle. Ordinates: a.p.d. from peak to 90% repolarization (a.p.d.$_{90}$), (ms). Abscissa: agonist concentration (mol l^{-1}). C, before administration of agonist. R, after wash-out of agonist. ●, effects of agonist alone; ■, in the presence of atenolol (10^{-6} mol l^{-1}); ▲, in the presence of ICI 118551 (10^{-6} mol l^{-1}). Significance of differences from control. ×, $P < 0.05$; +, $P < 0.01$; ++, $P < 0.001$.

pirbuterol did not increase $\dot{V}_{max}$ or overshoot, nor did the antagonists on their own. Indeed, ICI 118551, at the highest concentrations used, caused small decreases in $\dot{V}_{max}$ of -5.6% at 10^{-6} mol l^{-1} (n.s.) and of -11.6% at 4×10^{-6} mol l^{-1} ($P < 0.001$). Since the concentration employed for blockade of agonists was 10^{-6} mol l^{-1}, the intrinsic effect of ICI 118551 on $\dot{V}_{max}$ is unlikely to have influenced the results significantly.

Effects of α-adrenoceptor stimulation

Repolarization. Noradrenaline alone at a concentration of 10^{-7} mol l^{-1}, shortened a.p.d.$_{50}$ and a.p.d.$_{90}$ by means of 12 ms for both ($P < 0.001$) (Fig. 6*D*), but lengthened them significantly after combined β_1- and β_2-adrenoceptor blockade (Fig. 6*E*), the

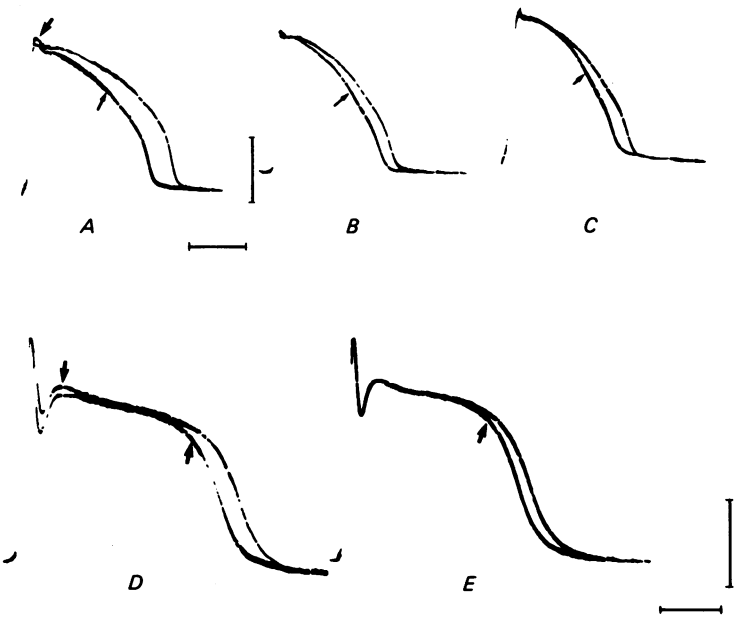

Fig. 8. Effects of isoprenaline (*A*), adrenaline (*B*), and of isoprenaline in the presence of atenolol (*C*) on papillary muscle action potentials. Vertical bar, 50 mV; horizontal, 50 ms. Arrows indicate the tracings recorded in the presence of the drugs. *D*, effects of isoprenaline (10^{-7} mol l^{-1}) alone, and *E*, in the presence of atenolol (10^{-6} mol l^{-1}) on action potentials recorded from distal Purkinje cells. Calibrations: vertical bar, 50 mV; horizontal bar, 50 ms. Arrows indicate tracing taken in the presence of isoprenaline, for comparison with the pre-drug control. A.p.d. shortening was attentuated, but not abolished, by selective β_1-adrenoceptor blockade.

mean increases in $a.p.d._{50}$ and $a.p.d._{90}$ being +16 and +21 ms respectively at 10^{-7} mol l^{-1}. St 587 alone significantly lengthened $a.p.d._{50}$ and $a.p.d._{90}$ by means of +9 and +12 ms respectively at 10^{-6} mol l^{-1} ($P < 0{\cdot}001$), but BHT 933 had no effect. On wash-out of noradrenaline and St 587 all parameters returned to their control values. Here again the prolongation of a.p.d. by St 587, and by noradrenaline in the presence of the β-blockers, was abolished by prazosin, but not by WY 25309. In contrast to its intrinsic bradycardic effect on the sinus node, even at 10^{-5} mol l^{-1} St 587 had no effect at all on the papillary muscle action potentials in the presence of prazosin at 10^{-6} mol l^{-1}.

Contractions

β-receptors. Adrenergically stimulated increases in the force of papillary muscle contractions were mediated by β_1-adrenoceptors. The effects of increasing concentrations of isoprenaline, rimiterol and pirbuterol on developed tension are depicted in Fig. 9.

α-receptors. As illustrated in Fig. 6*F* and Fig. 9 noradrenaline alone at 10^{-7} mol l^{-1} increased developed tension 3-fold. In the presence of combined β_1- and β_2-adrenoceptor blockade the peak tension was only slightly augmented, but the duration of contraction was substantially increased (Fig. 6*G*). The effect of St 587

on contractions was similar to that of noradrelaine after β-blockade, the quantitative results being as follows. In the presence of atenolol and ICI 118551 (10^{-6} mol l^{-1}), noradrenaline at 10^{-8}, 10^{-7} and 10^{-6} mol l^{-1} caused mean increases of papillary muscle contraction of 7·4, 15·9 ($P < 0.01$) and 46·7% ($P < 0.001$) respectively, lengthening the time-to-peak tension by 2·2, 8·7 ($P < 0.001$) and 16·3 ms ($P < 0.001$).

TABLE 1. Effects of β_1- and β_2-adrenoceptor excitation on the depolarization of papillary muscle

Concn. (mol l^{-1})	0	10^{-9}	10^{-8}	10^{-7}	10^{-6}	Recovery
			Overshoot potential (mV)			
Isoprenaline	36·4	35·1	32·4	39·5***	—	34·8+++
Adrenaline	32·5	32·9	31·9	34·7***	—	32·1+++
Rimiterol	31·5	30·9	32·4	36·4***	—	31·8+++
Pirbuterol	31·0	—	30·8	30·5	29·8	30·6
Isoprenaline + atenolol	29·5	30·0	32·5	34·1***	—	30·4+++
			Maximum rate of depolarization (V s^{-1})			
Isoprenaline	172·3	174·3	178·9	198·5***	—	168·1+++
Adrenaline	175·3	178·6	179·5	189·2***	—	174·8+++
Rimiterol	132·8	133·7	138·9	151·2	—	136·7***
Pirbuterol	172·5	—	169·4	167·2	155·3	174·5
Isoprenaline + atenolol	152·6	125·8	162·7	157·2	—	149·8

Significance of differences from the initial control is indicated by asterisks. Plus signs indicate significance of differences observed in the recovery period in drug-free solution from observations at the highest agonist concentration. *** or +++ = $P < 0.001$.

Concentrations of St 587 alone of 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} mol l^{-1} increased contractions by 2·2, 11·2, 15·8 ($P < 0.001$) and 35·8% ($P < 0.001$) respectively, and prolonged the time-to-peak by 0·4, 1·0, 6·0 ($P < 0.001$) and 16·1 ms ($P < 0.001$). The positive inotropic action and prolongation of the duration of contraction by St 587, and by noradrenaline in the presence of the β-blockers was abolished by prazosin but not by WY 25309.

Purkinje cells

β-receptors. The effects of the agonists and antagonists on Purkinje cells were in general similar to those on papillary muscle. Representative tracings of the effects of isoprenaline, alone and in the presence of atenolol, are shown in Fig. 8*D* and *E*. As in papillary muscle, β_1-adrenoceptor blockade did not abolish the shortening of a.p.d. by isoprenaline, implying that functional β_2-adrenoceptors are present in

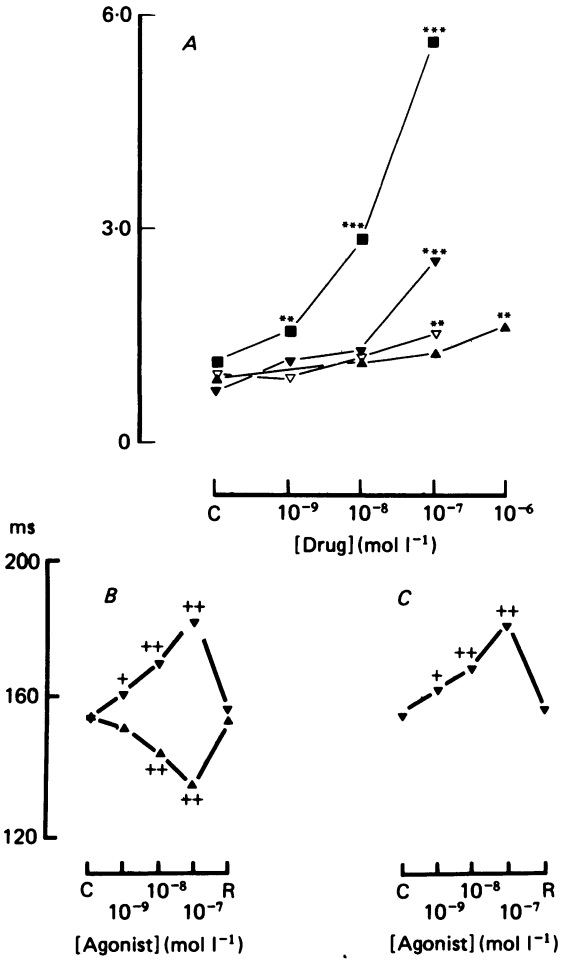

Fig. 9. Effects of agonists on contractions of papillary muscles and on a.p.d. from peak to 90% repolarization (a.p.d.$_{90}$), in Purkinje fibres. *A*, developed tension in response to isoprenaline (■), rimiterol alone (▼), rimiterol in the presence of atenolol (10^{-6} mol l^{-1}) (▽) and pirbuterol alone (▲). Ordinate: tension (g). Abscissa: drug concentrations on logarithmic scale. C, pre-drug control. *B*, effects on a.p.d. of noradrenaline alone (▲) and in the presence of atenolol and ICI 118551 (10^{-6} mol l^{-1}) (▼). *C*, effect of St 587 alone. Ordinates: a.p.d.$_{90}$ (ms). Abscissa: agonist concentrations (mol l^{-1}) on logarithmic scale. C, pre-drug control; R, recovery, after wash-out of drug for 1 h. The significance of differences is indicated thus: ** or +, $P < 0.01$; *** or ++, $P < 0.001$; bars showing S.E. of means have been omitted for clarity.

Purkinje cells also. This conclusion was confirmed by the fact that pirbuterol alone shortened a.p.d., but not in the presence of ICI 118551. In contrast, the maximum rate of depolarization was increased in a dose-dependent manner by isoprenaline (from 326 to 352 V s^{-1} at a concentration of 10^{-7} mol l^{-1}) (Fig. 10*C*), as was the overshoot potential (from 42·9 to 46·2 mV). That these latter effects were mediated mainly by β_1-adrenoceptors was indicated by the observation that they were abolished by atenolol but only attenuated by ICI 118551, and that pirbuterol had no significant effect except a small one at the highest concentration (Fig. 10 *D*). ICI

118551 alone, however, did depress $\dot{V}_{max}$ to some extent ($-1{\cdot}2\%$ at 10^{-6} mol l^{-1} (n.s.) and $-10{\cdot}2\%$ at 4×10^{-6} mol l^{-1}) ($P < 0{\cdot}01$) in Purkinje fibres, as already observed in papillary muscle. Since 10^{-6} mol l^{-1} was the concentration employed for blockade of agonists, this non-specific action cannot explain the attenuation by ICI 118551 of the increase by isoprenaline of $\dot{V}_{max}$ which was reduced from $+7{\cdot}8\%$ to $+3{\cdot}6\%$

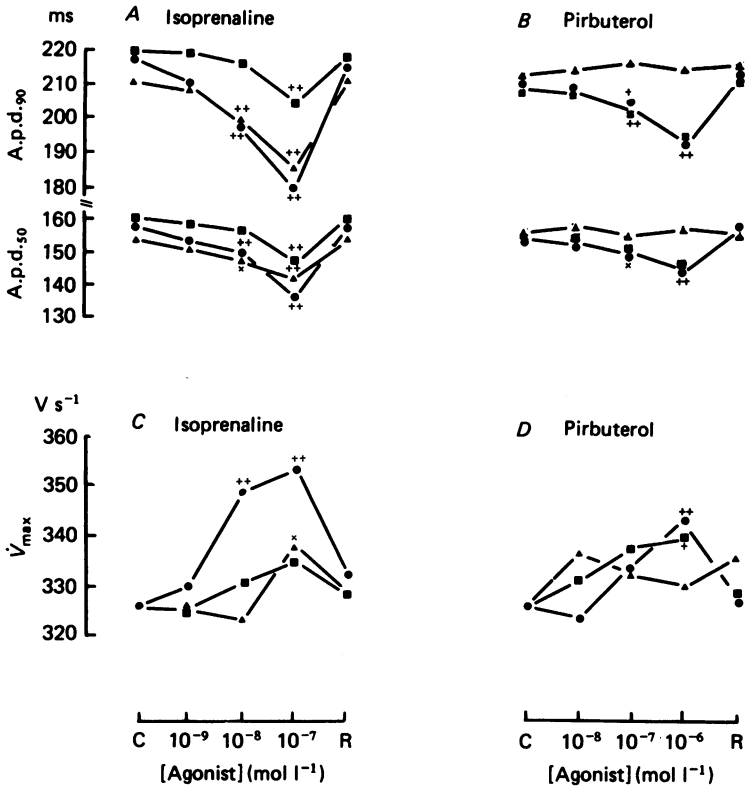

Fig. 10. Effects of isoprenaline (*A* and *C*) and pirbuterol (*B* and *D*) on $a.p.d._{50}$ and $a.p.d._{90}$ (*A* and *B*) and on the maximum rate of depolarization, $\dot{V}_{max}$ (*C* and *D*) in Purkinje cells. Ordinates: *A* and *B*, action potential duration from peak to 50% ($a.p.d._{50}$) and 90% ($a.p.d._{90}$) repolarization (ms). *C* and *D*, maximum rate of depolarization $\dot{V}_{max}$ (V s^{-1}). Abscissa: agonist concentration (mol l^{-1}), C, before administration. R, after wash-out of agonist (1 h). ●, effects of agonist alone. ■, in the presence of atenolol (10^{-6} mol l^{-1}). ▲, in the presence of ICI 118551 (10^{-6} mol l^{-1}). Significance of differences from pre-drug control: ×, $P < 0{\cdot}05$; +, $P < 0{\cdot}01$; ++, $P < 0{\cdot}001$.

at an isoprenaline concentration of 10^{-7} mol l^{-1}. Dose–response curves for isoprenaline and pirbuterol, alone and in the presence of antagonists, on $a.p.d._{50}$ and $a.p.d._{90}$ in Purkinje cells, presented in Fig. 10*A* and *B*, indicate clearly that both β_1- and β_2-adrenoceptor stimulation shortened a.p.d.

α-receptors. The effects of noradrenaline and St 587 in distal Purkinje cells were similar to those observed in papillary muscles, but were even more striking and are illustrated in Fig. 9 *B* and *C* respectively. These effects of St 587, and of noradrenaline in the presence of β-blockers, were completely abolished by prazosin at 10^{-6} mol l^{-1} but not by WY 25309.

Changes in resting potential

The results so far have been consistent with the view that the adrenergic positive inotropic effect and increased rate of depolarization and overshoot potential were mediated primarily in Purkinje fibres and exclusively in papillary muscle by β_1-adrenoceptors, whereas the shortening of a.p.d. was mediated by both β_1- and β_2-adrenoceptors. The small problem remains, however, of how to explain that in Purkinje fibres ICI 118551 did attenuate the increase in $\dot{V}_{max}$ by isoprenaline (Fig. 10*C*), and that pirbuterol did significantly increase $\dot{V}_{max}$ even in the presence of atenolol (Fig. 10*D*).

The shortening of a.p.d. could have involved an increase of potassium current relative to that of other ions, and if this had continued into diastole it could have made the resting potential more negative. In Purkinje cells the relation between $\dot{V}_{max}$ and initial resting potential is very steep (Weidmann, 1955), so that a negative shift of 2 mV could be responsible for an appreciable increase in $\dot{V}_{max}$. The changes of resting potential induced by β_1- and β_2-adrenoceptor agonists, alone and in the presence of antagonists, have been presented in Table 2. Although most of the changes were too small to be statistically significant, the trend is clear, and the highest concentrations of both β_1- and β_2-adrenoreceptor agonists produced statistically significant negative shifts of resting potential, abolished or attenuated by the appropriate antagonist. Confidence in the validity of the measurements is reinforced by the very small values in the recovery column, indicating that resting potential returned to control levels on wash-out of the drugs. Each entry in Table 2 is the difference between the means of measurements of resting potential in twenty-four fibres from four preparations. The standard errors of the means were within the range 0·3–0·9 mV. It is notable that in papillary muscle, in which pirbuterol had no effect on $\dot{V}_{max}$ it also had no effect on resting potential. In Purkinje fibres pirbuterol at 10^{-6} mol l^{-1} significantly increased resting potential by 1·41 mV, and significantly increased $\dot{V}_{max}$ by 15 V s^{-1}. It still increased $\dot{V}_{max}$ by 11·6 V s^{-1} in the presence of atenolol, but not in the presence of ICI 118551, which indicated that the effect was probably secondary to the more negative resting potential induced by β_2-adrenoceptor stimulation, and not to a direct effect of β_2-stimulation on $\dot{V}_{max}$.

DISCUSSION

The advent of highly selective agonists and antagonists has made it possible to differentiate between the electrophysiological consequences of stimulation of individual types of adrenoceptor. The results are relevant to two currently controversial topics, the influence of adrenoceptors on normal and abnormal cardiac rhythm, and the interpretation of the Q–T interval of the electrocardiogram.

Cardiac rhythm

β-adrenoceptors. The existence in the rabbit sinus node of β_2-adrenoceptors mediating increases in heart rate was demonstrated by the effects of the selective agonist, pirbuterol, and antagonist, ICI 118511, in confirmation of previous indirect evidence (Vaughan Williams *et al.* 1975). Brodde *et al.* (1982) found both β_1- and β_2-adrenoceptor binding sites in the rabbit heart, but Costin *et al.* (1983) concluded

that there were β_1-adrenoceptors only. The apparent conflict of evidence may readily be explained, however, because even the highest concentration of pirbuterol we used caused less tachycardia than isoprenaline, suggesting that insufficient β_2-adrenoceptors were present to produce a maximal response. Costin *et al.* (1983) found that the maximal response to another β_2-selective agonist, procaterol (Hedberg & Mattsson, 1981) 'was only $19{\cdot}5 \pm 2{\cdot}2$% of the mean maximum response to isoprenaline'. The authors attributed even this effect to stimulation of β_1-adrenoceptors, but

TABLE 2. Resting potential changes (mV)

Concn. (mol l^{-1})	10^{-9}	10^{-8}	10^{-7}	10^{-6}	Recovery
		Papillary muscle			
Isoprenaline	+0·42	−0·13	−2·17	—	+0·15
+atenolol	+0·48	+1·48	−0·84	—	+0·67
Adrenaline	−0·72	−0·52	−1·77**	—	+0·05
Rimiterol	−0·23	−0·59	−2·21**	—	−0·18
+atenolol	+0·34	−0·35	+0·1	—	−0·09
+ICI 118551	−0·7	−0·22	−1·31*	—	−0·29
Pirbuterol		−0·43	+1·09	−0·48	+0·62
		Purkinje fibres			
Isoprenaline	−0·2	−0·75	−2·42**	—	−0·24
+atenolol	−0·08	−0·4	−0·45	—	−0·02
+ICI 118551	+0·12	−0·17	−0·79	—	+0·06
Pirbuterol		−0·35	−0·45	−1·41**	−1·14
+ICI 118551		−0·1	+0·57	+0·27	+0·54

* $P = < 0{\cdot}05$; ** $P = < 0{\cdot}01$.

did not test whether it was abolished by a β_1-selective antagonist. In calculating agonist concentration ratios in the presence of the selective antagonists atenolol and ICI 118551 they estimated EC_{50} values (agonist concentrations producing 50% of a maximal response), but since the β_2-agonist could achieve only 20% of a maximal response the conclusion that only β_1-adrenoceptors were mediating the response was not surprising.

Our results indicated that increases of $\dot{V}_{max}$ and overshoot in sino-atrial cells were mediated by β_1-, not by β_2-adrenoceptors, implying that the second inward current (i_{si}) was controlled by β_1-adrenoceptors only. Brown *et al.* (1979) suggested that adrenaline accelerated heart rate by inducing a positive shift in the activation curve of i_f, a current activated by hyperpolarization, resulting in a steepening of the slope of the slow diastolic depolarization. A very similar current in the rabbit sino-atrial node, i_h, also activated by hyperpolarization, was described independently by Yanagihara & Irisawa (1980), but the latter authors concluded that it could not be responsible for the pace-making function of the node because its voltage-control range was negative to the maximum diastolic potential of dominant pace-maker cells, and its activation had 'too long a time constant to show dynamic changes during each cardiac cycle. It is unlikely that i_h has a significant effect on the normal action potential pattern'.

Our own experiments have indicated that shortening of the a.p.d. is a major component of adrenergically induced tachycardia, and that not only β_1- but also β_2-adrenoceptors mediated both the increases in slope of the diastolic depolarization, and the increases in the velocity of repolarization and in the maximum diastolic potential. The latter findings suggest that β_2-adrenoceptor stimulation could have increased outward potassium current (i_K). These effects were not exclusively due to β_2-stimulation, however, because they still occurred, though to a reduced extent, in response to isoprenaline after blockade of β_2-adrenoceptors by ICI 118551.

Isenberg (1977) found that after injection of calcium chloride from a micropipette into cardiac Purkinje fibres the duration of action potentials was shortened, and concluded that intracellular calcium concentration, $[Ca^{2+}]_i$, controlled potassium permeability. If such a mechanism were to exist in sinus node cells, a rise in $[Ca^{2+}]_i$ associated with increased i_{si} might be considered responsible for activating outward potassium current. Our evidence was not consistent with such a causal connexion, however, because pirbuterol alone, and isoprenaline in the presence of β_1-adrenoceptor blockade, accelerated repolarization in the absence of any increase in $\dot{V}_{max}$ or overshoot potential. The evidence of Kass & Wiegers (1982) indicated that increased $[Ca^{2+}]_i$ was not the cause of action potential shortening by noradrenaline in calf Purkinje fibres.

It is clear that the rabbit resembles man in the distribution of β_1- and β_2-adrenoceptors in the atrium, and that in neither species could the 'cardioselective' β-blockers be expected to reduce heart rate to as great an extent as non-selective compounds. It would, therefore, be appropriate to describe drugs such as atenolol and metoprolol as 'β_1-adrenoceptor selective' rather than 'cardioselective'.

α-adrenoceptors

Noradrenaline administered intravenously to man causes a bradycardia, the standard explanation for which is that the vasoconstriction induced provokes a vagal reflex (Laurence & Bennett, 1980). Our results have shown that selective α_1-, but not α_2-adrenoceptor stimulation causes bradycardia *in vitro*, the electrophysiological mechanism being delayed repolarization in sino-atrial cells, an effect which could be of significance in patients under β-blockade. It also explains the anomalous initial bradycardia often observed in experiments on pithed rats administered noradrenaline I.V.

The existence of cardiac α-adrenoceptors has long been recognized (Govier, Mosal, Whittington & Broom, 1966; Posner, Farrar & Lambert, 1971; Giotti *et al.* 1973; Rosen, Hordof, Ilvento & Danilo, 1977) but the agonists used were only partially selective for α-adrenoceptors and did not distinguish between α_1- and α_2-adrenoceptors. Effects on the tissue in which the occurrence of fibrillation is lethal, the ventricular myocardium, were not studied. It has been suggested that stimulation of α-adrenoceptors may precipitate or exacerbate arrhythmias associated with ischaemia (Sheridan *et al.* 1980), and that ischaemia lasting more than 15 min (the time at which myocardial damage becomes detectable (Braunwald, 1976)) increases α-adrenoceptor number (Corr, Shayman, Kramer & Kipnis, 1981), which could be explained if α-adrenoceptors were in a steady state and if ischaemic failure of internalization preceded impairment of replenishment. Binding studies do not

identify the source of the receptors, however, which could have been on blood vessels, and the anti-arrhythmic effect of α-adrenoceptor antagonists (Stewart, Burmeister & Lucchesi, 1980) could have been due to their non-adrenergic actions or to abolition of constriction in small vessels not causing gross changes in blood flow. Since stimulation of myocardial α-adrenoceptors *prolonged* a.p.d. in all cardiac tissues, including Purkinje cells and ventricular muscle, the effect should have been anti-arrhythmic rather than arrhythmogenic (Vaughan Williams, 1980).

Corr & Sharma (1982) reported that β-blockers were ineffective in ischaemic or post-ischaemic arrhythmias. In ischaemia β-adrenoceptor stimulation could fail if conditions became unfavourable for the existence of the GTP complex (Rodbell, 1980) in the form appropriate for the production of cyclic AMP, which mediates β-adrenoceptor effects (Kukovetz, Poch & Wurm, 1975), in which case the existence of an α-adrenoceptor-mediated action not requiring cyclic AMP (Brodde, Motomura, Endoh & Schumann, 1978) could confer some evolutionary advantage, perhaps by delaying inactivation of inward calcium current, rather than by increasing its magnitude. In severe ischaemia $[Ca^{2+}]_i$ rises, leading to contractures (Nayler, Yepez & Poole-Wilson, 1978) and transient depolarizations, possibly initiated by electrogenic sodium–calcium exchange (Mullins, 1981), in which event a further rise in $[Ca^{2+}]_i$ caused by α-adrenoceptor stimulation could be deleterious. It is of interest that, after β-blockade, α-adrenergically induced inward calcium current can be blocked by nifedipine (Miura, Inui & Immamura, 1978), emphasizing that the β_1-adrenoceptor stimulated mechanism for raising $[Ca^{2+}]_i$ is pharmacologically distinct from that blocked by nifedipine or verapamil (Harman & Poole-Wilson, 1981; Towart & Schramm, 1984).

Ventricular adrenoceptors and Q–T interval

Increased cardiac frequency induced by pacing causes the Q–T interval to shorten, but the slope of the relation between inter-beat (R–R) interval and Q–T is less steep than when frequency is increased by exercise (Vaughan Williams, 1982). Both β_1- and β_2-adrenoceptors were involved in the shortening of action potential duration in papillary muscles and Purkinje cells, so that in exercise there would be two distinct influences tending to shorten the Q–T interval, the effect of higher frequency and the direct action of adrenergic stimulation in shortening a.p.d. After β-blockade the latter effect is largely cancelled by concentrations of drug insufficient to block sympathetic action on the sinus node, and the slope of the relation between R–R and Q–T in exercise is less steep (Vaughan Williams, Hassan, Floras, Sleight & Jones, 1980). Thus in interpreting the effects of drug treatments on the Q–T interval it is advisable to measure Q–T at unchanged heart-rate (Birkhead, Vaughan Williams, Gwilt, Tanqueray & Cazes, 1983). Furthermore, since both circulating noradrenaline and adrenaline may rise, the effects of exertion on Q–T interval can only be interpreted correctly if account is taken of the differential effects of α-, β_1- and β_2-adrenoceptor stimulation on a.p.d. In this context Arnold, Page, Attwell, Cannell & Eisner (1982) stated 'both adrenaline and noradrenaline lengthen the ventricular action potential. A slow rise of catecholamine level following the onset of exercise, and a slow fall afterwards, cannot therefore be the cause of the slow changes of Q–T interval.' It is not, of course, permissible to extrapolate directly from rabbits to

man, but if human cardiac tissues are similar to those of rabbits in their responses to adrenergic stimulation, as seems probable from previous work (Raine & Vaughan Williams, 1980, 1981), β-adrenoceptor activation could well be responsible for a shortening of the Q–T interval independently of any change in heart rate.

We have not studied the ionic mechanisms responsible for the effects observed, and it is appropriate merely to mention two possible explanations for the shortening of a.p.d. and hyperpolarization induced by β-adrenoceptor stimulation. One is that electrogenic sodium–potassium pumping is stimulated; the other that outward potassium current is increased. The two are not mutually exclusive, and our evidence that both β_1- and β_2-adrenoceptors mediate shortening of a.p.d. as well as hyperpolarization is consistent with either explanation. A possibility worthy of investigation is that β_1-adrenoceptors might increase potassium permeability and that β_2-receptors stimulate the pump. Pump stimulation could not be the *only* factor involved, however, because Gadsby (1983) found that in voltage-clamped canine cardiac Purkinje cells, with the sodium–potassium pump inactivated by acetylstrophanthidin or by zero extracellular potassium, isoprenaline still caused outward current to increase. It was evident that the effect was mediated by β-adrenoceptor agonists since it was produced by isoprenaline, adrenaline and noradrenaline in the presence of phentolamine, but the participation of β_2-adrenoceptors was not excluded.

Finally, the evidence that increases in inward calcium current were mediated by β_1-, not by β_2-adrenoceptors was supported by the finding that the β_2-agonist pirbuterol did not augment contractions, and that the positive inotropic effect of isoprenaline was blocked by atenolol. After both β_1- and β_2-adrenoceptor blockade, noradrenaline only slightly increased peak developed tension, but substantially prolonged both a.p.d. and contraction, again suggesting that the effect of α-adrenoceptor stimulation was to delay removal of intracellular calcium, $[Ca]_i$, rather than to increase its magnitude.

Gifts of drugs from ICI, Riker 3M, Pfizer, Boehringer Ingelheim and Wyeth are gratefully acknowledged.

REFERENCES

ARNOLD, L., PAGE, J., ATTWELL, D., CANNELL, M. & EISNER, D. A. (1982). The dependence on heart rate of the human ventricular action potential duration. *Cardiovascular Research* **16**, 547–551.

BILSKI, A. J., HALLIDAY, S. E., FITZGERALD, J. D. & WALE, J. L. (1983). The pharmacology of a β_2-selective adrenoceptor antagonist ICI 118551. *Journal of Cardiovascular Pharmacology* **5**, 430–437.

BIRKHEAD, J. S., VAUGHAN WILLIAMS, E. M., GWILT, D. J., TANQUERAY, A. & CAZES, C. (1983). Heart rate and QT interval in subjects adapted to beta-blockade: bradycardia and hypotension as uncorrelated adaptations. *Circulation Research* **17**, 649–655.

BLINKS, J. R. (1964). The nature of the antagonism by methoxamine of the chronotropic and inotropic effects of catecholamines. *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie u. Pharmakologie* **248**, 73–84.

BRAUNWALD, E. (1976). Salvage of the ischaemic myocardium. In *Pathophysiology and Therapeutics of Myocardial Ischaemia*, ed. LEFER, A. M., KELLIHER, G. K. & ROVETTO, M. J., pp. 265–306. New York: Spectrum Publications.

BRODDE, O.-E., KARAD, K., ZERKOWSKI, H.-R., ROHM, N. & REIDEMEISTER, J. CHR. (1983). Coexistence of β_1- and β_2-adrenoceptors in human heart and lung. *British Journal of Pharmacology* **78**, 72*P*.

BRODDE, O.-E., LEIFERT, F.-J. & KREHL, H.-J. (1982). Coexistence of β_1- and β_2-adrenoceptors in the rabbit heart: quantitative analysis of the regional distribution by (−)-[^{3}H]dihydroalprenolol binding. *Journal of Cardiovascular Pharmacology* **4**, 34–43.

BRODDE, O.-E., MOTOMURA, S., ENDOH, M. & SCHUMANN, H. J. (1978). Lack of correlation between the positive inotropic effect evoked by α-adrenoceptor stimulation and the levels of cyclic AMP and/or cyclic GMP in the isolated ventricle strip of the rabbit. *Journal of Molecular and Cellular Cardiology* **10**, 207–219.

BROWN, H. F., DIFRANCESCO, D. & NOBLE, S. J. (1979). How does adrenaline accelerate the heart? *Nature* **280**, 235–236.

BRYAN, L. J., COLE, J. J., O'DONNELL, S. R. & WANSTALL, J. C. (1981). A study designed to explore the hypothesis that Beta-1 adrenoceptors are 'innervated' receptors and Beta-2 adrenoceptors are 'hormonal' receptors. *Journal of Pharmacology and Experimental Therapeutics* **216**, 395–400.

CARLSSON, E., ABLAD, B., BRANDSTROM, A. & CARLSSON, B. (1972). Differentiated blockade of the chronotropic effects of various adrenergic stimuli in the cat heart. *Life Sciences* **11**, 953–958.

COOKE, N. J., KERR, J. A., WILLEY, R. F., HOARE, M. V., GRANT, I. W. B. & CROMPTON, G. K. (1974). Response to rimiterol and salbutamol aerosols administered by intermittent positive-pressure ventilation. *British Medical Journal* **2**, 250–252.

CORR, P. & SHARMA, A. D. (1982). Alpha- *versus* beta-adrenergic influences on dysrhythmias induced by myocardial ischaemia and reperfusion. In *Advances in β-blocker Therapy*. II, ed. ZANCHETTI, A. *Excerpta Medica* ICS **593**, 163–180.

CORR, P. B., SHAYMAN, J. A., KRAMER, J. B. & KIPNIS, R. J. (1981). Increased alpha-adrenergic receptors in ischaemic cat myocardium. *Journal of Clinical Investigation* **67**, 1232–1236.

COSTIN, B. I., O'DONNELL, S. R. & WANSTALL, J. C. (1983). Chronotropic responses of rabbit isolated atria to β-adrenoceptor agonists are mediated by only β_1-adrenoceptors. *Journal of Pharmacy and Pharmacology* **35**, 752–754.

DAVEY, M. J. (1980). Pharmacology of Prazosin. *Journal of Cardiovascular Pharmacology* **2**, S287.

DUKES, I. D. & VAUGHAN WILLIAMS, E. M. (1984*a*). Noradrenaline-induced bradycardia in isolated rabbit sinus node is due to α_1-adrenoceptor stimulation. *Journal of Physiology* **351**, 44*P*.

DUKES, I. D. & VAUGHAN WILLIAMS, E. M. (1984*b*). Multiple modes of action of propafenone. *European Heart Journal* (in the Press).

GADSBY, D. C. (1983). β-adrenoceptor agonists increase membrane K^+-conductance in cardiac Purkinje fibres. *Nature* **306**, 691–693.

GIOTTI, A., LEDDA, F. & MANNAIONI, P. F. (1973). Effects of noradrenaline and isoprenaline in combination with α- and β-receptor blocking substances, on the action potential of cardiac Purkinje fibres. *Journal of Physiology* **229**, 99–113.

GOVIER, W. C. (1968). Myocardial alpha adrenergic receptors and their role in the production of a positive inotropic effect by sympathomimetic agents. *Journal of Pharmacology and Experimental Therapeutics* **159**, 82–90.

GOVIER, W. C., MOSAL, N. C., WHITTINGTON, P. & BROOM, H. (1966). Myocardial alpha and beta adrenergic receptors as demonstrated by atrial functional refractory-period changes. *Journal of Pharmacology and Experimental Therapeutics* **154**, 255–263.

HARMAN, M. C. & POOLE-WILSON, P. A. (1981). Comparison of two techniques for inducing slow action potentials in depolarized mammalian ventricular myocardium. *Journal of Physiology* **316**, 33*P*.

HAUSWIRTH, O., NOBLE, D. & TSIEN, R. W. (1968). Adrenaline: mechanism of action on the pacemaker potential in cardiac Purkinje fibers. *Science* **162**, 916–917.

HEDBERG, A. & MATTSSON, H. (1981). Beta adrenoceptor interaction of full and partial agonists in the cat heart and soleus muscle. *Journal of Pharmacology and Experimental Therapeutics* **219**, 798–808.

HEITZ, A., SCHWARTZ, J. & VELLY, J. (1983). β-adrenoceptors of the human myocardium: determination of β_1 and β_2 subtypes by radioligand binding. *British Journal of Pharmacology* **80**, 711–717.

INNEMEE, H. C., DE JONGE, A., VAN MEEL, C. A., TIMMERMANS, P. B. M. W. M. & VAN ZWEITEN, P. A. (1981). The effect of selective α_1- and α_2-adrenoceptor stimulation on intraocular pressure in the conscious rabbit. *Naunyn-Schmiedebergs Archives of Pharmacology* **316**, 294–298.

ISENBERG, G. (1977). Cardiac Purkinje fibres. $[Ca^{2+}]_i$ controls the potassium permeability via the conductance components of g_{K1} and g_{K2}. *Pflügers Archiv* **371**, 77–85.

JOHANSSON, L.-H. & PERSSON, H. (1983). β_2-adrenoceptors in guinea-pig atria. *Journal of Pharmacy and Pharmacology* **35**, 804–807.

KASS, R. A. & WIEGERS, S. E. (1982). The ionic basis of concentration-related effects of noradrenaline on the action potential of calf cardiac Purkinje fibres. *Journal of Physiology* **322**, 541–558.

KOBINGER, W. & PICHLER, L. (1977). Pharmacological characterization of BHT 933 (2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo-[5,4-*d*]-azepin-dihydrochloride) as a hypotensive agent of the 'clonidine-type'. *Naunyn-Schmiedebergs Archives of Pharmacology* **300**, 39–46.

KUKOVETZ, W. R., POCH, G. & WURM, A. (1975). Quantitative relations between cyclic AMP and contraction as affected by stimulators of adenylate cyclase and inhibitors of phosphodiesterase. *Advances in Cyclic Nucleotide Research* **5**, 395–414.

LANDS, A. M., ARNOLD, A., MCAULIFF, J. P., LUDUENA, F. P. & BROWN, T. G. (1967). Differentiation of receptor systems activated by sympathomimetic amines. *Nature* **214**, 597–598.

LANGER, S. Z. (1974). Presynaptic regulation and catecholamine release. *Biochemical Pharmacology* **123**, 1793–1800.

LANGER, S. Z. (1980). Presynaptic regulation of the release of catecholamines. *Pharmacological Reviews* **23**, 337–382.

LATTIMER, N., RHODES, K. F., WARD, T. J., WATERFALL, J. F. & WHITE, J. F. (1982). Selective α_2-adrenoceptor antagonist activity of novel substituted benzoquinolizines. *British Journal of Pharmacology* **75**, 154*P*.

LAURENCE, D. R. & BENNETT, P. N. (1980). *Clinical Pharmacology*, p. 589. Edinburgh: Churchill-Livingstone.

MIURA, Y., INUI, J. & IMAMURA, H. (1978). Alpha-adrenoceptor mediated restoration of calcium-dependent potential in the partially depolarized rabbit papillary muscle. *Naunyn-Schmiedebergs Archives of Pharmacology* **301**, 201–205.

MOORE, P. F., CONSTANTINE, J. W. & BARTH, W. E. (1978). Pirbuterol, a selective beta$_2$ adrenergic bronchodilator. *Journal of Pharmacology and Experimental Therapeutics* **207**, 410-418.

MULLINS, L. J. (1981). *Ion Transport in Heart*. New York: Raven Press.

NAYLER, W. G., YEPEZ, C. E. & POOLE-WILSON, P. A. (1978). The effect of β-adrenoceptor and Ca^{2+} antagonist drugs on the hypoxia-induced increase in resting tension. *Cardiovascular Research* **12**, 666–674.

PIERCE, V. & WATERFALL, J. F. (1982). Selectivity of substituted benzoquinolizines for α_2-adrenoceptors in pithed rats. *British Journal of Pharmacology* **76**, 263*P*.

POSNER, P., FARRAR, E. L. & LAMBERT, C. R. (1971). Inhibitory effects of catecholamines in canine cardiac Purkinje fibers. *American Journal of Physiology* **321**, 1415–1420.

PRICHARD, B. N. C. (1971). Panel discussion. *Post-graduate Medical Journal* **47**, suppl., p. 112.

RAINE, A. E. G. & VAUGHAN WILLIAMS, E. M. (1980). Adaptational responses to prolonged beta adrenoceptor blockade in adult rabbits. *British Journal of Pharmacology* **70**, 205–218.

RAINE, A. E. G. & VAUGHAN WILLIAMS, E. M. (1981). 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. *Circulation Research* **48**, 804–812.

REINHARDT, D. & WAGNER, J. (1974). Beta-adrenolytic, antiarrhythmic and local anaesthetic effects of phenylephrine. *Naunyn Schmiedebergs Archives of Pharmacology* **284**, 245–261.

RODBELL, M. (1980). The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* **284**, 17–22.

ROSEN, M. R., HORDOF, A. J., ILVENTO, J. P. & DANILO, P. (1977). Effects of adrenergic amines on electrophysiological properties and automaticity of neonatal and adult canine Purkinje fibres. *Circulation Research* **40**, 390–400.

SHERIDAN, D. J., PENKOSKE, P. A., SOBEL, B. E. & CORR, P. B. (1980). Alpha adrenergic contributions to dysrhythmia during myocardial ischaemia and reperfusion in cats. *Journal of Clinical Investigation* **65**, 161–171.

STEWART, J. R., BURMEISTER, W. E., BURMEISTER, J. & LUCCHESI, B. F. (1980). Electrophysiologic and antiarrhythmic effects of phentolamine in experimental coronary artery occlusion and reperfusion in the dog. *Journal of Cardiovascular Pharmacology* **2**, 77–91.

TOWART, R. & SCHRAMM, M. (1984). Recent advances in the pharmacology of the calcium channel. *Trends in Pharmacological Sciences* **5**, 111–113.

VAUGHAN WILLIAMS, E. M. (1977). Automated measurement of potentials. *Journal of Physiology* **272**, 1–2*P*.

VAUGHAN WILLIAMS, E. M. (1980). *Antiarrhythmic Action*. London: Academic Press.

VAUGHAN WILLIAMS, E. M. (1982). Q–T and action potential duration. *British Heart Journal* **47**, 513–514.

VAUGHAN WILLIAMS, E. M. & DUKES, I. D. (1983). The absence of effect of chemical sympathectomy on cardiac hypertrophy induced by hypoxia in young rabbits. *Cardiovascular Research* **17**, 379–389.

VAUGHAN WILLIAMS, E. M., HASSAN, M. O., FLORAS, J. S., SLEIGHT, P. & JONES, J. V. (1980). Adaptation of hypertensives to treatment with cardio-selective and non-selective beta blockers. Absence of correlation between bradycardia and blood-pressure control, and reduction in slope of the QT/RR relation. *British Heart Journal* **44**, 437–487.

VAUGHAN WILLIAMS, E. M., RAINE, A. E. G., CABRERA, A. A. & WHYTE, J. M. (1975). The effects of prolonged β-adrenoceptor blockade on heart weight and cardiac intracellular potentials in rabbits. *Cardiovascular Research* **9**, 579–592.

WEIDMANN, S. (1955). The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. *Journal of Physiology* **127**, 213–224.

WILSON, C. (1984). Comparison of β-adrenoceptors in guinea-pig, rat and human atria. *British Journal of Pharmacology* (in the Press).

YANAGIHARA, K. & IRISAWA, H. R. (1980). Inward current activated during hyperpolarization in the rabbit sinoatrial node cell. *Pflügers Archiv* **385**, 11–19.