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Cardiovascular effects of bevantolol, a selective β_1-adrenoceptor antagonist with a novel pharmacological profile

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1 Bevantolol was more potent in blocking the chronotropic than the hypotensive effects of isoprenaline in pithed rats.

2 Bevantolol itself induced bradycardia, so that it was not possible to estimate the pA_2 from non-parallel dose-response curves relating isoprenaline concentration to tachycardia.

3 Bevantolol caused hypertension in pithed rats, an effect attenuated by phentolamine, implying that bevantolol may be an α-adrenoceptor agonist.

4 Bevantolol potentiated the pressor effects of noradrenaline, the maximum potentiation equalling that produced by prior chemical sympathectomy with guanethidine, implying that bevantolol may block noradrenaline uptake.

5 In isolated atria bevantolol-induced bradycardia was associated with a positive shift in take-off potential, a reduction in the maximum rate of depolarization (V_{max}), and a lengthening of action potential duration (APD). No change in the slope of the slow diastolic depolarization occurred except at the highest concentration (18 μmol l^{-1}).

6 In atrial and ventricular muscle bevantolol reduced V_{max} and overshoot potential, implying reduction of fast inward sodium current (Class I antiarrhythmic action).

7 In pithed rats bevantolol lengthened the P-R interval in the ECG, and produced atrioventricular (A–V) block, and bundle-branch block. In isolated A–V nodal preparations, intranodal conduction time was greatly increased, implying restriction of inward current through calcium channels responsible for nodal depolarization.

8 Bevantolol had no negative inotropic effect in pithed rats, or in isolated atria, and did not alter the positive inotropic effect of raised extracellular calcium concentration, implying absence of restriction of current through calcium channels controlling contraction of the myocardium.

Introduction

Bevantolol is a recently introduced β-adrenoceptor antagonist at present undergoing clinical trial. Very few papers have been published about its pharmacological, and none about its electrophysiological, effects. Hastings *et al.* (1977) found, with isoprenaline as agonist, that bevantolol had twice the potency of propranolol as a β-adrenoceptor antagonist in guinea-pig isolated atria, and that the atrial:tracheal selectivity ratio was 32 (propranolol = 0.65). In man, with inhaled albuterol as agonist to induce bronchial relaxation, Mackay *et al.* (1981) observed dose-ratios of 1.02 and 2.77 after oral administration of 75 and 150 mg respectively of bevantolol, doses which reduced exercise tachycardia by 25% and 29%. The blockade of airway relaxation was equivalent to that induced by 100 and 200 mg of practolol respectively, but less than that induced by 40 mg of propranolol orally.

No experiments in pithed animals devoid of vascular reflexes have been reported, but in anaesthetized dogs, Gross *et al.* (1979) observed that 1 mg kg^{-1} of bevantolol i.v. reduced the tachycardic responses to 0.3 μg kg^{-1} of isoprenaline by 70–80% with little effect on the hypotensive responses, but the effects of other agonist and antagonist doses were not studied and cardiac output and peripheral resistance were not measured. In anaesthetized dogs, intravenous doses of 0.2, 0.6, 2.0 and 6.0 mg kg^{-1} of bevantolol reduced heart rate by 20, 23, 22 and 16 beats min^{-1} respectively, i.e. the bradycardia was not dose-related; mean

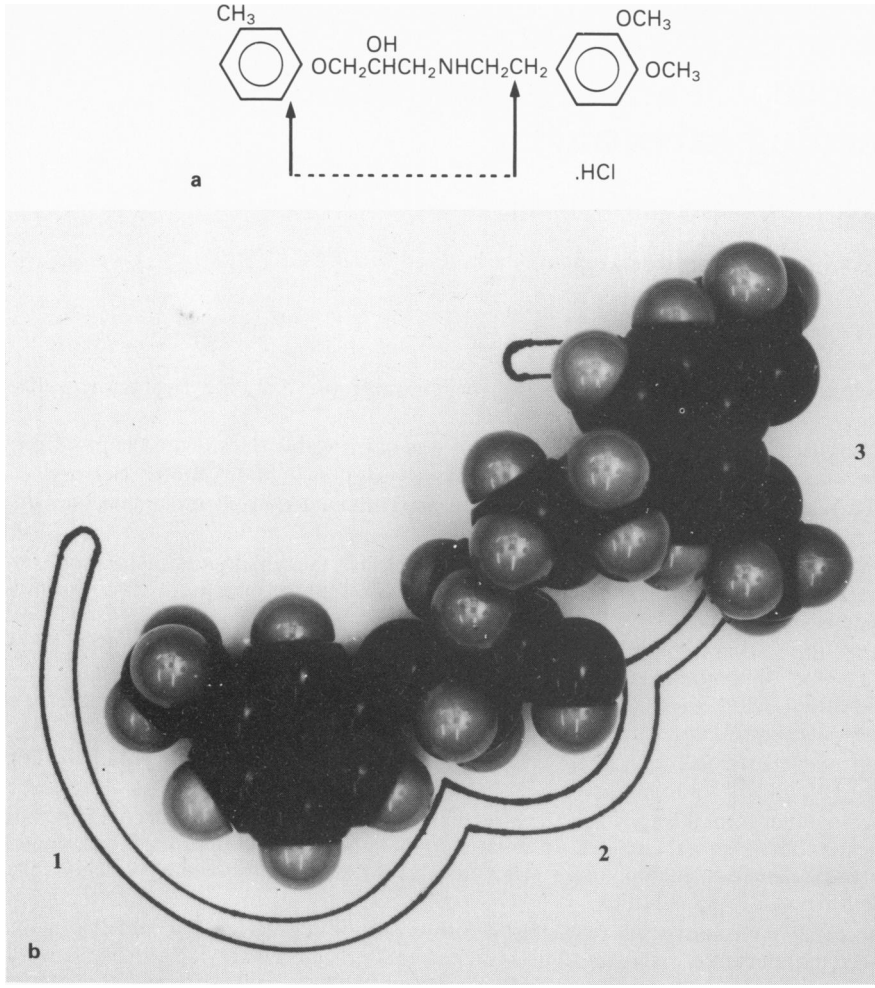

Figure 1 (a) Structure of bevantolol. The section between the arrows is common to many β-adrenoceptor blocking drugs. (b) The double lines represent the outline of a section through an imaginary β_2-adrenoceptor cleft, the length of which is limited by end-walls at 1 and 3, restricting entry of molecules with bulky substitutents at the 4 position in the ring (1), or on the nitrogen (3). It is supposed that there is an attachment site (2) accommodating the beta-hydroxyl.

blood pressure was reduced by 2, 2, 3 and 10 mmHg. In contrast, Baukema and Bovenkirk (unpublished) reported that bevantolol 1.0 mg kg^{-1} increased stroke volume in dogs (after coronary ligation) and 10 mg kg^{-1} raised mean blood pressure from 110 to 140 mmHg, and increased total peripheral resistance by 80%, in anaesthetized dogs.

Bevantolol was found to be an effective anti-anginal drug in a small series of patients (Steen *el al.* 1979). Other studies (unpublished) had indicated that oral dosage of 100–400 mg daily lowered blood pressure in 70% of hypertensive patients, and that the plasma elimination half-life was approximately 140 min. Of 30 patients with stable, non-life-threatening arrhythmias, half returned to sinus rhythm on treatment with intravenous bevantolol (maximum dosage 3.2 mg kg^{-1}), but four exhibited bradycardia. Such effects would be expected with any β-adrenoceptor blocking compound, but it is always of interest to investigate what properties such drugs possess in addition to blockade of β-receptors. The experiments described here show that bevantolol is strikingly different from other β-blockers in several respects. The structure of bevantolol (Figure 1a) is quite similar to that of many other β-blockers, the most important difference being the dimethoxyphenethyl in place of the usual isopropyl or terbutyl attached to the nitrogen, and a *meta*-methyl as the only substituent of the terminal ring.

Methods

Pithed rats

Rats of either sex weighing 300–400 g were anaesthetized with pentobarbitone sodium 60 mg kg^{-1} i.p. and pithed. They were artificially ventilated via a Y cannula in the trachea, positive pressure being applied during one-third only of each cycle of the pump (frequency 1 Hz). Mean arterial blood pressure (MAP) was recorded with a mercury manometer, and the ECG was monitored intermittently (lead II). In a separate series of experiments ($n = 6$) rats were treated with guanethidine 50 mg kg^{-1} daily for four days prior to pithing.

Rabbit isolated atria

Rabbits of either sex weighing 900–1300 g were stunned and their hearts were quickly removed. The atria were separated from the ventricles and suspended vertically in a 50 ml water-jacketed bath through which 95% O_2 and 5% CO_2 was bubbled. Spontaneous contractions were recorded with a strain gauge and displayed on a Devices paper chart. For comparison of the magnitude of contractions the atria were paced at 2.7 Hz. The bathing solution contained (mmol l^{-1}) NaCl 125, KCl 5.6, $CaCl_2$ 2.16, $NaHCO_3$ 25, $MgCl_2$ 1.0, NaH_2PO_4 0.8 and glucose 11. The pH was 7.4 and the temperature 32°C.

Intracellular potentials

(1) *Sinus node* Rabbit atria were prepared as above, and further dissected to leave the right atrium plus interatrial septum. This was then transferred to a bath (volume 12 ml), and pinned with endocardial aspect uppermost to its silastic base. Dissection was continued to leave a portion of the crista terminalis, and the posterior atrial wall between the venae cavae containing the sinus node (Dukes & Vaughan Williams, 1984a). The solution was oxygenated externally to the bath, and flowed continuously through it at 15 ml min^{-1} and at 36.5°C.

The sinus node region was explored with microelectrodes, and action potentials were accepted as 'sinus node potentials' if there was a slow diastolic depolarization merging smoothly into an action potential upstroke, and if the maximum rate of depolarization (V_{max}) of the latter was 7.5 V s^{-1} or less. Cells with slow diastolic depolarizations but faster V_{max} were considered transitional cells. The 'take off' potential was estimated by a computed extrapolation backwards from the point of V_{max} to the midpoint of transition from the slow diastolic depolarization. Single microelectrode impalements could usually be maintained throughout the course of an experiment.

(2) *Atrium* Atria were suspended horizontally in a bath of 12 ml with their endocardial aspect uppermost, between an anchoring hook and a strain gauge. They were paced continuously by twice threshold stimuli, of 1 ms duration, at a frequency 10% faster than the initial spontaneous frequency (range 2.1 to 2.5 Hz). The stimulating electrodes were always placed on the anterior wall of the left atrium 2–3 mm from the interatrial septum. Intracellular potentials were recorded from several regions in the neighbourhood of the crista terminalis and care was taken to record from the same regions before and after exposures to bevantolol.
(3) *Ventricle and proximal and distal Purkinje fibres* After separation of the ventricles from the atria, the left ventricular free wall was removed and the right ventricular wall was cut from the septum anteriorly and peeled back, revealing the anterior papillary muscles, the moderator band and other free-running strands of Purkinje fibres. A thread was tied to one of the chordae tendineae and the tricuspid leaflet to which it was connected was severed from its origin. The thread was attached to a strain gauge. Stimuli of twice threshold strength, and at a frequency just fast enough to 'capture' spontaneously beating preparations (usually 1.6 to 1.8 Hz) were applied to the bundle

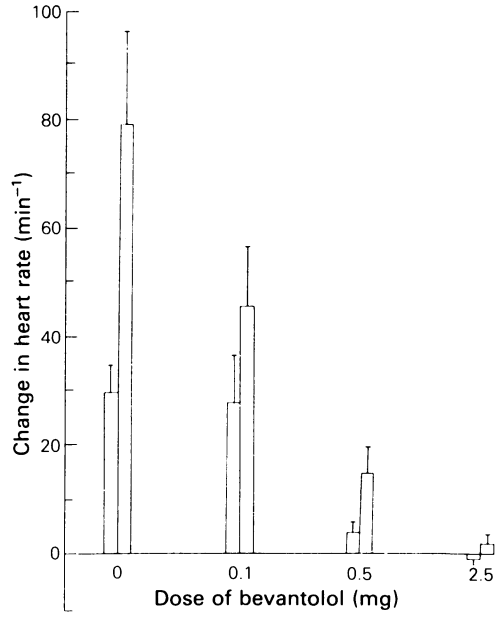

Figure 2 Mean chronotropic responses in four pithed rats to 20 and 50 ng isoprenaline i.v. before and after cumulative doses of bevantolol of 0.72, 3.6 and 18 μmol kg^{-1} i.v. In contrast to the results in Figure 5, bevantolol abolished the chronotropic response to isoprenaline, implying blockade of β_1-adrenoceptors. Bars indicate s.e.mean.

of His. Intracellular recordings were obtained from another papillary muscle left slack (V), from the proximal part of right bundle branch (BB), and from the fine Purkinje fibre network (P) at the base of the papillary muscles. (AV set-up, Figure 9.)

The atrial and ventricular preparations were kept at 32°C. Control records were made in atria 1 h, and in ventricles 2 h, after the preparations had been set up. Recordings were again taken after 40 min exposure to each concentration of bevantolol, higher concentrations replacing the lower without intermediate washout. Finally 'recovery' records were made after one hour's washing with drug-free solution.

Action potentials were recorded with 3 M KCl-filled glass microelectrodes coupled to a high input impedance d.c. amplifier with variable capacity compensation (WPI). During the experiments, action potentials and contractions were displayed on a storage oscilloscope (Tektronix 5103N) and recorded at will on tape (Racal Store 4). The stored records were played back into transient recorders, from which they were transferred to the computer (HP9830) which measured and analysed them statistically by a programme which incorporated Student's t test (Vaughan Williams, 1977). The stimuli were recorded on a separate channel, and were used on playback to trigger the transient recorders receiving the data. The distance between the stimulating and recording sites was measured in each experiment, and entered into the computer programme, which calculated conduction velocity from the time between the stimulus and the foot of the recorded action potential.

Results

Pithed rats

Heart rate Bevantolol injected intravenously in doses of 0.72, 3.6 and 18.0 μmol kg^{-1} caused a dose-related sinus bradycardia (mean depressions of -8.3, -9.0, and -15.0% respectively) from a mean control value of 196 ($\pm$ 14.0) beats min^{-1}. These doses of bevantolol progressively blocked the chronotropic responses to intravenous injections of 20 and 50 ng isoprenaline (Figure 2). Apart from the sinus bradycardia, bevan-

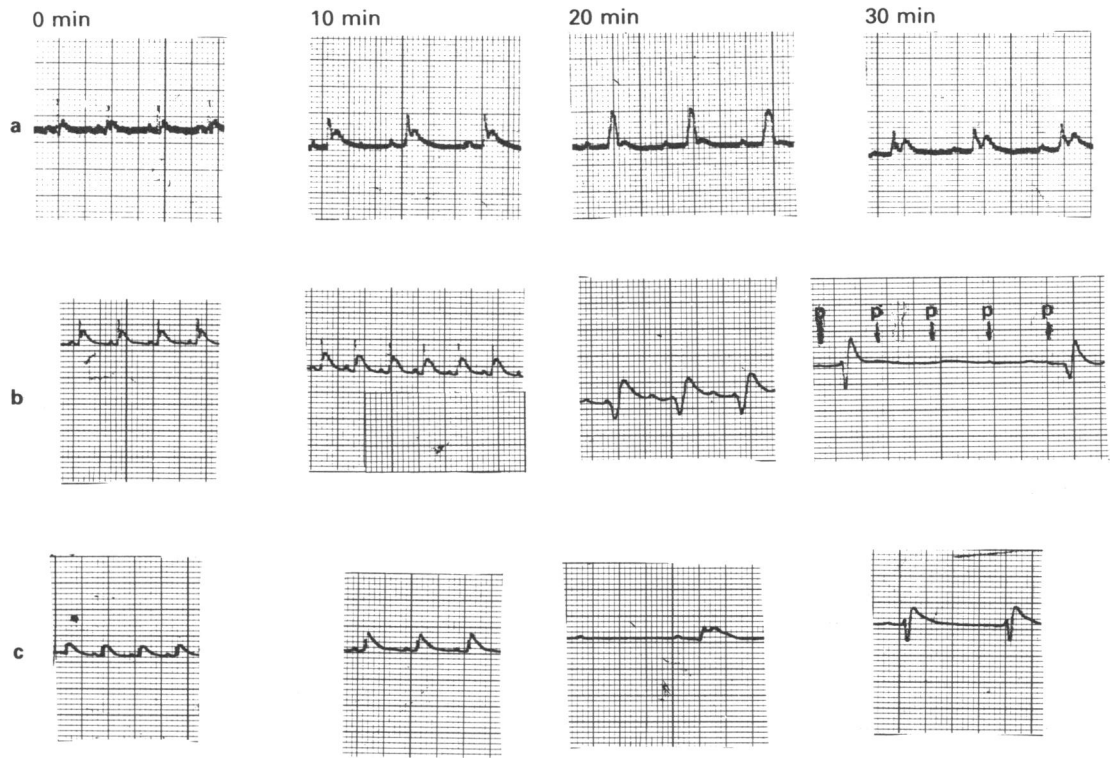

Figure 3 Electrocardiogram (Lead II) of 3 pithed rats (a, b, c), before (0) and 10, 20 and 30 min after the administration of 18 μmol kg^{-1} bevantolol i.v. The drug caused bradycardia, and delayed A–V conduction, leading eventually to A–V block and/or bundle branch block. The arrows marked P indicate the P waves.

tolol induced striking alterations in the ventricular complexes recorded on the ECG (Figure 3). In the rat the intracellular ventricular action potential repolarizes rapidly with very little plateau, so that there is no isoelectric interval in the surface electrocardiogram following the QRS and a T-wave is usually undetectable. Increasing doses of bevantolol progressively induced four changes: (1) the QRS was widened; (2) the repolarization phase was delayed, implying a prolonged action potential duration; (3) P–R interval lengthened until 2 to 1 (Figure 3c), 3 to 1 or even 4 to 1 (Figure 3b) atrioventricular (A–V) block occurred; (4) right or left bundle branch block was induced (Figure 3a, and Figure 3b and c).

Blood pressure Bevantolol 0.72, 3.6 and 18.0 μmol kg^{-1} caused dose-related increases in mean arterial blood pressure (MAP) to mean peaks of 69, 115 and 120 mmHg ($n = 4$). The hypertensive responses to bevantolol were characterized by a sharp rise in pressure, followed by a brief fall associated with bradycardia, and then a large long-lasting hypertension, maintained at its peak for 2–3 min. It was clear that bevantolol could not have had any significant negative inotropic effect on a heart capable of sustaining pressures in excess of 120 mmHg. In a typical experiment, 18 μmol kg^{-1} of bevantolol increased MAP by 60 mmHg, a second injection of 18 μmol kg^{-1} after 30 min inducing a similar response. If, however, an injection of 10 mg of phentolamine i.v. was interposed between the two injections of bevantolol, the second injection caused a rise in blood pressure of only 12 mmHg, implying that bevantolol might stimulate α-adrenoceptors. The hypertensive responses declined slowly during 7–10 min and MAP did not return to the original level so that the baseline mean blood pressure rose progressively from a mean control value of 55 mmHg to 65, 68 and 75 mmHg at 10 min after 'recovery' from the three doses (0.72, 3.6 and 18 μmol l^{-1}) respectively.

In the pithed rat, injections of noradrenaline repeated at intervals of 30–40 min have increasing hypertensive effects, possibly because presynaptic nerve endings, which provide the main mechanism for noradrenaline removal, become saturated or degenerate. There is, however, no increase in baseline MAP

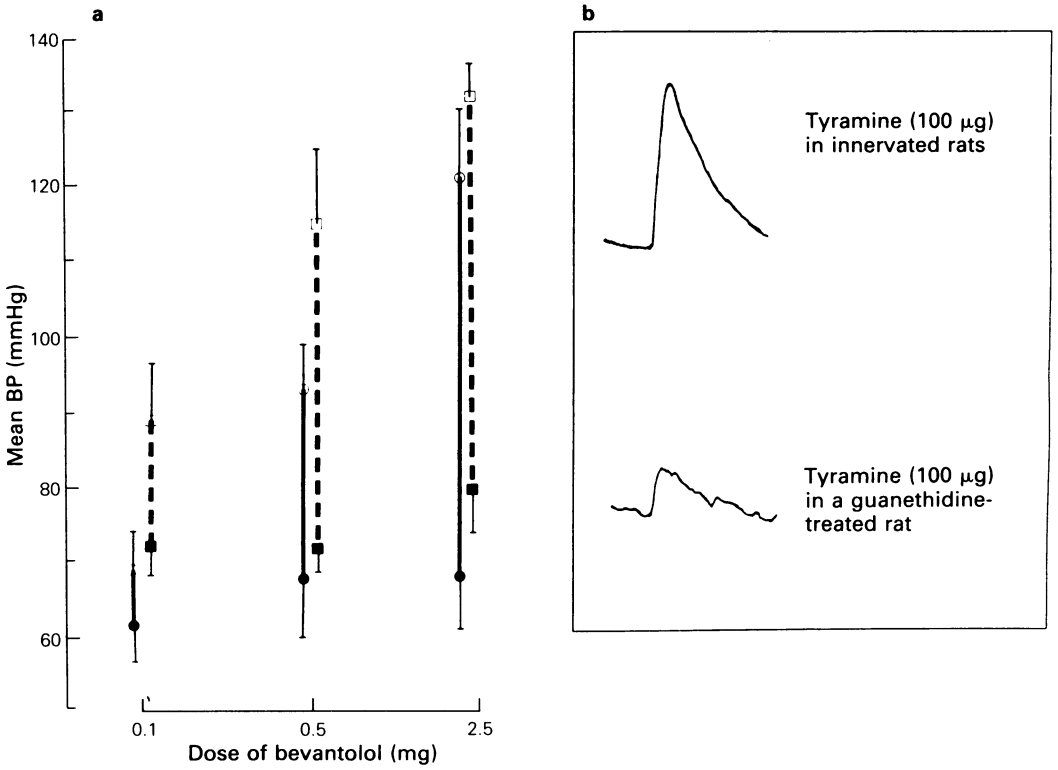

Figure 4 (a) Pressor responses to three doses of bevantolol i.v. in untreated pithed rats (circles and solid columns) and in rats pretreated with guanethidine (squares and dashed columns). The filled symbols indicate the initial pressure before bevantolol, and the open symbols the MAP at the peak of the response to bevantolol. Means and s.e.mean from four experiments. (b) Pressor responses to 100 μg tyramine i.v. in an untreated pithed rat (above) and in a rat pretreated with guanethidine (below).

after recovery from each injection. Noradrenaline 20 ng and 8 min subsequently, 50 ng, was injected before, and 10 min after, doses of bevantolol of 0.72, 3.6 and 18.0 μmol kg^{-1} administered after half hour intervals. Bevantolol not only raised the baseline blood pressure, but increased the peak responses to the injected noradrenaline to a greater extent than when repeated injections of noradrenaline had been given alone.

It seemed possible that bevantolol might be blocking noradrenaline uptake by presynaptic sympathetic nerve terminals. Accordingly a group of rats was pretreated with guanethidine 50 mg kg^{-1} daily for four days. As shown in Figure 4b this procedure greatly reduced the pressor response to tyramine, and has been shown in previous studies (Johnson & O'Brien, 1976) to abolish the effect of electrical stimulation of adrenergic nerves. In the guanethidine-treated animals, mean arterial pressure was raised and the effect of noradrenaline was greatly potentiated, presumably because presynaptic uptake had been eliminated. Bevantolol still caused acute hypertensive responses and a rise in basal mean arterial pressure.

Increasing doses of bevantolol in normal rats potentiated responses to the low dose of noradrenaline by 15, 100 and 146% (Table 1, Row 3), in comparison with the pre-bevantolol control response, a potentiation greater than that seen after repeated doses of noradrenaline alone. In the guanethidine-treated animals, before bevantolol, the low dose of noradrenaline was potentiated by 138%, i.e. by almost as much as by the highest dose of bevantolol in the animals with sympathetic nerves intact. This suggests the possibility that the potentiation by bevantolol of the responses to noradrenaline is due not only to an additive postsynaptic stimulation of α-adrenoceptors, but also to blockade by bevantolol of the presynaptic uptake of noradrenaline. Comparison of rows 1 and 3 in Table 1 indicates that prior chemical sympathectomy greatly attenuated the potentiation by bevantolol of the pressor responses to noradrenaline.

Depressor effects of isoprenaline The lower two doses of bevantolol, of 0.72 and 3.6 μmol kg^{-1}, did not reduce the falls of blood pressure produced by 20 and 50 ng isoprenaline i.v. (Figure 5). This contrasts with the effects of bevantolol on the heart rate responses to isoprenaline (Figure 2), indicating that bevantolol is relatively selective for β_1-adrenoceptors. The largest dose of bevantolol did, however, slightly (NS) attenuate the β_2 responses, in spite of the higher basal MAP which could have facilitated a more substantial fall of blood pressure. In the guanethidine-treated rats, although the basal MAP was consistently higher, as already noted, the responses to isoprenaline were not significantly altered, as would be expected from the fact that isoprenaline is a poor substrate for the presynaptic uptake pathway.

Table 1 Comparison of potentiation of pressor responses to noradrenaline by chemical sympathectomy and by bevantolol

	Bevantolol (μmol kg^{-1})							
	0		*0.72*		*3.6*		*18.0*	
	I	*G*	*I*	*G*	*I*	*G*	*I*	*G*
A *Responses to 20 ng noradrenaline (NA)*								
% increase due to sympathectomy	—	138	—	143	—	50	—	8
Rise in MAP after 20 ng NA (mmHg)	13	31	15	36	26	39	32	34
% increase due to bevantolol	—	—	15	16	100	26	146	11
B *Responses to 50 ng noradrenaline (NA)*								
% increase due to sympathectomy	—	91	—	103	—	32	—	15
Rise in MAP after 50 ng NA (mmHg)	28	53	30	61	45	60	46	53
% increase due to bevantolol	—	—	9	16	64	13	67	1

Row 2 shows the absolute increases in blood pressure in response to injections of 20 ng (A) and 50 ng (B) of noradrenaline i.v. in normally innervated rats (I) and guanethidine pretreated rats (G). In row 1 the responses of the chemically sympathectomized rats are expressed as percentage increases above the responses of the innervated rats. Thus, in the absence of bevantolol, chemical sympathectomy potentiated the responses to the low and high doses of noradrenaline by 138% and 91% respectively.

In row 3 the responses after bevantolol are expressed as percentage increases above the responses before bevantolol. It is clear that chemical sympathectomy occluded the potentiation by bevantolol of the responses to noradrenaline. N.B. The percentages were calculated from unrounded mean values.

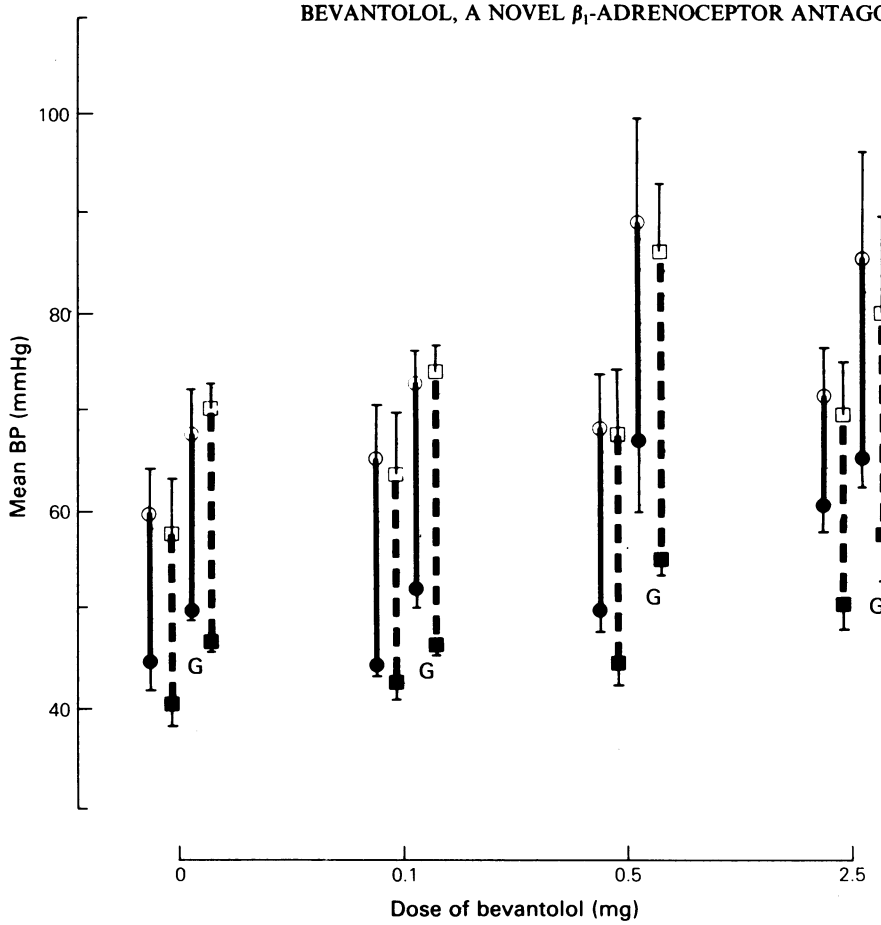

Figure 5 Falls in mean arterial pressure in response to injection of 20 ng (circles and solid columns) and 50 ng (squares and dashed columns) isoprenaline i.v. to untreated pithed rats, and to rats pretreated with guanethidine (G). The initial pressure (open symbols) was raised both by bevantolol and by chemical sympathectomy, but the depressor responses (closed symbols) to isoprenaline were not reduced significantly in either group. Means and s.e.mean from four experiments.

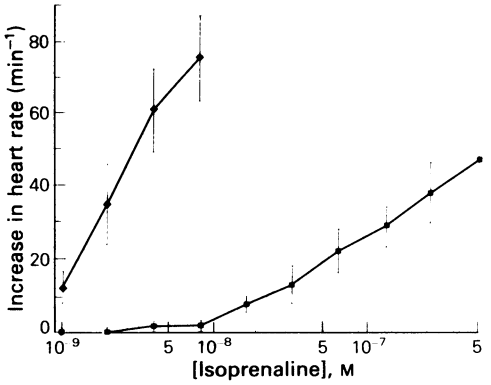

Figure 6 Effects of isoprenaline on the spontaneous frequency of rabbit isolated atria before and after exposure to bevantolol 1.0 μmol l^{-1}. Ordinate scale: increase in heart rate. Abscissa scale: concentration of isoprenaline on a logarithmic scale. Bevantolol itself caused bradycardia, and so a pA_2 could not be assessed from the non-parallel dose-response curves.

Rabbit isolated atria

Spontaneous heart rate Bevantolol caused a dose-related bradycardia, the mean spontaneous frequency of isolated atria being reduced from 147 ± 6.5 beats min^{-1} by 10.2% and 35.7% at concentrations of 1 and 10 μmol l^{-1} respectively. This must have been due to a direct action of bevantolol, unrelated to blockade of endogenously released noradrenaline, because atenolol has virtually no effect on heart rate in this preparation (Dukes & Vaughan Williams, 1984b). This explains why the shift in the dose-response curve relating tachycardia in response to logarithmically increasing concentrations of isoprenaline (Figure 6) is not shifted in a parallel manner to the right by bevantolol. It is, therefore, impossible to give an accurate figure for pA_2. The ratio for the concentrations of isoprenaline required to increase heart rate by 30 beats min^{-1} before and after exposure to bevantolol 1.0 μmol l^{-1} was 79, but for a tachycardia of

15 beats min^{-1} it was only 12.6. Had the slopes been parallel at these points the pA_2's would have been 7.9 and 7.1 respectively.

Effects of changes in extracellular calcium concentrations [Ca]$_o$ Over a range of calcium concentrations from about half to twice normal (1.08 to 4.32 mmol l^{-1}) the sinus node frequency increases with increasing $[Ca]_o$, but there is usually considerable hysteresis, in that frequency is always lower when $[Ca]_o$ is returned to the original concentration from a different concentration (Millar & Vaughan Williams, 1981). Bevantolol not only itself caused bradycardia, but also depressed the tachycardia induced in the controls by raising $[Ca]_o$ to twice the normal value (Figure 7a). In complete contrast, bevantolol itself had no negative inotropic action on paced isolated atria and did not alter the relation between $[Ca]_o$ and the force of contraction (Figure 7b).

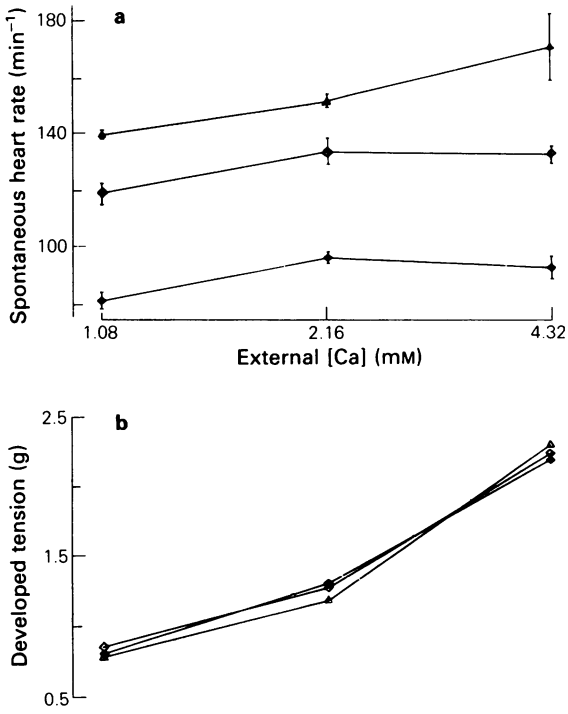

Figure 7 The effect of bevantolol on the chronotropic and inotropic responses of rabbit isolated atria to changes in extracellular calcium concentration $[Ca]_o$. (△) Control; (◇) bevantolol 1 μM: (◆) bevantolol 5 μM. Bevantolol caused bradycardia (a) especially at the highest $[Ca]_o$, but itself had no negative inotropic effect (b) and did not alter the responses to raising the calcium concentration.

Intracellular potentials

Sinus note potentials Drugs can cause bradycardia by altering sinus node potentials in various ways (Millar & Vaughan Williams, 1982). Bevantolol had no effect on the maximal diastolic potential, but depressed the maximum rate of rise and peak amplitude of the sinus

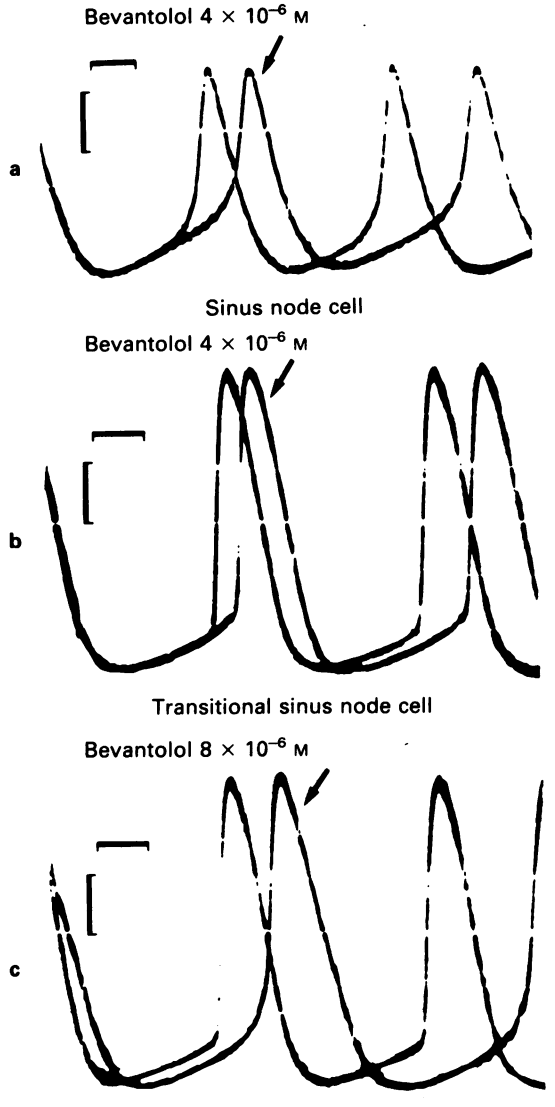

Figure 8 Effects of bevantolol on intracellular potentials recorded from the rabbit sino-atrial node. The arrows indicate tracings taken in the presence of bevantolol superimposed on control tracings. (a) Sinus node cell: (b & c) trasitional sino-atrial cells. Vertical and horizontal calibrations, 20 mV and 50 ms.

Table 2 Effects of bevantolol on sinus node potentials

	Bevantolol (mol l^{-1})				
	0	2×10^{-6}	4×10^{-6}	8×10^{-6}	*Recovery*
Heart rate (beats min^{-1})	154.7 (6.6)	150.7 (5.2)	133.6 (4.9) ***	126.4 (7.8) ***	148.5 (4.6) †††
Peak amplitude (mV)	3.1 (1.7)	3.4 (1.8)	1.2 (2.2) *	−1.1 (3.2) **	2.4 (2.1) †
Maximum diastolic potential (mV)	−65.01 (0.67)	−64.33 (0.90)	−64.52 (0.58)	−64.75 (0.76)	−64.81 (0.70)
Slope of slow diastolic depolarization (mVs^{-1})	134.66 (8.74)	134.25 (6.70)	134.22 (10.64)	81.29 (7.29) ***	126.81 (6.88) †††
Take-off potential (mV)	−50.00 (1.53)	−47.93 (1.96) *	−44.41 (2.61) ***	−43.26 (2.51) ***	−48.22 (3.11) †††
Maximum rate of depolarization (Vs^{-1})	3.48 (0.47)	2.82 (0.46)	2.35 (0.17) **	1.83 (0.46) ***	2.61 (0.23) †††
Mean rate of repolarization (Vs^{-1})	0.43 (0.05)	0.36 (0.04) *	0.33 (0.03) ***	0.31 (0.05) ***	0.34 (0.03) ††
Total duration of repolarization (ms)	168.3 (1.8)	176.6 (2.1) **	198.4 (2.2) ***	209.4 (1.8) ***	188.6 (2.4) †††

Mean results (± s.e.mean) from four experiments.
Statistical significance from initial controls. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.
Statistical significance of recovery results from the effects of the highest dose; † $P<0.05$; †† $P<0.01$; ††† $P<0.001$.
The bradycardia caused by bevantolol was due to delayed repolarization, and a more positive take-off potential and a slower MRD. The slope of the diastolic depolarization was not altered except by the highest concentration of bevantolol.

Table 3 Effects of bevantolol on transitional sino-atrial cell potentials

	Bevantolol (mol l^{-1})				
	0	2×10^{-6}	4×10^{-6}	8×10^{-6}	*Recovery*
Heart rate (beats min^{-1})	180.6 (10.3)	170.4 (12.7)	156.5 (11.2) ***	141.5 (11.3) ***	159.2 (10.8) †††
Peak amplitude (mV)	11.4 (1.6)	11.3 (1.2)	9.5 (0.8) *	5.3 (0.5) ***	10.2 (0.5) †††
Maximum diastolic potential (mV)	−79.94 (0.68)	−79.56 (0.95)	−79.90 (0.78)	−79.31 (0.58) *	−80.21 (0.62)
Slope of slow diastolic depolarization (mVs^{-1})	51.43 (4.30)	51.53 (3.42)	49.24 (5.13)	44.90 (4.08) **	50.21 (4.57) ††
Take-off potential (mV)	−65.90 (1.86)	−65.12 (1.98) *	−63.86 (1.94) ***	−62.82 (1.98) ***	−64.48 (1.88) †††
Maximum rate of depolarization (Vs^{-1})	28.68 (1.43)	28.03 (1.79)	22.60 (1.68) **	12.16 (1.40) ***	23.58 (1.66) †††
Mean rate of repolarization (Vs^{-1})	0.58 (0.02)	0.54 (0.02) *	0.49 (0.02) ***	0.42 (0.02) ***	0.51 (0.03) ††
Total duration of repolarization (ms)	157.4 (5.2)	168.8 (7.1) **	184.9 (8.4) ***	205.9 (11.4) ***	173.0 (2.4) †††

Mean results (± s.e.mean) from four experiments.
Symbols as for Table 2.

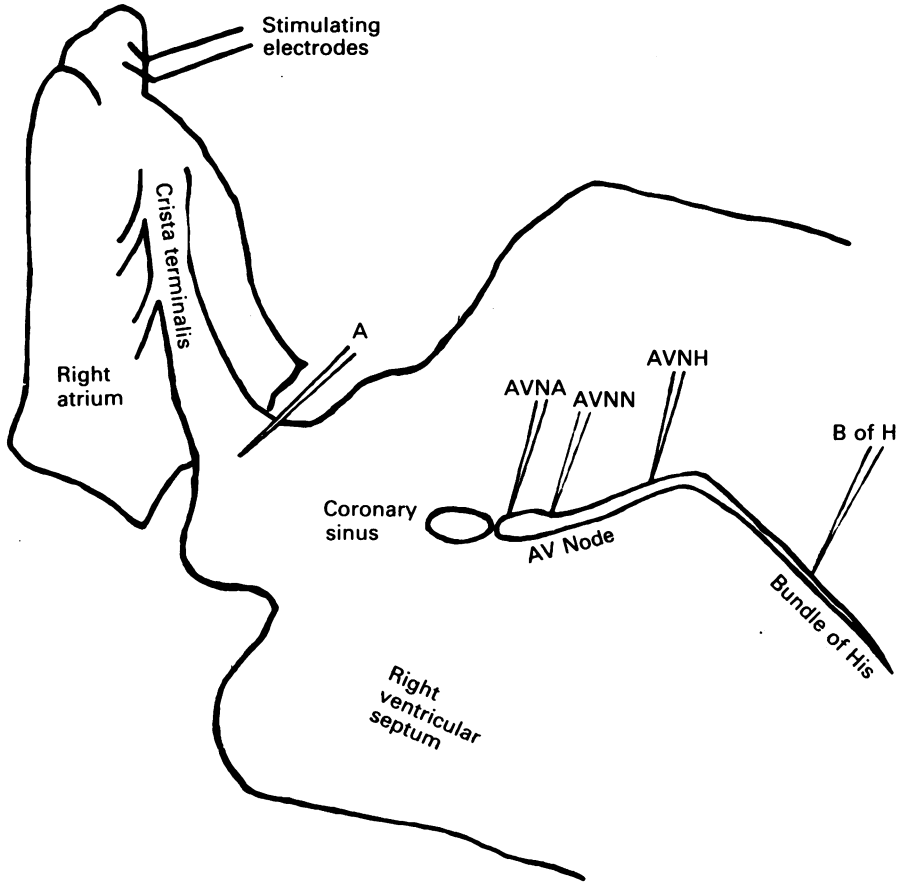

Figure 9 Diagram of the position of stimulating and recording electrodes in the rabbit isolated atrio-ventricular preparation. The distances between S and A, and between AVNH and B of H were greater than between AVNA and AVNH, but the greatest conduction delay induced by bevantolol occurred within the node itself (Table 4).

Table 4 Effects of bevantolol on conduction times measured from the point of stimulation on the right atrial appendage to various points in the atrioventricular conduction pathway

		Bevantolol (mol l^{-1})				
	Conduction time (ms)	*0*	*2×10^{-6}*	*4×10^{-6}*	*8×10^{-6}*	*Recovery*
	Atrium	10.0	9.1	16.2	20.3	12.8
		(1.4)	(1.5)	(1.2)	(1.6)	(1.3)
				***	***	†††
	A region	28.1	32.6	43.1	50.6	29.6
		(2.6)	(2.4)	(3.1)	(1.8)	(2.0)
			*	***	***	†††
AV node	N region	85.2	95.6	108.4	128.2	94.5
		(2.9)	(3.8)	(4.1)	(3.8)	(4.9)
			***	***	***	†††
	H region	88.7	108.5	128.4	145.1	119.2
		(6.2)	(3.7)	(4.1)	(3.8)	(4.1)
			***	***	***	†††
	Bundle of His	90.2	113.2	134.6	150.3	125.3
		(2.6)	(5.4)	(4.7)	(6.7)	(4.2)
			***	***	***	†††

Mean results (± s.e.mean) from four experiments.
Symbols as for Table 2.

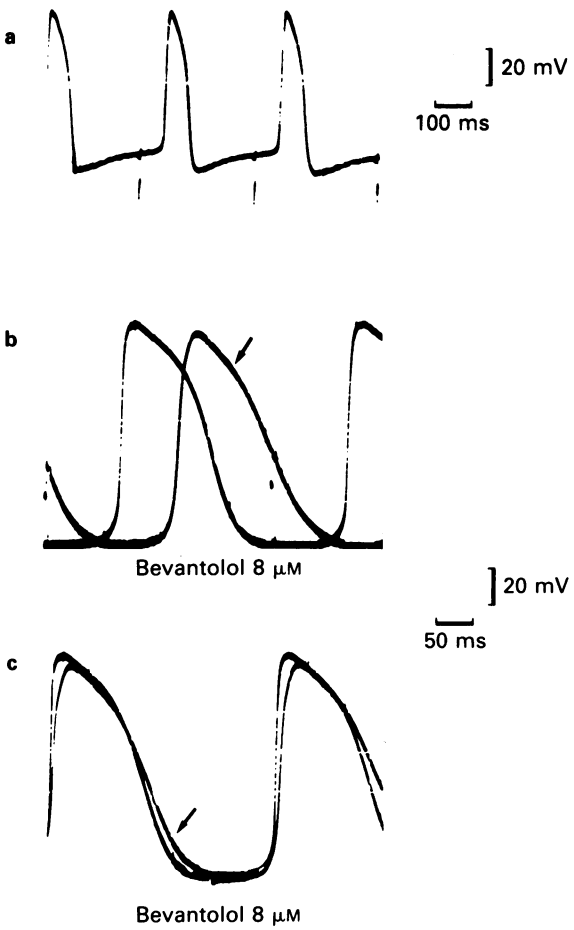

Figure 10 Intracellular potentials recorded from the paced atrio-ventricular node of a rabbit. (a) Control. (b & c) Tracings in the presence of bevantolol 8×10^{-6} mol l^{-1} superimposed on control tracings. (b) The two traces were synchronized on the stimulus to illustrate the large increase in conduction time. (c) Records superimposed at the first action potential upstroke to illustrate the depression of V_{max} and overshoot potential, and the small increase in APD.

node action potential, implying that the second inward current (i_{si}) was reduced. There was no significant change in the slope of the slow diastolic depolarization, except by the highest concentration used (8×10^{-6} mol l^{-1}). As can be seen from Figure 8 and Tables 2 and 3, the bradycardia was caused mainly by a positive shift in the voltage at which the upstroke of the action potential upstroke 'took off', most obviously apparent in transitional sinoatrial cells (Figure 8b and c) and by a prolongation of the repolarization time from the action potential peak to the maximum diastolic potential (last rows of Tables 2 and 3).

AV Node The position of stimulating and recording electrodes in the atrio-ventricular preparations is indicated in the diagram presented in Figure 9. The time taken for conduction of the action potentials from the stimulating electrode to the lower atrium, to the designated parts of the AV node itself, and to the bundle of His, before and after exposure to three concentrations of bevantolol are given in Table 4. The two higher concentrations of bevantolol added 6 and 10 ms respectively to conduction time in the atrium, and 4.7 and 2.7 ms respectively to conduction time from the AV node to the Bundle of His. In contrast, conduction time through the AV node itself was increased by the three concentrations of bevantolol by 99, 118 and 130 ms respectively, implying that AV-nodal depolarizing current was strongly inhibited. Representative intracellular recordings from AV nodal cells are presented in Figure 10, and show that the rate of depolarization was decreased and that APD was prolonged (by a mean of 15.2 ± 1.3 ms at the highest concentration, 8×10^{-6} mol l^{-1}).

Purkinje cells In the ventricular conduction pathway action potential duration is much longer in the pre-terminal Purkinje cells, than in the Bundle of His or terminal Purkinje cells, and drugs may affect these regions differentially (Wittig *et al.*, 1973; Vaughan Williams *et al.*, 1977). Bevantolol had no effect on resting potential in pre-terminal or terminal Purkinje cells (Tables 5 and 6), but reduced V_{max} and overshoot potential in both types of cell in a dose-related manner, implying reduction of fast inward sodium current (Class I action). APD_{50} was shortened in both tissues also but APD_{90} was lengthened in the terminal but shortened in the pre-terminal cells, in which inward sodium current persists during the plateau (Coraboeuf *et al.*, 1979; Attwell *et al.*, 1979; Carmeliet & Saikawa, 1982). The large effect on APD_{20} in the terminal Purkinje cells was associated with diminution of the transient outward current (the 'notch'), implying some reduction in outward potassium current, as is consistent with the lengthened APD in these cells. Representative records of Purkinje cell action potentials are presented in Figure 11.

Myocardium: Atrium Bevantolol had no effect on atrial resting potential or APD_{20}. V_{max} and overshoot potential were reduced in a dose-dependent manner, and recovered on drug washout (Table 7). APD_{50} and APD_{90} were prolonged, again in a dose-dependent manner, with recovery on washout. Thus in the atrium bevantolol appeared to restrict both fast inward current and repolarizing current, as does quinidine (Colatsky, 1982).

Ventricle The effects of bevantolol on the ventricle were broadly similar to those on the atrium. There was

Table 5 Effects of bevantolol on pre-terminal Purkinje cell action potential

	Bevantolol ($mol\,l^{-1}$)				
	0	*2×10^{-6}*	*4×10^{-6}*	*8×10^{-6}*	*Recovery*
APD_{20} (ms)	10.2 (0.8)	10.3 (0.9)	11.4 (0.9)	13.9 (1.6) *	10.8 (0.8) †
APD_{50} (ms)	207.5 (4.5)	197.2 (2.5) *	178.4 (5.8) ***	172.4 (4.1) ***	214.6 (3.9) †††
APD_{90} (ms)	250.3 (4.3)	245.7 (4.3)	241.8 (4.4) **	240.6 (1.0) ***	261.7 (3.6) †††
MRD (Vs^{-1})	299.0 (6.8)	291.1 (3.4) *	241.6 (9.3) ***	200.8 (8.9) ***	289.3 (6.7) †††
RP (mV)	−87.57 (1.3)	−87.59 (1.4)	−87.50 (1.6)	−87.51 (1.6)	−86.94 (1.7)
APA (mV)	121.6 (1.5)	120.6 (1.3)	119.8 (1.5)	117.2 (0.8) ***	120.3 (1.6) †††

Means (± s.e.mean) from four experiments.
Symbols as for Table 2.

Table 6 Effects of bevantolol on terminal Purkinje cell action potential

	Bevantolol ($mol\,l^{-1}$)				
	0	*2×10^{-6}*	*4×10^{-6}*	*8×10^{-6}*	*Recovery*
APD_{20} (ms)	6.8 (0.7)	43.0 (2.7) ***	53.5 (4.0) ***	59.0 (3.8) ***	21.2 (1.9) †††
APD_{50} (ms)	139.6 (3.1)	131.4 (3.7) **	125.1 (2.3) ***	129.5 (3.0) ***	141.8 (3.7) †††
APD_{90} (ms)	184.2 (3.8)	186.7 (3.1)	182.1 (4.0)	192.6 (3.0) ***	188.4 (3.7)
MRD (Vs^{-1})	250.4 (12.4)	226.4 (7.4) **	207.2 (8.5) ***	184.3 (8.3) ***	229.4 (8.7) †††
RP (mV)	−82.78 (0.6)	−82.73 (0.6)	−81.94 (0.6)	−82.92 (0.5)	−81.84 (0.5)
APA (mV)	123.3 (2.9)	119.1 (1.3)	114.8 (0.9)	110.3 (0.7) ***	118.6 (0.8) †††

Mean (± s.e.mean) from four experiments.
Symbols as for Table 2.

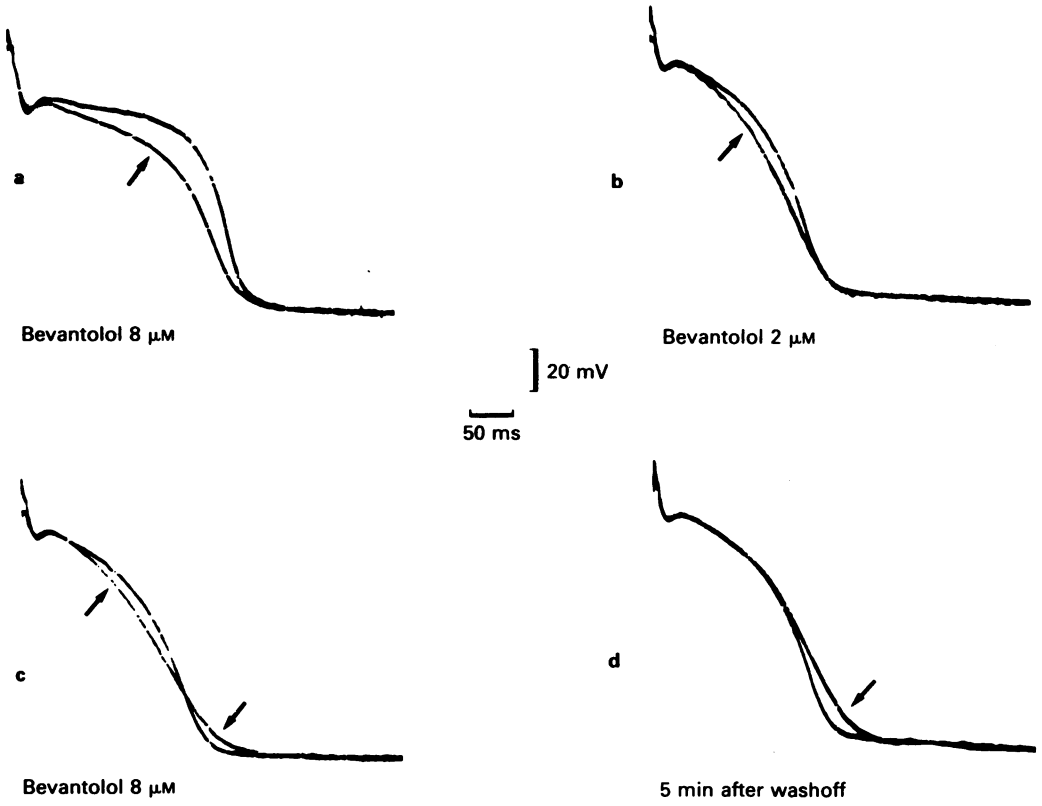

Figure 11 Intracellular records from Purkinje cells. Arrows depict tracings obtained in the presence of bevantolol, superimposed on the pre-drug control records. (a) Pre-terminal Purkinje cell. Bevantolol shortened APD_{50} and APD_{90} making the plateau markedly less positive. (b–d) Terminal Purkinje cell. Bevantolol at a concentration of 2×10^{-6} mol l^{-1}; (b) shortened APD_{50}, but had no effect on APD_{90}. At 8×10^{-6} mol l^{-1} (c) APD_{50} was still shorter, but APD_{90} was lengthened. (d) On washout the plateau was restored to normal, but the lengthening of APD_{90} persisted.

no effect on resting potential, and V_{max} and overshoot potential were reduced dose-dependently, with recovery on washout. Action potential duration at 20%, 50% and 90% levels was prolonged by the $2 \times$ and 4×10^{-6} mol l^{-1} concentrations, but the highest concentration caused less prolongation, implying that some additional effect on repolarization came into play at this level (e.g. block of plateau inward sodium current).

Discussion

We have confirmed the β-adrenoceptor blocking action of bevantolol, but were unable to assign a pA_2 value on the basis of inhibition of the chronotropic action of isoprenaline, because bevantolol itself caused bradycardia and in the spontaneously beating rabbit preparation the slopes of the isoprenaline dose-response curves before and after the drug were not parallel. The blocking effect of bevantolol in the pithed rat was much greater on the tachycardia than the hypotension induced by isoprenaline, implying β_1-adrenoceptor selectivity. In addition, bevantolol itself caused dose-related increases in blood pressure, blocked by phentolamine, and potentiated the hypertensive responses to noradrenaline, implying that bevantolol may be an α-adrenoceptor agonist. Bevantolol may also block presynaptic noradrenaline uptake, because in chemically sympathectomized rats the responses to noradrenaline alone were potentiated to the same degree as by the highest dose of bevantolol in normal animals.

Many β-adrenoceptor blocking drugs are iso-propyl or terbutyl propanolamines, with the OH at − 2, and a substituted phenoxy at −3. *Ortho*-substituted compounds (e.g. alprenolol, oxprenolol) block both β_1- and β_2-adrenoceptors, but the *para*-substituted com-

Table 7 Effects of bevantolol on myocardial action potentials

		Bevantolol ($mol\,l^{-1}$)				
		0	*2×10^{-6}*	*4×10^{-6}*	*8×10^{-6}*	*Recovery*
A. Atrium	APD_{50} (ms)	42.1 (0.7)	45.4 (0.3) *	53.4 (1.7) ***	63.2 (2.0) ***	44.3 (1.1) †††
	APD_{90} (ms)	88.2 (1.0)	96.8 (1.6) ***	110.2 (1.4) ***	119.9 (2.1) ***	92.1 (1.2) †††
	MRD (Vs^{-1})	126.7 (7.0)	113.5 (7.7) ***	102.9 (8.6) ***	86.0 (2.6) ***	119.5 (3.4) †††
	APA (mV)	102.30 (1.2)	97.20 (1.6) *	94.39 (1.6) ***	91.32 (1.3) ***	98.57 (1.4) †††
B. Ventricle	APD_{20} (ms)	38.3 (1.2)	43.2 (0.7) **	48.1 (0.6) ***	44.0 (1.3) **	40.1 (1.0) ††
	APD_{50} (ms)	82.7 (4.5)	91.2 (2.5) **	93.1 (5.8) ***	83.4 (4.1)	81.1 (3.9)
	APD_{90} (ms)	119.2 (2.4)	127.9 (3.0) ***	139.5 (1.4) ***	130.6 (1.0) ***	121.2 (1.1) †††
	MRD (Vs^{-1})	161.9 (10.7)	139.2 (5.4) ***	119.8 (4.0) ***	101.9 (4.2) ***	152.5 (5.6) †††
	APA (mV)	110.95 (2.0)	107.84 (1.9)	106.52 (1.3) **	102.67 (1.4) ***	108.55 (1.2) †††

Means (± s.e.mean) from four experiments.
Symbols as for Table 2.

pounds (e.g. practolol, atenolol) have less effect on β_2-adrenoceptors. It has been suggested that the β_1-receptor might be a cleft with open ends, but that the β_2-receptor might be of limited length, as depicted in section in Figure 1b, the two end walls being marked 1 and 3 (Vaughan Williams & Papp, 1970; Vaughan Williams *et al.*, 1973; Bagwell & Vaughan Williams, 1973). Since the ring can rotate about the oxygen bridge, an *ortho*-substituted compound could enter the cleft when the side-chain was perpendicular to the surface of the cell membrane, but the entry of a *para*-substituent, since it is in the axis of rotation, would always be obstructed by the barrier wall at 1. On this model a larger substituent at the amino end would also restrict entry to a β_2-receptor at 3, yet might still be able to enter an open-ended β_1-receptor. Bevantolol has only methyl at the *meta* position on the phenoxy group, so entry would not be restricted at 1, but the larger group at the amino end would account for its β_1-selectivity as a β-blocker provided the substituent does not also reduce β_1-activity, dimethoxyphenylethyl being optimal (Hoefle *et al.*, 1975).

Quite apart from its effects on adrenoceptors, bevantolol had direct actions on cardiac potentials. V_{max} and overshoot potential were reduced in atrium, ventricle and Purkinje cells in a dose-related manner, implying that fast inward current was reduced (Class I action). Action potential duration was increased in atrium and sino-atrial node, also in a dose-related manner, and all these effects were reversed on washout. It is of interest that selective α_1-adrenoceptor stimulation causes bradycardia by delaying repolarization in rabbit sinus node cells (Dukes & Vaughan Williams, 1984b). In ventricular muscle,

APD was lengthened by bevantolol, but the effect of 8×10^{-6} mol l^{-1} was less than that of 4×10^{-6} mol l^{-1}, as if a secondary shortening effect was added at the higher concentration. In the pre-terminal Purkinje cells, APD was actually shortened. A similar pattern has been observed previously with propafenone (Dukes & Vaughan Williams, 1984a). These results would be consistent with bevantolol delaying repolarization by reducing outward potassium current, as does quinidine, but also tending to shorten APD, especially in Purkinje cells, by blocking the plateau inward sodium current which is found predominantly in pre-terminal Purkinje cells. Prolongation of APD could thus contribute to an antiarrhythmic effect (Class 3), especially against atrial arrhythmias.

Bevantolol had no negative inotropic effect in isolated atria, and in pithed rats a very high mean arterial blood pressure was sustained after large doses of bevantolol, implying little impairment of cardiac contractility. The positive inotropic action of raised calcium concentrations was unaffected by bevantolol 5×10^{-6} mol l^{-1}. Thus it may be concluded that bevantolol did not block the calcium currents associated with excitation-contraction coupling in the myocardium (M). On the other hand, bevantolol caused bradycardia in association with a depressed V_{max} of the sino-atrial action potential upstroke, which took off from more positive voltages in the presence of bevantolol. Furthermore, V_{max} of AV nodal potentials was depressed, A–V conduction time was greatly increased in isolated preparations by bevantolol, and A–V block was induced in pithed rats. All these results would suggest that bevantolol blocked inward calcium currents responsible for depolarization in the sino-atrial and atrio-ventricular nodes (N). Thus the effects of bevantolol re-inforce the evidence already obtained (Dukes & Vaughan Williams, 1984c) that calcium currents in the nodes (N) are pharmacologically distinct from those involved in controlling the contractile process in the myocardium (M).

In conclusion, bevantolol is a β_1-adrenoceptor blocking drug with an unusual profile of additional properties. Its β_1-selectivity is not associated with substitution in the *para*-position of the ring, distinguishing it from atenolol, practolol and metoprolol. Like propranolol and oxprenolol, bevantolol restricts fast inward current (class 1 action), and, like sotalol, in atrial and ventricular muscle it prolongs APD (Class 3 action). In theory, therefore, it would be expected to have antiarrhythmic properties additional to that furnished by β-adrenoceptor blockade. On the other hand, bevantolol may have α-adrenoceptor agonist activity which could be arrhythmogenic (Sheridan *et al.*, 1980). This activity merits further study, especially in the CNS, since in pithed rats, bevantolol caused hypertension, but did not do so in the anaesthetized dogs studied by Hastings *et al.* (1977), implying that a peripheral vasoconstrictor action might be counteracted by some central effect. Baukema and Bovenkirk (unpublished), however, observed that bevantolol increased stroke volume and peripheral resistance in anaesthetized dogs, effects consistent with α-adrenoceptor agonist action causing a direct positive inotropic effect on the ventricular myocardium (Dukes & Vaughan Williams, 1984b), in addition to peripheral vasoconstriction. Although bevantolol appeared to block i_{si} in the nodes, it had no negative inotropic action, which could be advantageous in hypertensive patients with impaired myocardial function. It would be of great interest to learn whether prolonged treatment with bevantolol has antihypertensive actions similar to those of other β-blockers.

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