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Mode of action of (−)-pindolol on feline and human myocardium

Alberto J. Kaumann[1] & Brigitte M. Lobnig

Lehrstuhl fur Klinische Physiologie, Physiologisches Institut der Universitat Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf, FRG

1 (−)-Pindolol antagonized competitively and to a similar extent the positive inotropic effects of both (−)-noradrenaline and (−)-adrenaline in human ventricular preparations. An equilibrium dissociation constant K_D ($-\log$ mol l^{-1} = pK_D) of 9.2–9.3 was estimated regardless of disease present or agonist used.

2 (−)-Pindolol antagonized competitively the positive inotropic effects of (−)-adrenaline more than those of (−)-noradrenaline in human atrial preparations. pK_D values of (−)-pindolol were 9.6 against (−)-adrenaline and 9.1 against (−)-noradrenaline. The results are consistent with a moderate selectivity of (−)-pindolol for β_2- compared to β_1-adrenoceptors in human atrium.

3 (−)-Pindolol competed with [^{3}H]-(−)-bupranolol with a pK_D of 9.4 for β-adrenoceptors of human ventricle.

4 Positive inotropic effects of (−)-pindolol were not detected on human atrium or ventricle in a concentration range of 1–1000 nmol l^{-1}.

5 The affinity of (−)-pindolol estimated for human myocardial β-adrenoceptors, its moderate β_2-selectivity and its lack of intrinsic activity for contractile force agreed with similar characteristics in other species.

6 (−)-Pindolol caused marked positive chronotropic effects in kitten right atria with an intrinsic activity of 0.5 with respect to catecholamines. On kitten left atria it caused only weak positive inotropic effects with an intrinsic activity of 0.1. (−)-Pindolol (0.6–6000 nmol l^{-1}) did not cause positive inotropic effects in kitten papillary muscle.

7 The concentration-effect curve for (−)-pindolol on kitten right and left atria was biphasic. Its positive chronotropic and inotropic effects were not blocked by methysergide, suggesting that 5-hydroxytryptamine (5-HT)-receptors were not involved. Low concentrations of antagonists selective for β_1- and β_2-adrenoceptors blocked the high sensitivity component but not the low sensitivity component of the positive chronotropic and inotropic effects.

8 The biphasic nature of the positive chronotropic effects of (−)-pindolol in kitten agreed with previous observations made on guinea-pig right atria and support the concept that 3 receptors in the sinoatrial pacemaker contribute to these chronotropic effects: β_1, β_2 and a low-affinity receptor for (−)-pindolol which is neither β_1 nor β_2. The partial agonistic activity of (−)-pindolol in the heart appears to be mainly (kitten) or completely (man) restricted to the sinoatrial pacemaker.

Introduction

Pindolol is a high-affinity β-adrenoceptor blocking agent with sympathomimetic effects in tissues of rat (Barrett & Carter, 1970), cat (Kaumann & Blinks, 1980a,b) and guinea-pig (Walter *et al.*, 1984). What is the affinity of pindolol for β-adrenoceptors of human myocardium? Does pindolol exert sympathomimetic effects on human isolated myocardium? Human myocardium is known to contain both β_1- and β_2-adrenoceptors (Stiles *et al.*, 1983a,b; Brodde *et al.*, 1983; Gille *et al.*, 1985). In some tissues pindolol is slightly selective for β_2-adrenoceptors (Morris & Kaumann, 1984; Walter *et al.*, 1984). Does pindolol also exert some selectivity for β_2-adrenoceptors of human myocardium?

[1]Author for correspondence at: ICI Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG.

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To answer these questions we investigated the antagonistic action of pindolol of the positive inotropic effects of (−)-adrenaline and (−)-noradrenaline in isolated preparations of human right atrium and left ventricle. We chose (−)-pindolol because it is 200 to 300 times more potent than (+)-pindolol (Walter *et al.*, 1984; Morris & Kaumann, 1984), and because it is nearly exclusively the active species of the effects of racemic pindolol on human heart. We estimated the affinity of (−)-pindolol for β-adrenoceptors of human heart both by antagonizing the effects of catecholamines and by inhibiting the binding of [^{3}H]-(−)-bupranolol to ventricular β-adrenoceptors. As we were unable to detect any positive inotropic effects of (−)-pindolol in human atrial and ventricular preparations and because human sinoatrial pacemakers are not available, we used spontaneously beating right atria and paced left atrial strips of the kitten as models to study the nature of the positive chronotropic and inotropic effects of (−)-pindolol.

Methods

Isolated tissues of human heart

Myocardial tissues were excised from patients undergoing open heart surgery. The patients were selected as those not receiving β-adrenoceptor blocking agents or sympathomimetics for at least 1 week before the operation. The long-term medication of the patients comprised a variety of drugs (diuretics, digoxin, nitrates, etc). The right atrial appendage was excised for the introduction of cardiac bypass catheters in patients with coronary heart disease, aortic valve lesion and atrial septal defect. Left ventricular preparations were taken from patients with mitral valve lesion whose valve was replaced, and from patients with hypertrophic obstructive cardiomyopathy (HOCM) undergoing transaortal partial ablation of septal ventricular tissue. The tissues were transported to the laboratory in a sealed flask containing oxygenated solution at room temperature (20°C) of the following composition (mmol l^{-1}): Na^+ 120, K^+ 5, Ca^{2+} 2.25, Mg^{2+} 0.5, Cl^- 98.5, SO_4^{2-} 0.5, HCO_3^- 34, HPO_4^{2-} 1, EDTA 0.04, equilibrated with 95% O_2 and 5% CO_2. The water was deionized and double distilled in glass.

Dissection and setting up of the tissues was started within 5 min after surgical removal in a laboratory close to the operating theatres. The tissues were dissected without causing visible damage to atrial epicardium and ventricular endocardium. Strips, with a thickness <1 mm, were cut to facilitate diffusion of oxygen and drugs. Whenever possible, at least 3 strips were prepared from the atrium or ventricle of each patient. Only thin (width <1 mm) papillary muscles (from mitral valve lesion) were used without further dissection. Septal tissues of HOCM patients with subendocardial regions exhibiting sclerosis were not used because they were electrically inexcitable. The tissues were mounted in an apparatus (Blinks, 1965) containing 50 ml of the solution described above supplemented with (mmol l^{-1}): Na^+ 20, fumarate 5, pyruvate 5, L-glutamate 5, glucose 10.

Atrial and ventricular preparations were attached to strain-gauge transducers and driven at 2 s and 5 s intervals, respectively, with square-wave pulses of 5 ms duration and of just over threshold voltage. The length of atrial and ventricular tissues was adjusted to develop 20–30 and 50–70% of maximum force, respectively. The maximum catecholamine-induced force varied greatly for both atrial and ventricular preparations (4–22 mN).

To inhibit uptake of catecholamines and to block irreversibly myocardial α-adrenoceptors (Kaumann, 1970; 1972) the tissues were incubated for 2 h with 5 μmol l^{-1} phenoxybenzamine. This treatment causes irreversible potentiation of the effects of physiological catecholamines in atrial and ventricular tissues of man (Gille *et al.*, 1985).

To estimate equilibrium dissociation constants for (−)-pindolol ($-\log K_D = pK_D$, mol l^{-1}) we obtained a single concentration-effect curve for a catecholamine on atrial preparations and two successive concentration-effect curves on ventricular preparations, as validated previously (Gille *et al.*, 1985). On atria, simultaneous concentration-effect curves were determined usually on the first strip in the absence of (−)-pindolol and on the second and third strip in the presence of two different concentrations of (−)-pindolol. The degree of antagonism was estimated at the EC_{50} level and calculated as ratio of the concentrations (CR) of the chosen catecholamine in the presence and absence of (−)-pindolol resulting in log CR values.

For ventricle usually 3 or more strips were cut from one tissue. After the first concentration-effect curve for the chosen catecholamine had been determined, the 3 tissues were washed and the second and third strip were incubated with 2 different concentrations of (−)-pindolol. Ninety minutes after the incubation with (−)-pindolol, a second concentration-effect curve for the chosen catecholamine was determined in the absence (first strip) and presence (second and third strip) of (−)-pindolol. Log CR values were estimated as described above for EC_{50} levels (second and third strip) and corrected for desensitization (estimated on the first strip).

Kitten myocardial preparations

Experiments were carried out at 37°C on isolated left and right atria and papillary muscles of kittens (0.5–1.3 kg). The kittens were pretreated with

4 mg kg^{-1} reserpine s.c. 20 h before death. The anaesthetic used was chloroform; the hearts were rapidly removed and washed free of blood in the same solution as that used for human heart tissues. The muscles were set up in the apparatus with a 50 ml bath described by Blinks (1965). To study the chronotropic effects of (−)-pindolol, spontaneously beating right atria were suspended at a resting tension just sufficient for measurable development of tension. Two left atrial strips were dissected out and driven at 2 s intervals; their resting tension was adjusted to about ½ the level associated with maximum developed tension (after determination of a length tension curve) and the length of the strip was kept constant thereafter. Right ventricular papillary muscles and left ventricular strips (width <0.8 mm) were usually mounted in pairs and stretched to their optimal length (length at which maximal contractile force was developed). They were paced at 5 s intervals. Further conditions were the same as those used for human heart preparations.

Only a single concentration-effect curve was determined cumulatively for (−)-pindolol in each tissue. The incubation time for (−)-pindolol was 90 min for 0.6 nmol l^{-1}, 60 min for 2–20 nmol l^{-1}, and 40 min for 60 nmol l^{-1} and higher concentrations. Putative antagonists were incubated 90 min before a concentration-effect curve to (−)-pindolol was begun in the presence of the antagonist. After observing an equilibrium effect with the highest (−)-pindolol concentration (usually 20 μmol l^{-1}) the experiment was terminated by adding 0.2 mmol l^{-1} (−)-isoprenaline. Stimulant effects of (−)-pindolol were usually expressed as a percentage of the (−)-isoprenaline-induced increase in contractile force of paced tissues or beating rate in right atria.

Binding

Ventricular tissues from patients with a mitral lesion were transported and dissected on ice in the solution described under isolated tissues. Membrane particles were prepared as described by Kaumann & Birnbaumer (1974), as validated for human cardiac tissues (Kaumann *et al.*, 1982; Ferry *et al.*, 1985; Gille *et al.*, 1985). The protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Binding experiments were performed as described by Kaumann *et al.* (1982). The membrane suspension was incubated for 30 min at 37°C with the indicated concentrations of (−)-pindolol and 1.6 nmol l^{-1} [^{3}H]-(−)-bupranolol (Morris *et al.*, 1981) in a final volume of 200 μl incubation buffer containing (mmol l^{-1}): Tris-HCl (pH 7.6) 100, $MgCl_2$ 2, EGTA 1, ascorbic acid 0.2 and GTP 0.2. Binding observed in the presence of 0.2 mmol l^{-1} (−)-isoprenaline was considered to be non-specific. The reaction was stopped by the addition of 2 ml of ice-cold incubation medium. The membrane particles were collected by vacuum on Whatman GF/A glass-fibre filters and washed 7 times with 3 ml ice-cold washing solution (contained: 10 mmol l^{-1} Tris-HCl (pH 8.0) and 5 mmol l^{-1} $MgCl_2$). The filters were incubated with 0.5 ml Protocol (NEN) at 60°C for 30 min, then cooled on ice, acidified with 50 μl glacial acetic acid and treated with 8 ml of Econofluor (NEN). The radioactivity was measured by liquid scintillation counting. Counting efficiency and recovery were 46% and 65%, respectively.

The data of the binding inhibition experiments were fitted to equation (2) of Gille *et al.* (1985). Parameters were calculated after transforming radioactive counts (to obtain homoscedasticity) as described by Ehle *et al.* (1985).

Calculations and statistics of data from (−)-pindolol antagonism

The relationship between the degree of blockade of the positive inotropic effects of a catecholamine to (−)-pindolol concentration was analysed with the help of Schild plots (Arunlakshana & Schild, 1959).

The influence of agonists, tissues and disease on the affinity of (−)-pindolol for β-adrenoceptors was determined by two-way analysis of variance. The programme BMDP 3V (Dixon & Brown, 1979) was used. This programme performs a maximum-likelihood estimate of the effects. A detailed description of the method is found in the BMDP manual. All tests were performed at a 5% level. Significance was tested by a likelihood-ratio test, where the likelihood obtained with the full set of parameters was compared with the likelihood under the corresponding restrictions.

Drugs and materials

(−)-Noradrenaline L-tartrate and (−)-bisoprolol hemifumarate (Merck, Darmstadt, FRG); erythro-DL-(α-methyl-indan-4-yloxy) - 3-isopropylaminobutan-2-ol (ICI 118,551) hydrochloride (ICI, Macclesfield, UK); 1-[2((3-carbamoyl-4-hydroxy) phenoxy) ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2 - imidazolyl) phenoxy] - 2 - propranol methane sulphonate (CGP 20712A) and reserpine phosphate (CIBA-Geigy, Basle, Switzerland); [^{3}H]-(−)-bupranolol hydrochloride (specific activity 577 GBq mmol^{-1}; Sanol, Monheim, FRG); (−)-isoprenaline bitartrate (Sterling Winthrop, Rensselaer, N.Y., U.S.A.); phenoxybenzamine hydrochloride (Smith, Kline and French, Philadelphia, PA, U.S.A.); (−)-pindolol (prepared as a tartrate) and methysergide hydrogen maleate (Sandoz, Basle, Switzerland). Materials for the binding assays were the same as those of Morris *et al.* (1981). Other chemicals were obtained from local commercial sources.

Stock solutions of catecholamines (0.1 mol l^{-1}) contained 0.04 mmol l^{-1} EDTA and were adjusted to pH 4 with HCl; all dilutions were in 0.04 mmol l^{-1} EDTA.

Results

(−)-Pindolol does not cause inotropic stimulant effects on human cardiac preparations and on kitten papillary muscles

Atrial preparations which had not been exposed to any drug were incubated for 2 h with a single concentration of (−)-pindolol. In strips from 8, 6 and 5 patients 10, 100 and 1000 nmol l^{-1} (−)-pindolol, respectively, did not cause positive inotropic effects. Treatment with the β-adrenoceptor blocking agent (−)-bupranolol (10 nmol l^{-1}) (atria from 5 patients) or a 2 h pretreatment with 6 μmol l^{-1} phenoxybenzamine (see the following section) did not reveal positive inotropic responses to (−)-pindolol.

In untreated ventricular preparations 100 nmol l^{-1} (6 patients) and 1000 nmol l^{-1} (5 patients) (−)-pindolol did not cause positive inotropic effects. In addition, in ventricular preparations from 8 patients treated with 10 nmol l^{-1} (−)-bupranolol, 1000 nmol l^{-1} (−)-pindolol did not cause positive inotropic effects and in none of the phenoxybenzamine-pretreated ventricular preparations did it exert inotropic stimulation.

(−)-Pindolol (0.6–6000 nmol l^{-1}) did not cause positive inotropic effects in 6 right ventricular papillary muscles and in 6 left ventricular strips of kitten. These results agree with a lack of a positive inotropic effect of racemic pindolol described previously for the concentration range used (Kaumann & Blinks, 1980a).

(−)-Pindolol as a competitive antagonist in human cardiac tissues

Concentration-effect curves for the positive inotropic effects of catecholamines were shifted to the right by (−)-pindolol in a nearly parallel manner in both atrial and ventricular tissues (Figure 1). The maximum atrial response to a catecholamine was not significantly different in the absence or presence of (−)-pindolol at the concentrations used (not shown). The antagonism of the ventricular effects of catecholamines by (−)-pindolol were surmounted by higher cate-

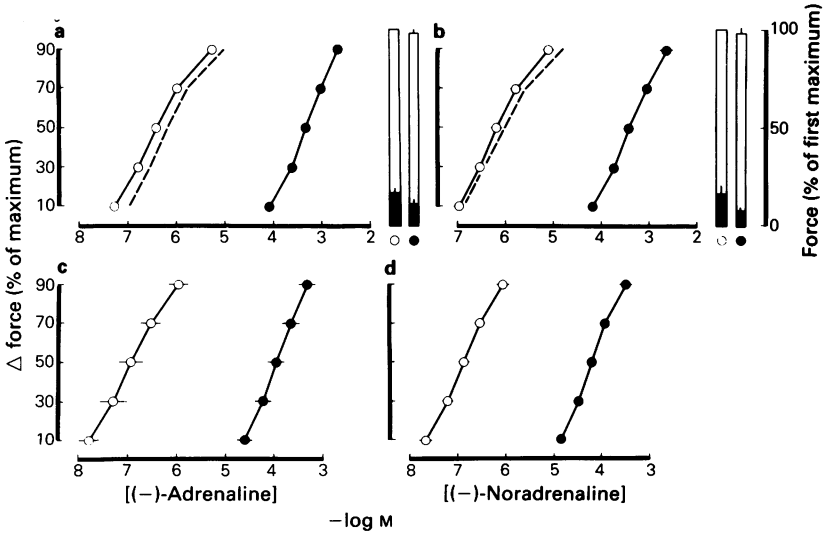

Figure 1 Antagonism by 300 nmol l^{-1} (−)-pindolol of the positive inotropic effects of catecholamines in human cardiac preparations. (a and b) Represent results on ventricular preparations from 14 patients with mitral valve lesion. Two successive concentration-effect curves were determined, the first in the absence (○) the second in the presence of (●) (−)-pindolol; for (−)-adrenaline $n = 8$ and for (−)-noradrenaline $n = 6$. Columns represent the basal force of contraction (solid portion) and maximum effects of catecholamines (open portion), vertical lines indicate s.e.mean. Broken lines represent a second curve determined in the absence of (−)-pindolol. (c and d) Represent results of atrial preparations from 10 patients with coronary heart disease. A single curve was determined on each tissue in the absence (○) or presence of (●) (−)-pindolol; $n = 4$ (−)-adrenaline, $n = 6$ (−)-noradrenaline. Horizontal bars through symbols of normalized concentration-effect curves represent s.e.means of n tissues (not shown if smaller than size of symbol).

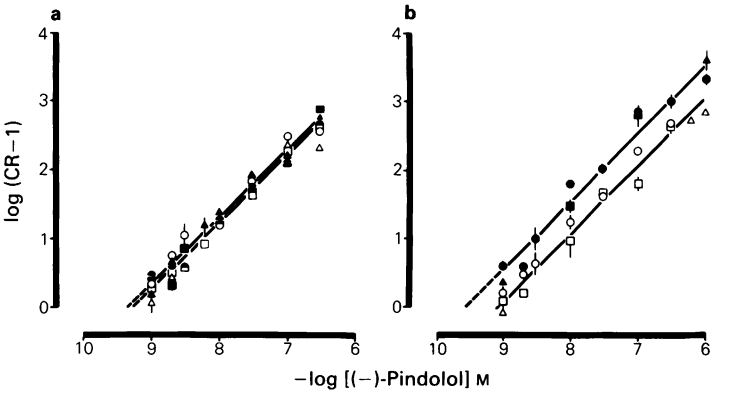

Figure 2 Comparison of Schild plots for (−)-pindolol as a function of different agonists (open symbols, (−)-noradrenaline; closed symbols, (−)-adrenaline) used in preparations from left ventricle (a) and right atrium (b) of patients with several diseases. In (a) symbols represent tissues from mitral lesion (■, □), residual mitral lesion (▲, △) and HOCM (●, ○). In (b) symbols represent tissues from coronary heart disease (●, ○), aortic valve lesion (■, □) and atrial septal defect (▲, △). To calculate slopes, one to three concentrations of (−)-pindolol per patient were used; data were pooled regardless of disease. Slopes are 0.96 against both (−)-adrenaline and (−)-noradrenaline in (a), and 1 against (−)-adrenaline and 0.99 against (−)-noradrenaline in (b). Vertical lines represent s.e.means; not shown if smaller than size of symbol. For further information and number of experiments see Table 1.

cholamine concentrations (Figure 1). This blocking pattern suggests that (−)-pindolol is a simple competitive antagonist.

Effect of (−)-pindolol on the positive inotropic effects of (−)-adrenaline and (−)-noradrenaline on atrial preparations

(−)-Pindolol was a more potent antagonist of the effects of (−)-adrenaline than of those of (−)-noradrenaline, regardless of disease. The slope of the Schild plots was not significantly different from 1, regardless of agonist (Figure 2b; Table 1), supporting a mode of simple competitive inhibition. Equilibrium dissociation constants (K_Ds, Table 1) were calculated and found to be independent of disease. However, K_D values of (−)-pindolol against (−)-adrenaline were consistently lower (i.e. higher affinity) than those against (−)-noradrenaline.

Effect of (−)-pindolol on the positive inotropic effects of (−)-adrenaline and (−)-noradrenaline in ventricular muscle

Slopes of Schild plots were not different from 1 regardless of the catecholamine used (Figure 2a, Table 1). K_D values for (−)-pindolol antagonism of the effects of (−)-adrenaline were not different from those against (−)-noradrenaline (Table 1).

Affinity of (−)-pindolol for ventricular β-adrenoceptors labelled with [^{3}H]-(−)-bupranolol

Figure 3 depicts the inhibition of binding of [^{3}H]-(−)-bupranolol to ventricular β-adrenoceptors by (−)-pindolol. The shape of the binding inhibition curve was nearly monophasic. From the experiment in Figure 3 and an additional experiment an average equilibrium dissociation constant ($-\log \text{mol}\,l^{-1}$) of 9.4 was estimated.

Stimulant effects of (−)-pindolol on kitten atria

(−)-Pindolol elicited positive chronotropic effects and weak inotropic effects in kitten atria (Figure 4). The intrinsic activity of (−)-pindolol with respect to (−)-isoprenaline was 0.5 on spontaneously beating right atria and 0.1 on paced left atrial strips. The concentration-effect curve for (−)-pindolol was biphasic on both right atria (Figures 4 and 5) and on left atria (Figures 4 and 6). As encountered before for racemic pindolol (Kaumann & Blinks, 1980a,b), the concentration-effect curve for (−)-pindolol was flat (covering 4 log units) and located substantially to the right of the corresponding curves for β-adrenoceptor occupancy by (−)-pindolol in right atria (Figure 4) and left atria (Figure 6).

Table 1 Equilibrium dissociation constants, K_D ($-\log K_D = pK_D$, $mol\,l^{-1}$), and slopes of Schild plots for (−)-pindolol

Tissue	*Disease*	*Agonist*	n	*Slope ± s.e.mean*	*pK_D ± s.e.mean*
	CHD	(−)-Adrenaline	17[a] (29[b])	0.98 ± 0.04 (10)[c]	9.51 ± 0.06
I	ASD	(−)-Adrenaline	2 (3)	1.18 (1)	9.51 ± 0.18
Atrium	AVL	(−)-Adrenaline	4 (6)	1.03 ± 0.14 (2)	9.60 ± 0.15
	CHD	(−)-Noradrenaline	17 (31)	1.10 ± 0.04 (13)	9.18 ± 0.06
	ASD	(−)-Noradrenaline	2 (4)	0.92 (1)	8.91 ± 0.16
	AVL	(−)-Noradrenaline	5 (11)	0.98 ± 0.05 (4)	8.99 ± 0.10
	MVL	(−)-Adrenaline	10 (26)	1.00 ± 0.04 (9)	9.23 ± 0.06
II	MVLR	(−)-Adrenaline	4 (16)	1.00 ± 0.12 (4)	9.33 ± 0.08
Ventricle	HOCM	(−)-Adrenaline	5 (18)	0.93 ± 0.08 (5)	9.27 ± 0.07
	MVL	(−)-Noradrenaline	14 (37)	1.04 ± 0.04 (10)	9.23 ± 0.05
	MVLR	(−)-Noradrenaline	4 (9)	0.91 ± 0.15 (3)	9.02 ± 0.09
	HOCM	(−)-Noradrenaline	5 (15)	0.97 ± 0.06 (5)	9.34 ± 0.07
III					
Atrium		(−)-Adrenaline	23 (36)	0.99 ± 0.04 (13)	9.55 ± 0.05
Atrium		(−)-Noradrenaline	24 (46)	1.06 ± 0.04 (18)	9.11 ± 0.05
Ventricle		(−)-Adrenaline	19 (60)	0.96 ± 0.04 (18)	9.27 ± 0.05
Ventricle		(−)-Noradrenaline	23 (61)	1.00 ± 0.04 (18)	9.22 ± 0.04

CHD = coronary heart disease, ASD = atrial septal defect, AVL = aortic valve lesion, MVL = mitral valve lesion, MVLR = mitral valve lesion recidive, HOCM = hypertrophic obstructive myopathy, [a] = number of patients, [b] = number of tissues, [c] = number of individual slopes, I, II, III = two-way analysis of variance of the blocking potency of (−)-pindolol. I = the effects of (−)-adrenaline in atrium are blocked significantly more than those of (−)-noradrenaline. Neither disease nor the interaction of disease with agonist was significant. II = the influence of agonist and disease in ventricle. None of the main effects or interactions was significant. III = the effects of tissue and agonist. The main effect of agonist and the interaction of tissue and agonist was significant, indicating that the effects of (−)-adrenaline are blocked more potently than those of (−)-noradrenaline, but only on atrium.

Effect of β_1- and β_2-adrenoceptor blocking agents and methysergide on the stimulant effects of (−)-pindolol in kitten atria

Concentrations of the β_1-selective antagonists (−)-bisoprolol and CGP 20172A that saturate β_1-adrenoceptors without causing significant antagonism of β_2-adrenoceptors partially blocked the high-sensitivity component of the positive chronotropic effects of (−)-pindolol (Figure 5a). However, the antagonism was smaller than expected from the affinity of (−)-bisoprolol and CGP 20172A for β_1-adrenoceptors (Table 2). A concentration of ICI 118,551 that saturates β_2-adrenoceptors also caused antagonism of the high-sensitivity component of the positive chronotropic effects of (−)-pindolol (Figure 5c). However, the antagonism by ICI 118,551 was smaller than expected from the affinity of the drug for β_2-adrenoceptors (Table 2).

A combination of ICI 118,551 with (−)-bisoprolol (Figure 5c) or with CGP 20712A (Figure 5b) caused substantially less blockade than expected from an exclusive involvement of β_1- and β_2-adrenoceptors (Table 2) in the chronotropic effect of (−)-pindolol.

Methysergide did not block the effects of (−)-pindolol on the sinoatrial node and actually appeared to sensitize right atria to (−)-pindolol (Figure 5d) even in the presence of both ICI 118,551 and CGP 20712A (Figure 5b).

The combination of (−)-bisoprolol and ICI 118,551 caused blockade of the high-sensitivity component of the positive inotropic effects of (−)-pindolol on kitten left atria. After joint blockade of both β_1- and β_2-adrenoceptors the positive inotropic curve for (−)-pindolol became monophasic (Figure 6a). Methysergide did not affect the positive inotropic effects of (−)-pindolol (Figure 6b). The lack of blockade by methysergide of the stimulant atrial effects of (−)-pindolol contrasts with the considerable antagonism by methysergide of the positive inotropic and chronotropic effects of 5-hydroxytryptamine on kitten atria (Table 2).

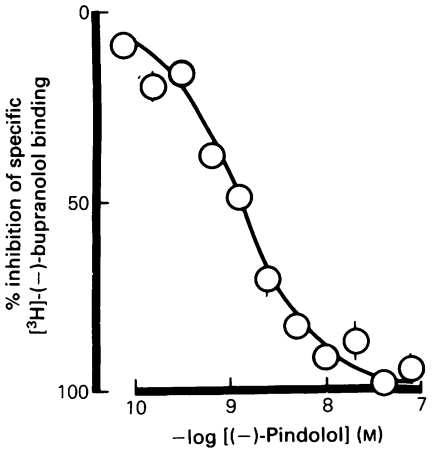

Figure 3 Inhibition of binding of [^{3}H]-(−)-bupranolol by (−)-pindolol. Membrane particles were derived from left ventricular myocardium of patients with lesions of the mitral valve. Protein concentration was 300 mg l^{-1}. Binding of [^{3}H]-(−)-bupranolol was 42 and 19.5 fmol mg^{-1} in the absence of competing ligand and in the presence of 0.2 mmol l^{-1} (−)-isoprenaline, respectively. Each point represents mean, with vertical lines indicating s.e.mean, of quadruplicates (s.e.mean not shown if smaller than symbol). The equilibrium dissociation constant (K_D) of (−)-pindolol estimated by non-linear regression was 9.43 ± 0.11. In an experiment on membranes from another patient with mitral lesion we estimated a K_D of 9.39 ± 0.10 for (−)-pindolol.

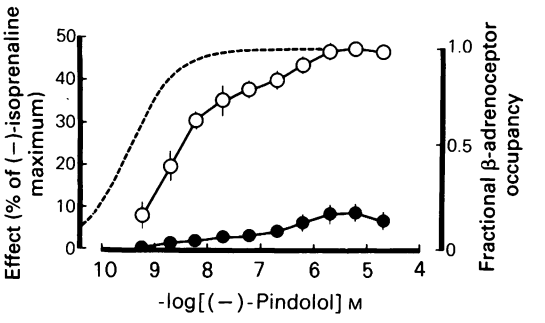

Figure 4 Tissue difference in the stimulant effects of (−)-pindolol on kitten atria. (○) Effect on rate of spontaneously beating right atria (n = 6); frequency was 127 ± 12 beats min^{-1} (mean ± s.d.) in the absence and 213 ± 25 beats min^{-1} in the presence of 0.2 mmol l^{-1} (−)-isoprenaline. (●) Effect on force of paced left atrial strips (n = 12). Vertical lines indicate s.e.mean of n tissues. The broken line depicts the curve for β-adrenoceptor occupancy by (−)-pindolol (P), calculated from [P]/([P] + K_D), where K_D is the equilibrium dissociation constant estimated from the antagonism of the positive chronotropic effects of (−)-isoprenaline (Table 3).

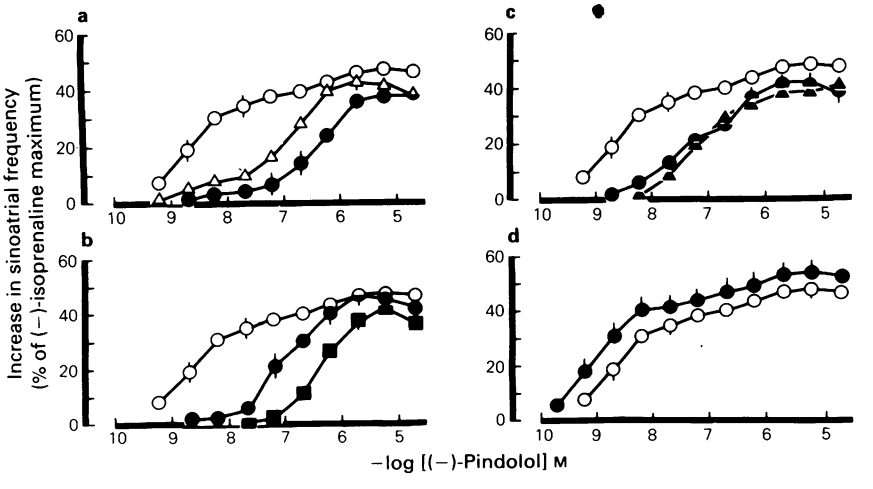

Figure 5 Influence of blocking agents, alone or in combination, on the stimulant chronotropic effects of (−)-pindolol in kitten right atria. (○) Represent the effects of (−)-pindolol in the absence of an antagonist; the curves were taken from Figure 4. Antagonists used: (a) 0.1 μmol l^{-1} (−)-bisoprolol (△, n = 6), 0.5 μmol l^{-1} CGP 20712 A (○, n = 4); (b) 0.1 μmol l^{-1} ICI 118,551 + 0.5 μmol l^{-1} CGP 20712A (■, n = 4), 0.1 μmol l^{-1} ICI 118,551 + 0.5 μmol l^{-1} CGP 20712A + 1 μmol l^{-1} methysergide (●, n = 4); (c) 0.1 μmol l^{-1} ICI 118,551 (●, n = 3), 0.1 μmol l^{-1} ICI 118,551 + 0.1 μmol l^{-1} (−)-bisoprolol (▲, n = 6); (d) 1 μmol l^{-1} methysergide (●, n = 4). Vertical lines show s.e.mean of n atria (not shown if smaller than symbol).

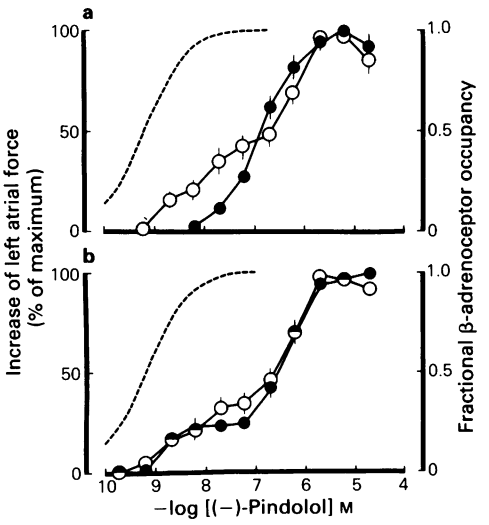

Figure 6 Influence of blocking agents on the stimulant inotropic effects of (−)-pindolol on kitten left atrial strips. In (a) a single concentration-effect curve for (−)-pindolol determined in the absence of blocking agent (○, atria from 12 kittens), or in the presence of both (−)-bisoprolol and ICI 118,551 (●, atrial from 6 kittens). (b) Shows results on atria from 4 kittens; on 4 atrial halves a control curve for (−)-pindolol was determined in the absence of methysergide (○), on the other corresponding 4 halves the curve was determined in the presence of methysergide (●). Vertical lines represent s.e.mean (not shown if smaller than symbol). The broken lines depict curves for the β-adrenoceptor occupancy by (−)-pindolol (P), calculated from [P]/([P] + K_D), where the corresponding K_D value was taken from Table 3 (see also legend to Figure 4).

Discussion

The affinity of (−)-pindolol for cardiac β-adrenoceptors in man and other mammals

We found good agreement between our affinity estimates of (−)-pindolol for β-adrenoceptors of human myocardium and those for the myocardium of other species (Table 3). This finding is virtually independent of the method or system used provided the affinity estimates are obtained under equilibrium conditions. Similar accordance has been observed for the affinity of the β-adrenoceptor antagonists (−)-bupranolol (Kaumann, 1983; Kaumann *et al.*, 1982) and (−)-propranolol (Gille *et al.*, 1985) for β-adrenoceptors of a variety of species including man. It also appears that the estimates of molecular weight of human myocardial β-adrenoceptors are similar to those in other species (Stiles *et al.*, 1983b). These similar values of affinity characteristics and structure suggest that cardiac β-adrenoceptors in man and other mammals are identical. However, the β-adrenoceptor system that mediates increases in contractile force of human myocardium differs from that of other species in 2 respects: (1) catecholamines are 50 times less effective in enhancing myocardial contractile force in man than in other species (Kaumann *et al.*, 1982). (2) (−)-Pindolol is an inotropic partial agonist in feline atrium but not in human atrium (present work). These differences are presumably due to a less efficient activation in man than in other species of some of the components of the biochemical cascade causing an increase in contractile force of the heart.

The affinity of (−)-pindolol for β_1- and β_2-adrenoceptors of human myocardium

(−)-Pindolol antagonized the positive inotropic effects of (−)-adrenaline consistently more than those of (−)-noradrenaline in human atrium. We interpret this as a result of an important contribution of β_2-adrenoceptors to the increase of atrial force caused by (−)-adrenaline and as a result of a higher affinity of (−)-pindolol for β_2- than for β_1-adrenoceptors. The greater affinity of (−)-pindolol estimated against (−)-adrenaline than against (−)-noradrenaline is consistent with this interpretation. Up to ½ of the atrial β-adrenoceptor population of man has been estimated from binding of ligands to membranes of fresh cardiac tissues, to have characteristics of β_2-adrenoceptors (Stiles *et al.*, 1983a; Brodde *et al.*, 1983; Heitz *et al.*, 1983). The affinity of (−)-noradrenaline is 10 times lower for β_2- than for β_1-adrenoceptors, while the affinity of (−)-adrenaline is similar for β_1- and β_2-adrenoceptors (Lemoine *et al.*, 1985a,b). It is therefore likely that atrial inotropic effects of (−)-adrenaline are mediated through both β_1- and β_2-adrenoceptors while the effects of (−)-noradrenaline occur mostly through β_1-adrenoceptors.

We assume that (−)-pindolol has a higher affinity for β_2- than for β_1-adrenoceptors. For theoretical reasons, it is not possible to detect a differential affinity of (−)-pindolol from binding inhibition experiments in a system containing both receptor subtypes because the difference in affinity is too low. Nevertheless, an overall binding constant of (−)-pindolol for lung β-adrenoceptors of guinea-pig (80% β_2, Lemoine *et al.*, 1985a,b) and calf (75% β_2, Morris & Kaumann, 1984) is a good approximation of affinity for β_2-adrenoceptors and agrees closely with the estimate for tracheal β_2-adrenoceptors obtained by Walter *et al.* (1984). On average the equilibrium dissociation constant of (−)-pindolol in these 3 systems is 9.5 (Table 3). On the other hand, with the exception of cat sinoatrial β-adrenoceptors known to

Table 2 Comparison of the observed antagonism of the positive chronotropic effects of (−)-pindolol with the antagonism expected from blockade of β_1- and β_2-adrenoceptors and 5-hydroxytryptamine (5-HT)-receptors in kitten right atria

	Concentration ($nmol\,l^{-1}$)	*Receptor*	($nmol\,l^{-1}$)	*Concentration-ratio* *Expected*	*Observed*[f]
(−)-Bisoprolol (Bis)	100	[a]β_1	2	50	
		[a]β_2	100	2	
		$\beta_1 + \beta_2$		100[e]	30
(±)-CGP 22017A (CG)	500	[b]β_1	0.2	2500	
		[b]β_2	2,500	1	
		$\beta_1 + \beta_2$		2500[e]	50
				<2	
ICI 118,551 (ICI)	100	[c]β_1	100	2	
		[c]β_2	0.3	300	
		$\beta_1 + \beta_2$		600[e]	20
Methysergide	1000	[d]5-HT_3		50	−3
Bis + ICI	100 + 100	$\beta_1 + \beta_2$		20,000[e]	30
CG + ICI	500 + 100	$\beta_1 + \beta_2$		1,000,000[e]	200

K_D Equilibrium dissociation constant. [d]From Kaumann & Lemoine (1985). [b]From Kaumann (1986) and Lemoine *et al.* (1985b). [c]From Lemoine *et al.* (1985a). [d]From Kaumann (1985). [e]Product of the concentration-ratios expected from β_1- and β_2-adrenoceptors (Paton & Rang, 1965). [f]The concentration-ratio of (−)-pindolol was taken at the 15 beats min^{-1} level from the experiments depicted in Figure 5. Neither the basal beating rate nor the maximum rate attained with 0.2 $mmol\,l^{-1}$ (−)-isoprenaline was changed by the antagonists. In a pool of 31 atria, treated with various antagonists, 0.2 $mmol\,l^{-1}$ (−)-isoprenaline enhanced the rate from 130 ± 14 beats min^{-1} to 217 ± 21 beats min^{-1} (mean ± s.d.).

have a considerable involvement of β_2-adrenoceptors (Carlsson *et al.*, 1972; Kaumann & Marano, 1982; Kaumann & Lemoine, 1985), the average equilibrium dissociation constant of (−)-pindolol for cardiac β-adrenoceptors is 9.2 (Table 3) and an approximation for β_1-adrenoceptors (see also Walter *et al.*, 1984). Thus, evidence from various species and systems suggests that (−)-pindolol is β_2-selective. The affinity of (−)-pindolol estimated on human atrium against (−)-adrenaline (β_2) and (−)-noradrenaline (β_1) is consistent with a 2–3 fold β_2-selectivity of the antagonist.

Table 3 Estimation of equilibrium dissociation constants ($-\log mol\,l^{-1}$) for the (−)-pindolol-β-adrenoceptor complex by various methods in several species

Species	*Inotropic* *Atrium*	*Ventricle*	*Chronotropic sinoatrial node*	*Adenylate cyclase*	*Tracheal relaxation*	*Binding* *Lung*	*Ventricle*
Man	9.6[a]–9.1[b]	9.1[a]–9.0[b]	–	–	–	–	9.4[c]
Guinea-pig	–	–	9.5[d]	–	9.6[d]	9.4[e]	9.1[e]–4.9[f]
Cat	9.2[g]	9.0[g]	9.5[h]	9.3[i,j]	–	–	9.1[k]
Rat	–	–	9.1[l]	–	–	–	–
Calf	–	–	–	–	–	9.5[m]	9.3[m]

[a]This paper, against (−)-adrenaline. [b]This paper, against (−)-noradrenaline. [c]This paper, against [^{3}H]-(−)-bupranolol. [d]Walter *et al.* (1984) against both (−)-adrenaline and (−)-noradrenaline. [e]Walter *et al.* (1984) against [^{3}H]-(−)-bupranolol (high affinity site for (−)-pindolol). [f]Walter *et al.* (1984) against [^{3}H]-(−)-bupranolol (low affinity site for (−)-pindolol). [g]Lemoine & Kaumann (unpublished) against (−)-isoprenaline ((±)-pindolol, corrected for (−)-pindolol). [h]Kaumann & Blinks (1980b) against (−)-isoprenaline ((±)-pindolol, corrected for (−)-pindolol). [i]Kaumann *et al.* (1979) against (−)-isoprenaline. ((±)-pindolol, corrected for (−)-pindolol). [j]Kaumann & Birnbaumer (1974) against (−)-isoprenaline ((±)-pindolol, corrected for (−)-pindolol). [k]Morris *et al.* (1981) against [^{3}H]-(−)-bupranolol. [l]Kaumann *et al.* (1979) against (−)-isoprenaline ((±)-pindolol, corrected for (−)-pindolol). [m]Morris & Kaumann (1984) against [^{3}H]-(−)-propranolol.

In human ventricular myocardium, however, the effects of (−)-adrenaline and (−)-noradrenaline are antagonized by (−)-pindolol with the same relatively low potency. The affinity estimate of (−)-pindolol against both (−)-adrenaline and (−)-noradrenaline accords with the affinity for β_1-adrenoceptors, suggesting that most ventricular inotropic effects of the catecholamines are mediated through β_1-adrenoceptors. Human ventricle contains a small fraction of β_2-adrenoceptors (Stiles *et al.*, 1983a) responsible for the submaximal inotropic effect of (−)-adrenaline (Kaumann *et al.*, 1985a) as revealed with highly β_1-selective antagonists. Because of the small fraction of β_2-adrenoceptors in human ventricle and the small β_2-selectivity we did not observe a differential antagonism of the effects of (−)-adrenaline and (−)-noradrenaline.

Nature of the stimulant inotropic effect of (−)-pindolol in kitten left atrium

In agreement with previous results with racemic pindolol (Kaumann & Blinks, 1980a), (−)-pindolol did not cause positive inotropic effects in kitten papillary muscle, but caused some effects in kitten left atria which we have now analysed. Combined blockade of β_1- and β_2-adrenoceptors abolished only the high-sensitivity component of the concentration-effect curve to (−)-pindolol, suggesting that the bulk of the positive inotropic effect of (−)-pindolol is not mediated through β_1- or β_2-adrenoceptors. Concentration-effect curves of (−)-pindolol are located at considerably higher concentrations than the β-adrenoceptor occupancy curve calculated from antagonism by (−)-pindolol of the atrial effects of catecholamines. This dissociation between blockade and stimulation by (−)-pindolol agrees with a previously observed pattern with racemic pindolol (Kaumann, 1973; 1983; Bilski & Wale, 1976; Kaumann & Blinks, 1980b) and further suggests that the bulk of the inotropic stimulant effects of (−)-pindolol are not mediated through β_1- or β_2-adrenoceptors. Both 5-hydroxytryptamine (5-HT) and pindolol are indoles that could conceivably interact with a common receptor, as for instance described in brain tissue (Nahorski & Willcocks, 1983). Are 5-HT-receptors involved in the stimulant inotropic effects of (−)-pindolol? The answer is no because 1 μmol l^{-1} methysergide, a concentration causing a 1.7 log unit shift of the concentration-effect curve for the positive inotropic response to 5-HT in kitten left atria (Kaumann, 1985), did not antagonize the effects of (−)-pindolol.

Although the mechanism by which the low-sensitivity stimulant effects of (−)-pindolol on kitten left atria occur is still unknown, it is possible that a low-affinity receptor for (−)-pindolol, postulated by Walter *et al.* (1984), may be involved.

Nature of the stimulant chronotropic effects of (−)-pindolol in kitten sinoatrial node

As described for racemic pindolol (Kaumann & Blinks, 1980a,b), (−)-pindolol is a strong partial agonist (intrinsic activity 0.5) exhibiting a biphasic concentration-effect curve on kitten right atria. Our results with subtype-selective blocking agents are consistent with an involvement of both β_1- and β_2-adrenoceptors in the high-sensitivity stimulant component of (−)-pindolol.

However, the results depicted in Figure 5 and Table 2 show that although the antagonists always caused a blockade greater than expected for the β-subtype for which they exhibit low affinity, the blockade was consistently lower than expected for the corresponding β-subtype for which they exhibit high affinity. Hence, the stimulant chronotropic effects of (−)-pindolol cannot solely be due to an interaction with β_1- and β_2-adrenoceptors.

The β-adrenoceptor occupancy curve calculated for (−)-pindolol from antagonism of the effects of catecholamines is located at lower concentrations than the curve for its chronotropic stimulant effects. A similar dissociation between stimulation and blockade has been previously observed with racemic pindolol (Kaumann, 1973; 1983; Bilski & Wale, 1976; Kaumann & Blinks, 1980b). The dissociation between stimulation and blockade further does not tally with the assumption that all of the stimulating effects of (−)-pindolol are mediated through β_1- and β_2-adrenoceptors.

Sinoatrial 5-HT-receptors mediate positive chronotropic effects of 5-HT; in the cat these effects are antagonized by methysergide (Trendelenburg, 1960). However, the stimulant chronotropic effects of (−)-pindolol are unlikely to be mediated through sinoatrial 5-HT-receptors as 1 μmol l^{-1} methysergide, a concentration causing a 1.7 log unit shift of the concentration-effect curve for 5-hydroxytryptamine in kitten atria (Kaumann, 1985), did not block the effects of (−)-pindolol. Surprisingly, and for reasons that are not known, methysergide appeared to sensitize the sinoatrial node to the stimulant effects of (−)-pindolol. This suggests that methysergide and (−)-pindolol do not compete for the same receptor. A similar conclusion was reached for the stimulant chronotropic effects of (−)-pindolol on guinea-pig atria (Walter *et al.*, 1984).

The potentiating effect of methysergide on the stimulant chronotropic effects of (−)-pindolol is puzzling. Methysergide has been shown to be an allosteric regulator of 5-HT_2-receptors in a variety of smooth muscle systems (Kaumann & Frenken, 1985; Kaumann *et al.*, 1985b). Perhaps methysergide acts on some allosteric site in the sinoatrial node that

facilitates the stimulant chronotropic effects of (−)-pindolol.

We suggest that the bulk of the stimulant chronotropic effect of (−)-pindolol is mediated by a receptor which is distinct from β_1- and β_2-adrenoceptors or from sinoatrial 5-HT-receptors. We adhere to and extend to kitten sinoatrial pacemaker, the proposal of Walter *et al.* (1984) that the majority of the stimulant chronotropic effect of (−)-pindolol is mediated in guinea-pig atria through receptors with low affinity for (−)-pindolol. A low-affinity site for (−)-pindolol has been detected in radiolabelled β-adrenoceptors in rat glioma cells (Maguire *et al.*, 1976) and guinea-pig ventricle (Walter *et al.*, 1984), and may well correspond to the receptor that predominantly mediates the stimulant chronotropic and inotropic effects of (−)-pindolol.

Pindolol and the human heart

We did not detect any stimulant inotropic effect of (−)-pindolol in isolated preparations of human right atrium and left ventricle. This is not surprising because concentrations up to 6 μmol l^{-1} of racemic pindolol (Kaumann & Blinks, 1980a) or (−)-pindolol (this paper) do not increase contractile force in kitten papillary muscle and cause only slight positive inotropic effects in kitten left atria. However, it would seem that these negative findings may contradict an increase in stroke volume and cardiac output measured under the influence of pindolol in patients with severe orthostatic hypotension (Man in't Veld & Schalekamp, 1981). We interpret the increase in cardiac output caused by pindolol merely as a result of the increase in heart rate, also measured by Man in't Veld & Schalekamp. The increase in heart rate may cause a positive staircase effect, i.e. the higher the heart rate, the stronger the ventricular contractions and the greater the cardiac output. Data of Clark *et al.* (1982) support our explanation because the dependence on (−)-pindolol concentration of increases in heart rate and myocardial contractile force is similar in spinal cats. An *in vitro* observation of Ferry *et al.* (1985) supports the staircase concept in man. They increased the pacing rate of isolated ventricular preparations of man between 12 and 120 contractions per minute and observed a proportional increase in contractile force with pacing frequency.

In conclusion, as in the kitten, (−)-pindolol appears to be only a partial agonist in the human sinoatrial pacemaker but not on human ventricle. The possibility has to be taken seriously that, in addition to β_1- and β_2-adrenoceptors, a low-affinity receptor also mediates the stimulant chronotropic effects of (−)-pindolol in man, as observed in guinea-pig and kitten.

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