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Contribution of β_1- and β_2-adrenoceptors of human atrium and ventricle to the effects of noradrenaline and adrenaline as assessed with (−)-atenolol

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1 (−)-Atenolol was used as a tool to assess the function of β_1- and β_2-adrenoceptors in human heart. Right atrial and left ventricular preparations from patients undergoing open heart surgery were set up to contract isometrically. Membrane particles were prepared for β-adrenoceptor labelling with [^{3}H]-(−)-bupranolol and adenylate cyclase assays.

2 The positive inotropic effects of (−)-noradrenaline were antagonized to a similar extent by (−)-atenolol in atrial and ventricular preparations. (−)-Atenolol consistently antagonized the effects of (−)-adrenaline to a lesser extent than those of (−)-noradrenaline in atrial preparations. In ventricular preparations (−)-atenolol antagonized the effects of low concentrations of (−)-adrenaline to a lesser extent than those of high concentrations.

3 pK_B values (M) of (−)-atenolol, estimated with non-linear analysis from the blockade of the positive inotropic effects of the catecholamines, were 7.4 for β_1-adrenoceptors and 6.0 for β_2-adrenoceptors.

4 (−)-Atenolol inhibited the binding of [^{3}H]-(−)-bupranolol to ventricular β_1-adrenoceptors with a pK_D (M) of 5.9 and to ventricular β_2-adrenoceptors with a pK_D of 4.6.

5 (−)-Atenolol inhibited the catecholamine-induced adenylate cyclase stimulation in the atrium and ventricle with pK_B values of 5.8–6.4 for β_1- and pK_B values of 4.7–5.7 for β_2-adrenoceptors. The binding and cyclase assays suggest a partial affinity loss for (−)-atenolol inherent to membrane preparations.

6 β_1-Adrenoceptors mediate the maximum positive inotropic effects of (−)-noradrenaline in both the atrium and ventricle of man. β_2-Adrenoceptors appear to be capable of mediating maximal positive inotropic effects of (−)-adrenaline in atrium. In contrast, ventricular β_2-adrenoceptors mediate only submaximal effects of (−)-adrenaline.

Introduction

Evidence from binding assays increasingly shows that β_1- and β_2-adrenoceptors coexist in the human ventricle (Stiles *et al.*, 1983; Gille *et al.*, 1985; Kaumann & Lemoine, 1987) and right atrium (Brodde *et al.*, 1983; Heitz *et al.*, 1983). The adenylate cyclase appears to be coupled preferentially to β_2-adrenoceptors in membrane particles obtained from human atrium and ventricle (Robberecht *et al.*, 1983; Gille *et al.*, 1985; Kaumann & Lemoine, 1987). However, in human ventricle, although both β_1- and β_2-adrenoceptors mediate positive inotropic effects of (−)-adrenaline and (−)-noradrenaline, β_1-adrenoceptors are predominantly involved, as expected from their greater relative density (Kaumann & Lemoine, 1987). What is the contribution of β_1- and β_2-adrenoceptors of human atrium to the positive inotropic effects of (−)-adrenaline and (−)-noradrenaline? To answer this question we have used the β_1-selective antagonist (−)-atenolol (Conway *et al.*, 1976) as a tool because it is devoid of both cardiodepressant and cardiostimulating effects (Kaumann & Blinks, 1980).

As (−)-enantiomers of β-adrenoceptor blocking agents tend to be more β_1-selective than their corresponding (+)-enantiomers (Lemoine & Kaumann, 1983; Morris & Kaumann, 1984) we used (−)-atenolol. We estimated the affinity of (−)-atenolol for both β_1- and β_2-adrenoceptors from the antagonism of both the positive inotropic effects and the stimulation of adenylate cyclase by (−)-adrenaline and (−)-noradrenaline and from the inhibition of

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binding of [^{3}H]-(−)-bupranolol. Simultaneously with these affinities we estimated the relative contribution of β_1- and β_2-adrenoceptors to the positive inotropic effects of the catecholamines and their stimulation of adenylate cyclase.

Methods

Cardiac tissues were excised from patients undergoing open heart surgery. Fentanyl was used for the induction of anaesthesia, enflurane (ethrane) as anaesthetic gas and pancuronium as muscle relaxant. Right atrial strips were from patients with mitral valve lesions or coronary heart disease. Left ventricular strips were from patients with mitral valve lesions. Although many patients were treated with a variety of drugs (diuretics, digitalis etc.), none received β-adrenoceptor blocking agents or sympathomimetics at least one week before surgery. The tissues were transported to the laboratory in a sealed vial containing oxygenated solution at room temperature; composition (mM): 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 twice distilled in glass.

Isolated tissue preparations

Dissection and setting up of the tissues were as described by Gille *et al.* (1985). We used only tissues which could be divided into at least three strips. After dissection, tissues were mounted in an apparatus (Blinks, 1965) containing the solution described above supplemented with (mM): Na^+ 15, fumarate 5, pyruvate 5, L-glutamate 5 and glucose 10. Right atrial strips and ventricular strips 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. To block both neuronal uptake and α-adrenoceptors the tissues were incubated for 2 h with 5 μM phenoxybenzamine followed by a wash (Gille *et al.*, 1985).

We determined either a single concentration-effect curve (atrium) or two successive concentration-effect curves (atrium and ventricle) for the positive inotropic effects of a catecholamine, as described by Gille *et al.* (1985). Single curves for a catecholamine were determined simultaneously on at least three atrial strips from the same tissue, one curve in the absence, the other curves in the presence of different concentrations of (−)-atenolol (Figure 1).

Two successive curves for a catecholamine were determined as follows: after the first curve the tissues were washed and incubated with different concentrations of (−)-atenolol for 1 h, except one strip which was used as control. A second curve was determined thereafter (Figures 2–4). Equieffective concentration-effect ratios (CR) for catecholamines in the presence and the absence of (−)-atenolol were estimated and corrected for desensitization.

Membrane particles

The tissues were transported and dissected on ice. Membrane particles were prepared as described by Kaumann & Birnbaumer (1974) as validated for human heart tissues (Kaumann *et al.*, 1982; Gille *et al.*, 1985) and stored at −80°C until use. The protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Binding [^{3}H]-(−)-bupranolol was used because the affinity of [^{3}H]-(−)-bupranolol for membrane bound β-adrenoceptors matches the affinity of (−)-bupranolol as antagonist of the effects of catecholamines in isolated tissues (Morris *et al.*, 1981). The affinity of [^{3}H]-(−)-bupranolol for human ventricular β_1- and β_2-adrenoceptors is known from binding experiments (Kaumann & Lemoine, 1987) and agrees with affinity estimates of unlabelled (−)-bupranolol (Lemoine & Kaumann, 1982). The membrane suspension was incubated at 37°C for 0.5 h with the indicated concentrations of (−)-atenolol (Figure 6), with 2.2 nM [^{3}H]-(−)-bupranolol in the presence or absence of the β_2-selective antagonist ICI 118, 551 (75 nM). Non-specific binding was defined as bound radioligand in the presence of 0.2 mM (−)-isoprenaline. The incubation buffer (pH 7.6) contained (mM): Tris · HCl 50, $MgCl_2$ 4, EGTA 2, ascorbate 0.2 and GTP 0.2. Free radioligand was separated from bound by rapid vacuum filtration (<10 s) through Whatman GF/A glass fibre filters. Filters were washed 7 times with 4 ml washing solution (pH 7.6) containing (mM): Tris · HCl 10 and $MgCl_2$ 5. The filters were treated with 0.5 ml Protosol (NEN) for 30 min at 60°C, then collected on ice, acidified with 50 μl glacial acetic acid and counted in 8 ml Econofluor (NEN) (Kaumann, 1978). Efficiency of liquid scintillation counting was 43% as measured with [^{3}H]-toluol.

Adenylate cyclase assay Assays were carried out by the same method of Kaumann & Birnbaumer (1974) with slight modifications. Incubations were made in a final volume of 60 μl containing (mM): Tris · HCl 100 (pH 7.4), $MgCl_2$ 2, EGTA 1, ascorbate 0.1, [^{3}H]-cyclic AMP 1 (5000 c.p.m. per assay tube), α-[^{32}P]-ATP 0.1 (100–150 c.p.m. pmol^{-1}), GTP 0.01, creatine phosphate 20 plus the enzymes (u ml^{-1}): creatine phosphokinase 15 and myokinase 9.8. The reaction was begun by the addition of the membrane suspension and continued for 10 min at 37°C. The

reaction was terminated by the addition of a solution containing 1% sodium dodecyl sulphate, 40 mM ATP and 10 mM cyclic AMP. [^{3}H]-cyclic AMP (recovery 75–85%) and [^{32}P]-cyclic AMP were isolated by double chromatography (Salomon *et al.*, 1974) and quantified by dual channel (cross over < 1%) liquid scintillation counting.

Calculations and statistics

The antagonism by (−)-atenolol (B) of the positive inotropic effects of catecholamines was analysed by use of equation (1) (Lemoine & Kaumann, 1983). Equation (1) can be used if there is evidence of spare receptors (Stephenson, 1956) and if the stimuli from β_1- and β_2-adrenoceptors are additive (Kaumann & Marano, 1982).

$$\log (CR - 1) = \log [B] - \log \frac{(\sigma_1 K_{B1} + \sigma_2 K_{B2})[B] + K_{B1} \cdot K_{B2}}{[B] + \sigma_2 K_{B1} + \sigma_1 K_{B2}} \quad (1)$$

where σ_1- and σ_2-values are fractional stimuli of a catecholamine mediated through β_1- and β_2-adrenoceptors, and K_{B1} and K_{B2} are the equilibrium dissociation constants of the antagonist for the corresponding subtypes. In the experiments on right atrial strips the concentration-effect curves for both catecholamines were parallel and the concentration-ratios (CR) were measured at 50% effect levels. On ventricular preparations, concentration-effect curves were parallel for (−)-noradrenaline but not for (−)-adrenaline. Therefore, CRs for (−)-noradrenaline were measured at 50% effect levels, whereas CRs for (−)-adrenaline were measured at 80% effect levels (mainly β_1) and 30% effect levels (mainly β_2).

The inhibition of [^{3}H]-(−)-bupranolol (L*) binding to both β_1- and β_2-adrenoceptors by (−)-atenolol in the absence and presence of 75 nM ICI 118,551 (Figure 6) was analysed by the equation (Ehle *et al.*, 1985):

$$B_s = B_0 - B_0 \cdot \sum_{i=1}^{2} \frac{f_i \cdot [L]}{[L] + K_{Li} \cdot (1 + [L^*]/K_{L^*i} + [ICI]/K_{ICIi})} \quad (2)$$

where B_0 and the function B_s represent the specific binding by [L*] in the absence and presence of competing ligands L respectively, indices i = 1, 2 indicate the corresponding β-adrenoceptor subtypes, f_i is the receptor fraction ($f_1 + f_2 = 1$), [ICI] is the concentration of ICI 118,551 (0 or 75 nM), K_{L^*i} is the equilibrium dissociation constant of [^{3}H]-(−)-bupranolol (β_1: 1.6 nM, β_2: 0.8 nM, Kaumann & Lemoine, 1987), and K_{ICIi} is the equilibrium dissociation constant of ICI 118,551 (β_1: 100 nM, β_2: 1 nM, Lemoine *et al.*, 1985).

The contribution of β_1- and β_2-adrenoceptors to the adenylate cyclase stimulation by a catecholamine (CA) was analysed by the equation (Gille *et al.*, 1985):

$$S = S_{basij} + (S_{maxij} - S_{basij}) \times \left\{ \frac{f_{S1} \cdot [CA]}{[CA] + EC_{50,1} \cdot (1 + [B_{ij}]/K_{Bi1})} + \frac{f_{S2} \cdot [CA]}{[CA] + EC_{50,2} \cdot (1 + [B_{ij}]/K_{Bi2})} \right\} \quad (3)$$

where S, S_{bas} and S_{max} are the resultant, the basal and the maximum activity (pmol min^{-1} cyclic AMP mg^{-1} protein) of the adenylate cyclase, $f_{S1} = 1 - f_{S2}$ is the fraction of stimulation of adenylate cyclase. Indices 1 and 2 are for β_1- and β_2-adrenoceptors; indices i of the antagonists B represent (−)-atenolol (i = 1) and ICI 118,551 (i = 2) in Figures 7 and 8; indices j = 1, ..., 3 represent different concentrations of (−)-atenolol in Figure 7. Common fits were made by non-linear regression according to a set of 5 (Figure 7) or 3 (Figure 8) equations.

Materials

Adenosine 5′-triphosphate Tris salt (ATP), creatine phosphate, myokinase, adenosine 3′ : 5′-monophosphoric acid (cyclic AMP), guanosine 5′-triphosphate Tris salt (GTP) were from Sigma, St. Louis, MO, U.S.A.; α-[^{32}P]-ATP and 3,8-[^{3}H]-cyclic AMP were from the Radiochemical Centre, Amersham, U.K.; creatine phosphokinase from Calbiochem, La Jolla, CA, U.S.A.; (−)-bupranolol and [^{3}H]-(−)-bupranolol · HCl (specific activity 14.2 Ci · mmol^{-1}) from Sanol, Monheim, F.R.G.; (−)-isoprenaline bitartrate and (−)-noradrenaline bitartrate, from Sterling Winthrop, Rensselaer, NY, U.S.A.; (−)-adrenaline bitartrate from Serva, Heidelberg, F.R.G.; phenoxybenzamine · HCl from Smith, Kline & French, Philadelphia, PA, U.S.A.; ICI 118,551 · HCl (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylamino-buran-2-ol) and (−)-atenolol (free base) were from ICI, Macclesfield, U.K. (−)-Atenolol was prepared as the hydrochloride.

Results

Similar inotropic effects of catecholamines and blocking effects of (−)-atenolol on right atria from patients with coronary artery disease and mitral lesion

Neither the EC_{50} values of catecholamines for positive inotropic effects nor the shift of the

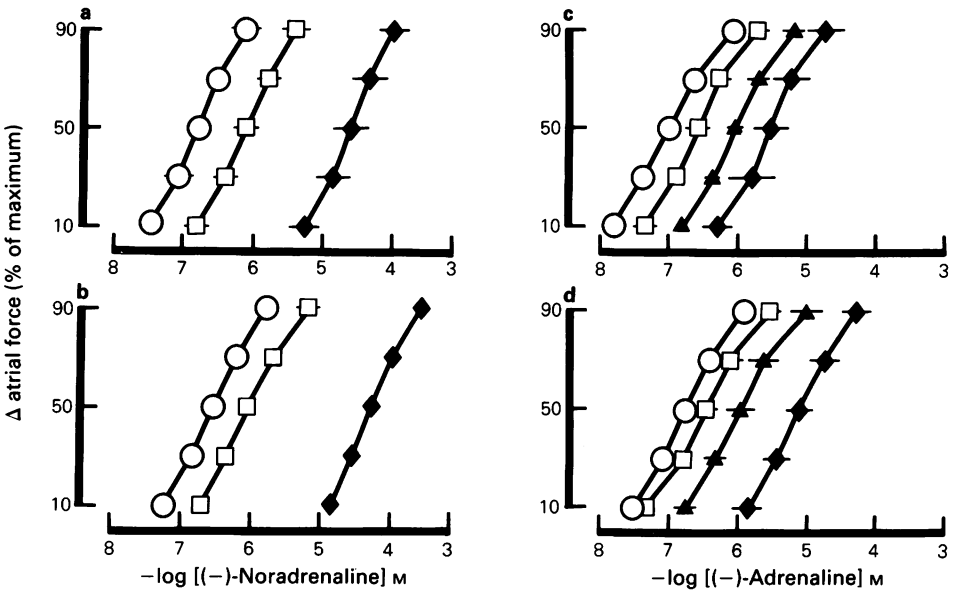

Figure 1 Comparison of the antagonism by (−)-atenolol of the positive inotropic effects of catecholamines in right atrial strips from patients with coronary heart disease (a, c) and mitral valve lesions (b, d). Only one cumulative concentration-effect curve for a catecholamine was determined on each preparation. Results in (a), (b), (c) and (d) were from 8, 8, 13 and 10 patients, respectively. Curves in the absence of (−)-atenolol (○) were determined in 8, 8, 13 and 10 preparations in (a), (b), (c) and (d), respectively. Curves in the presence of 0.1 μM (−)-atenolol (□) were determined in 7, 6, 10 and 8 preparations in (a), (b), (c) and (d), respectively. Curves in the presence of 2 μM (−)-atenolol (▲) were determined in 9 and 4 preparations in (c) and (d), respectively. Curves in the presence of 10 μM (−)-atenolol (◆) were determined in 7, 7, 3 and 6 preparations in (a), (b), (c) and (d), respectively. Horizontal lines represent s.e.mean.

concentration-effect curves by the concentrations of (−)-atenolol used differed significantly between the 2 diseases (Student's t test, $P < 0.05$). Concentration-effect curves for both catecholamines in the absence and presence of (−)-atenolol were parallel (Figure 1). The concentration-ratios of (−)-noradrenaline in the presence and absence of 10 μM (−)-atenolol were 3 to 4 times greater than concentration-ratios of (−)-adrenaline, suggesting a different involvement of β_1- and β_2-adrenoceptors (see also Figure 5).

Differential blockade of the inotropic effects of (−)-adrenaline and (−)-noradrenaline by (−)-atenolol in right atria

In order to investigate the ability of (−)-noradrenaline and (−)-adrenaline to surmount the antagonism by (−)-atenolol we used the method of 2 successive concentration effect-curves. Because concentration-ratios were not different between the 2 diseases we pooled data from patients with coronary heart disease with those from patients with mitral valve lesions. Maximum effects of both catecholamines in the presence of (−)-atenolol were not different from those obtained in its absence, except in the experiment with 10 μM (−)-atenolol (Figure 2d). The partially insurmountable antagonism appears to be related to the high degree of blockade caused by 10 μM (−)-atenolol, which requires (−)-noradrenaline concentrations greater than 0.6 mM to surmount it. The depression of the maximum response to (−)-noradrenaline is not due to a non-specific effect of 10 μM (−)-atenolol because it was not observed with (−)-adrenaline (Figure 2h). Concentration-effect curves for both catecholamines were parallel in the absence and presence of (−)-atenolol. Concentration-ratios of (−)-adrenaline were smaller than those of (−)-noradrenaline. These differences were small with low concentrations of (−)-atenolol (1.5 to 2 fold with 0.1 μM) and greater with higher concentrations (3 to 4 fold with 10 μM).

Differential blockade of the inotropic effects of (−)-adrenaline and (−)-noradrenaline by (−)-atenolol in ventricular tissues

As in the experiments with atrial myocardium, (−)-atenolol induced a parallel shift of concentration-

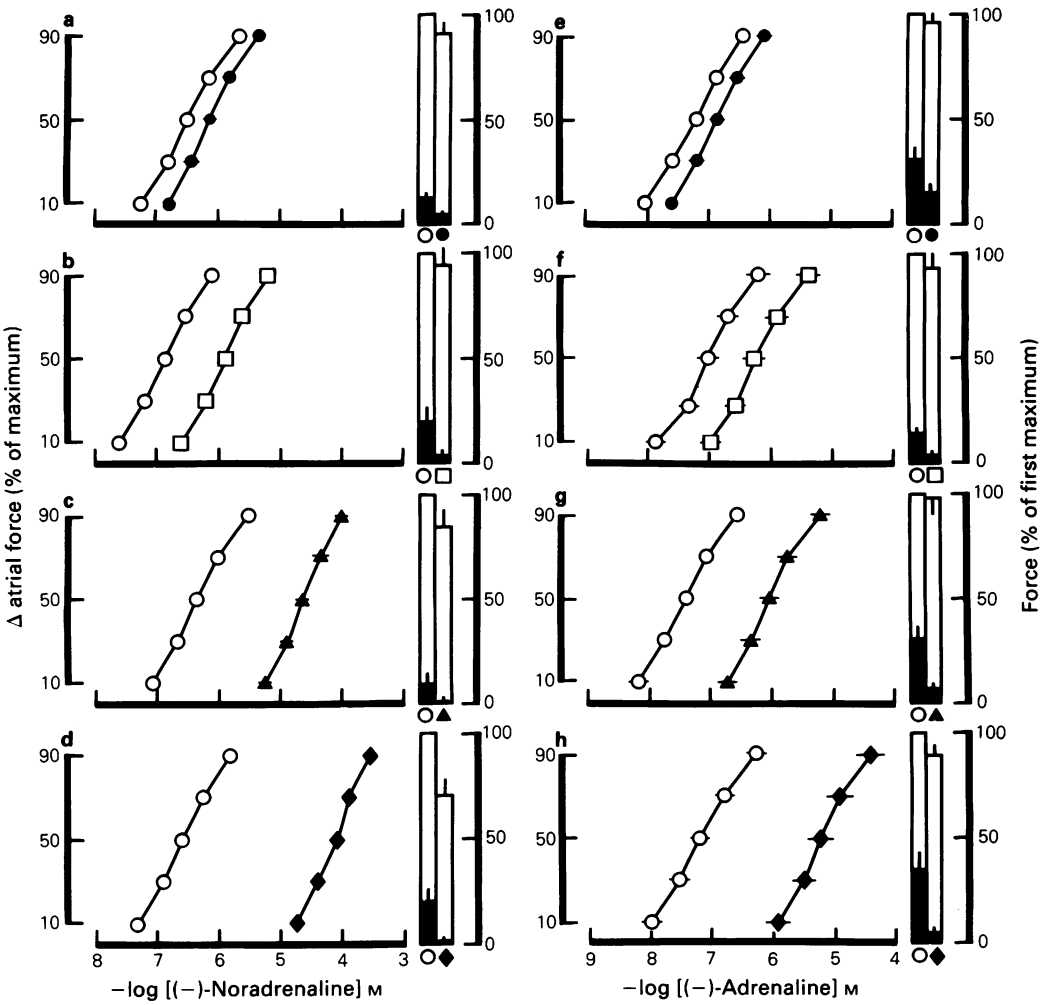

Figure 2 Antagonism by (−)-atenolol of the positive inotropic effects of (−)-noradrenaline and (−)-adrenaline on atrial strips from a pool of 18 patients with coronary heart disease and 22 patients with mitral valve lesion. Two successive concentration-effect curves for a catecholamine were determined on each preparation: the first curve (○) was determined in the absence of (−)-atenolol, the second curve was determined either in the absence of (−)-atenolol (a, e; ●) or in the presence of (−)-atenolol 0.1 μM (□), 2 μM (▲) and 10 μM (◆). The number of preparations was for (a) 24, (b) 10, (c) 10, (d) 11, (e) 16, (f) 9, (g) 8 and (h) 11. Columns on the right of each panel represent basal (solid portion) and maximal (open portion) developed force of contraction; basal force was measured in the absence of catecholamines, maximal force was measured in the presence of maximally effective concentrations of the catecholamine used. Horizontal lines (symbols) and vertical lines (columns) represent s.e.mean.

effect curves for (−)-noradrenaline (Figure 3). The concentration-ratios of (−)-noradrenaline were similar to those obtained in atrial myocardium. In contrast to the experiments on atrium, in the ventricle the concentration-effect curves for (−)-adrenaline became flat in the presence of (−)-atenolol (≥0.5 μM) (Figure 4). Maximum developed force induced by (−)-adrenaline was not different in the absence and presence of (−)-atenolol.

The contribution of β_1- and β_2-adrenoceptors to the inotropic effects of (−)-adrenaline and (−)-noradrenaline

The dependence of the catecholamine concentration-ratios on the (−)-atenolol concentration (Figure 5) was analysed by non-linear regression according to equation (1). The use of equation (1) seemed reasonable with the data from the atrium, because the

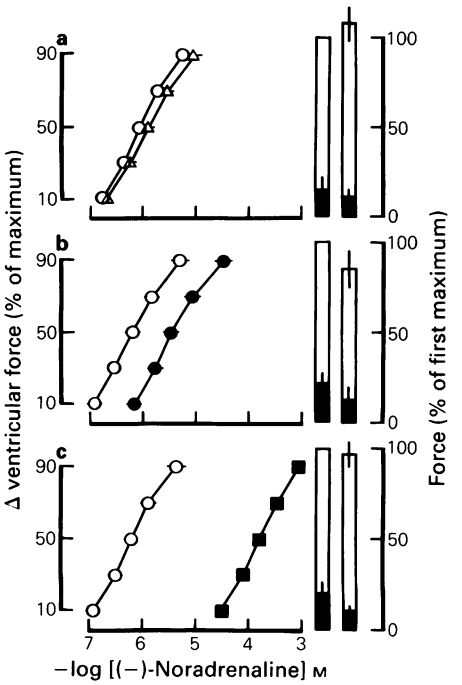

Figure 3 Antagonism by (−)-atenolol of the positive inotropic effects of (−)-noradrenaline on ventricular strips of 4 patients with mitral valve lesions. Two successive concentration-effect curves for (−)-noradrenaline were determined on each preparation (○). Curves in the absence (a, △; 4 strips) and in the presence of (−)-atenolol 0.1 μM (b, ●; 4 strips) and 10 μM (c, ■; 4 strips). For other details see legends of Figures 1 and 2.

spare receptor assumption is supported by the submicromolar potencies of the catecholamines (Figures 1–4) compared to supramicromolar affinities (Robberecht *et al.*, 1983; Kaumann & Lemoine, 1985; Lemoine *et al.*, 1985). The estimates of K_B-values for (−)-atenolol were 40 nM and 1000 nM for β_1- and β_2-adrenoceptors respectively. From the estimates of the fractional stimuli σ (see legend of Figure 5) we concluded, with help of the equation $EC_{50.1}/EC_{50.2} = \sigma_2/\sigma_1$ (Kaumann & Lemoine, 1985), that 3 fold and 20 fold higher concentrations of (−)-adrenaline and (−)-noradrenaline, respectively, are necessary to cause equieffective atrial responses through β_2-adrenoceptors compared to β_1-adrenoceptors.

In the ventricle β_2-adrenoceptors appear to mediate only up to 60% of the maximum positive inotropic effects of (−)-adrenaline (Kaumann & Lemoine, 1987), hence the assumption of spare receptors for the β_2-subtype was not fulfilled. The flat concentration effect-curves for (−)-adrenaline in the presence of (−)-atenolol appear to be a manifestation of submaximal β_2-mediated effects at low (−)-adrenaline concentrations and β_1-mediated effects (up to maximal) at high (−)-adrenaline concentrations. As a first approximation we analysed the data of Figure 4 by measuring concentration-ratios at the 30% effect levels (i.e. half maximal β_2-response) and at the 80% effect levels (i.e. half maximum of the remaining β_1-response) (see Figure 5b). Concentration-ratios for (−)-adrenaline measured at 80% matched the ratios for (−)-noradrenaline at 50% effect levels, supporting the hypothesis that in the presence of high (−)-atenolol concentrations β_1-adrenoceptors contribute to the mediation of the inotropic effects of (−)-adrenaline. Concentration-ratios of (−)-adrenaline measured at the 30% effect levels were significantly smaller than those measured at the 80% effect levels. As a first approximation, the data (Figure 5b) were analysed by non-linear regression according to equation (1). The estimated affinity parameters for (−)-atenolol were identical to those estimated for atrial β_1- and β_2-adrenoceptors.

Binding affinity of (−)-atenolol for ventricular β_1- and β_2-adrenoceptors

Two experiments were carried out with a pool of ventricular membranes using identical concentrations of [^{3}H]-(−)-bupranolol and (−)-atenolol (Figure 6). In the absence of ICI 118,551, [^{3}H]-(−)-bupranolol labelled both β_1- and β_2-adrenoceptors (Figure 6a). In the presence of ICI 118,551, [^{3}H]-(−) bupranolol labelled virtually only β_1-adrenoceptors (Figure 6b). From the latter experiment we estimated the equilibrium dissociation constant K_{L1} of (−)-atenolol and the maximum binding, $B_0(\beta_1)$, of 2.2 nM [^{3}H]-(−)-bupranolol to β_1-adrenoceptors. The binding of 2.2 nM [^{3}H]-(−)-bupranolol to both β_1- and β_2-adrenoceptors, B_0 (β_1 and β_2), was estimated from Figure 6a. From the ratio of $B_0(\beta_1)$ to $B_0(\beta_1 + \beta_2)$ independent information about the fraction f_1 of β_1-adrenoceptors was incorporated into a joint non-linear regression analysis of both experiments (Figure 6a and b). (The assumption was made that the parameters pK_{L1} and f_1 are identical in both experiments). Hence, the joint analysis increased the reliability of the parameter estimates of pK_{L1}, pK_{L2} and f_1. The estimated parameters were $pK_{L1} = 5.8$, $pK_{L2} = 4.6$ and $f_1 = 0.63$.

(−)-Atenolol as antagonist of adenylate cyclase stimulation by catecholamines in ventricular membranes

Concentration-effect curves for (−)-noradrenaline and (−)-adrenaline were determined in the absence

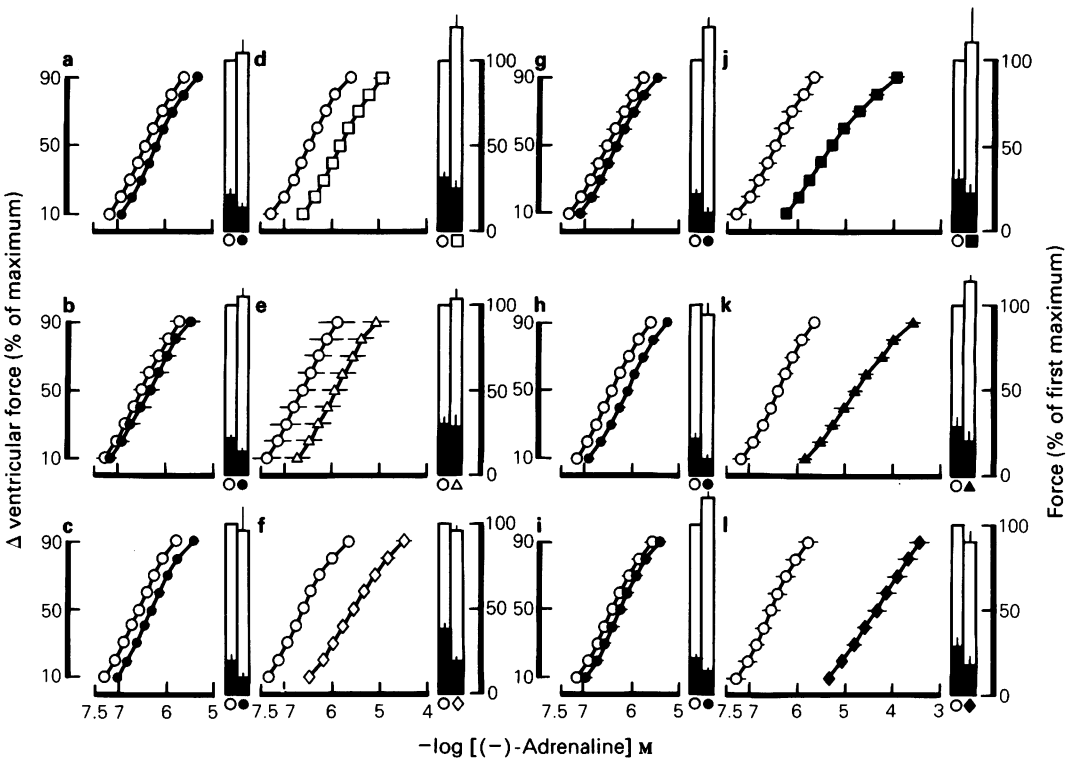

Figure 4 Antagonism by (−)-atenolol of the positive inotropic effects of (−)-adrenaline on ventricular strips of 12 patients with mitral valve lesions. Two successive concentration-effect curves for (−)-adrenaline were determined on each preparation. Two successive curves (first ○: all panels; second ●: a, b, c, g, h, i) were determined in the absence of (−)-atenolol on preparations from the corresponding patients in (d, e, f, j, k and l) respectively. A second curve was determined in the presence of (−)-atenolol 0.1 μM (□, 9), 0.2 μM (△, 6), 0.5 μM (◇, 5), 1 μM (■, 4), 2 μM (▲, 8) and 10 μM (◆, 8) (number of preparations in parentheses). For other details see legends of Figures 1 and 2.

and presence of (−)-atenolol. To obtain an independent estimate of the fractional cyclase stimulation through β_1- and β_2-adrenoceptors concentration-effect curves in the presence of the β_2-selective compound ICI 118,551 were also determined. The 5 concentration-effect curves for each catecholamine in Figure 7 were analysed jointly. The separation of β_1- and β_2-adrenoceptor-mediated components for adenylate cyclase stimulation by ICI 118,551 was more effective in the experiment with (−)-noradrenaline than in the experiment with (−)-adrenaline. This is because the β_1-selectivity of (−)-noradrenaline apparently becomes 2000 fold in the presence of ICI 118,551 (100 fold β_2-selective). On the other hand, the apparent selectivity of the non-selective agonist (−)-adrenaline in the presence of ICI 118,551 is identical to the 100 fold selectivity of ICI 118,551 for β_2-adrenoceptors. From the concentration-effect curves in the presence of ICI 118,551 we found a fractional cyclase stimulation through β_1-adrenoceptors of 0.36 by (−)-noradrenaline and of 0.39 by (−)-adrenaline, respectively. The β_1-selectivity of (−)-atenolol for adenylate cyclase coupled receptors was 5 fold and 8 fold in the experiments with (−)-noradrenaline and (−)-adrenaline, respectively (Figure 7).

(−)-Atenolol as antagonist of the adenylate cyclase stimulation by catecholamines in atrial membranes

In the regression analysis of the experiment with (−)-adrenaline we assumed that (−)-adrenaline is non-selective (for evidence see Lemoine *et al.*, 1985). Because small selectivities (<3 fold) cannot be reliably estimated from a concentration-effect curve for (−)-adrenaline, the estimation of K_B values for (−)-atenolol has a high degree of uncertainty, despite the relatively small asymptotic standard deviations. (−)-

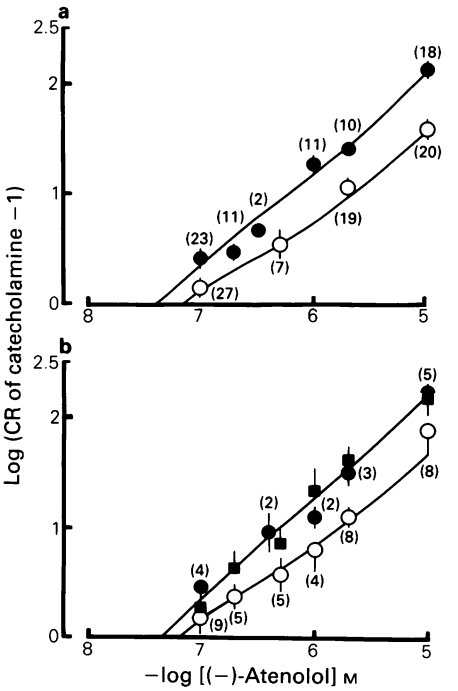

Figure 5 Non-linear analysis of the dependence of antagonism of the effects of (−)-noradrenaline and (−)-adrenaline on the concentration of (−)-atenolol. (a) Data from right atrial strips. (b) Data from ventricular strips. The analysis according to equation (1) was made with data from experiments shown in Figures 1–4 and from additional experiments. Concentration-ratios (CR) were measured at EC_{50} levels of concentration-effect curves for (−)-noradrenaline (●) in right atria and ventricle and for (−)-adrenaline (○) in right atria. In the ventricle, concentration-ratios for (−)-adrenaline were measured at EC_{30} levels (β_2, ○) and at EC_{80} levels (β_1, ■). pK_B values estimated for (−)-atenolol were 7.4 ± 0.1 (β_1) and 6.0 ± 0.2 (β_2) in atria and ventricle, respectively. Fractional stimuli ($\sigma_1 = 1 - \sigma_2$) were estimated to be 0.95 ± 0.03 for (−)-noradrenaline and 0.75 ± 0.08 for (−)-adrenaline in atrium and 0.97 ± 0.03 for (−)-noradrenaline in ventricle. From experiments with (−)-adrenaline in ventricular preparations 2 σ_1 values were estimated, 0.97 ± 0.08 and 0.80 ± 0.07 for concentration-ratios measured at EC_{80} levels and EC_{30} levels, respectively. Numbers next to the symbols indicate the number of preparations; numbers close to (○) also refer to (■).

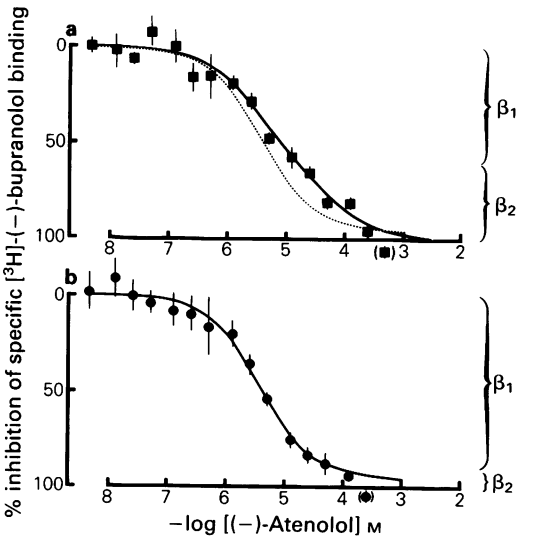

Figure 6 Inhibition of the binding of [^{3}H]-(−)-bupranolol by (−)-atenolol in the absence (a) and presence (b) of 75 nM ICI 118,551. Membrane particles were derived from 5 patients with mitral valve lesions. β-Adrenoceptors were labelled with 2.2 nM [^{3}H]-(−)-bupranolol, which occupied 57% of the β_1-adrenoceptors and 73% of the β_2-adrenoceptors, as calculated from the pK values of [^{3}H]-(−)-bupranolol (Kaumann & Lemoine, 1987) in the absence of ICI 118,551. In the presence of ICI 118,551 [^{3}H]-(−)-bupranolol occupied 44% of the β_1-adrenoceptors and 3% of the β_2-adrenoceptors. The protein content of each assay tube was 180 ± 6 μg. Each symbol represents the mean of quadruplicate determinations; vertical lines indicate s.e.mean. Non-specific binding in the presence of 0.2 mM (−)-isoprenaline was 37 ± 2%. pK_L values for (−)-atenolol, estimated by non-linear regression according to equation (2), were 5.93 ± 0.12 (β_1) and 4.57 ± 0.21 (β_2); the β_1-adrenoceptor fraction f_1 in the absence of ICI 118,551 was 0.63 ± 0.07. The stippled curve in (a) is identical to the curve in (b).

Atenolol was 3 fold and 15 fold β_1-selective against (−)-noradrenaline and (−)-adrenaline, respectively. As in the experiments in ventricular membranes, ICI 118,551 was also used in atrial membranes to obtain independent estimates of the fractional cyclase stimulation through the β_1- and β_2-adrenoceptors. The fractional stimulation by (−)-noradrenaline and (−)-adrenaline was 0.55 and 0.50 through β_1-adrenoceptors, respectively (Figure 8).

Discussion

β_2-Adrenoceptors and inotropic effects of (−)-adrenaline in human heart

The positive inotropic effects of low (−)-adrenaline concentrations were antagonized consistently less by (−)-atenolol than those of (−)-noradrenaline in both atrial and ventricular preparations. The binding selectivity for β_1-adrenoceptors of (−)-atenolol (this paper) and of (−)-noradrenaline (Kaumann & Lemoine, 1985) are both 20 fold. It is

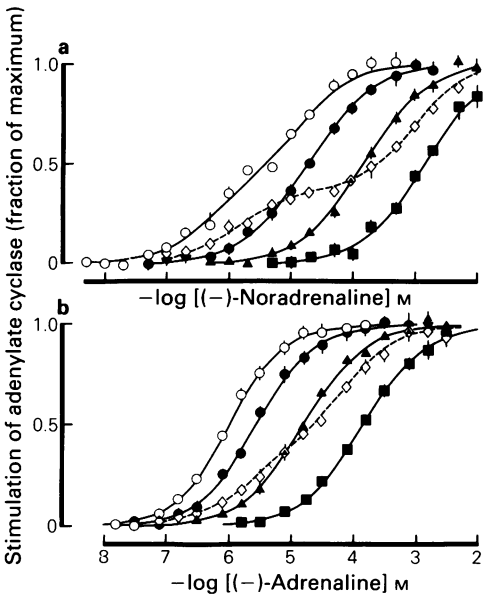

Figure 7 Antagonism by (−)-atenolol of the catecholamine-induced increase of adenylate cyclase activity of membranes from human ventricle. Effects of (−)-noradrenaline (a) in the absence (○) and in the presence of (−)-atenolol 4 μM (●), 40 μM (▲) and 400 μM (■). Effects of (−)-adrenaline (b) in the absence (○) and presence of (−)-atenolol 3.3 μM (●), 33 μM (▲) and 333 μM (■). Effects of the catecholamines in the presence of 0.1 μM ICI 118,551 (◇). Concentration-effect curves were calculated for a model of two non-interacting β-adrenoceptor subtypes, equation (3). Parameters, estimated by non-linear regression were: (a) for (−)-noradrenaline $pEC_{50.1} = 6.35 \pm 0.09$, $pEC_{50.2} = 4.88 \pm 0.07$; for (−)-atenolol $pK_{B1} = 6.44 \pm 0.11$, $pK_{B2} = 5.72 \pm 0.10$; for ICI 118,551 $pK_{B1} = 7.45 \pm 0.11$, $pK_{B2} = 8.86 \pm 0.06$; $36 \pm 5\%$ of maximum cyclase activity (78.9 ± 1.2 pmol min^{-1} mg^{-1} protein, basal: 32.2 ± 0.3) was mediated through β_1-adrenoceptors. Protein content per assay tube was 62.8 ± 0.7 μg. Membranes were from a 59 year old patient with mitral valve lesion. (b) For (−)-adrenaline $pEC_{50.1} = pEC_{50.2} = 6.00 \pm 0.10$; for (−)-atenolol $pK_{B1} = 6.16 \pm 0.18$, $pK_{B2} = 5.30 \pm 0.12$; for ICI 118,551 $pK_{B1} = 7.16 \pm 0.35$, $pK_{B2} = 8.84 \pm 0.16$; $39 \pm 5\%$ of maximum cyclase activity (44.5 ± 0.4 pmol min^{-1} mg^{-1} protein, basal 18.8 ± 0.1) was mediated through β_1-adrenoceptors. Protein content per assay tube was 46.9 ± 0.5 μg. Membranes were pooled from 3 patients (aged between 50 and 60 years) with mitral valve lesions. Symbols represent mean, vertical lines indicate s.e.mean (not shown if smaller than the size of the symbol), of triplicate determinations.

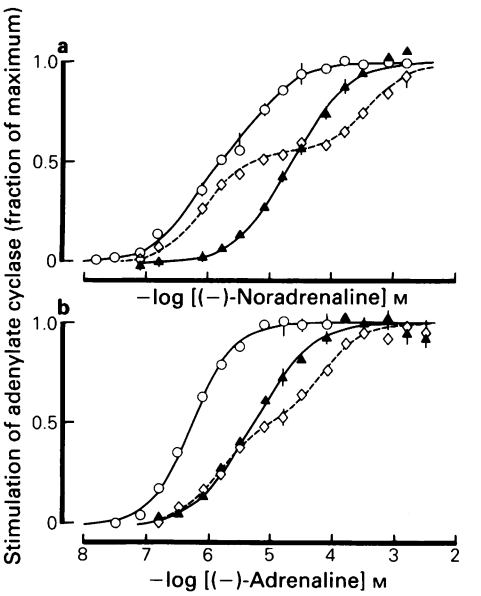

Figure 8 Antagonism by (−)-atenolol of the catecholamine-induced increase of adenylate cyclase activity of membranes from human right atria. The effects of (−)-noradrenaline (a) and (−)-adrenaline (b) were measured in the absence of antagonist (○), in the presence of 33 μM (−)-atenolol (▲) and in the presence of 0.1 μM ICI 118,551 (◇). Parameters were estimated by non-linear regression according to equation (3). (a) For (−)-noradrenaline $pEC_{50.1} = 6.30 \pm 0.20$, $pEC_{50.2} = 5.10 \pm 0.30$; for (−)-atenolol $pK_{B1} = 5.77 \pm 0.29$, $pK_{B2} = 5.26 \pm 0.21$; for ICI 118,551 $pK_{B1} = 6.89 \pm 0.31$, $pK_{B2} = 8.69 \pm 0.22$; $55 \pm 8\%$ of maximum cyclase activity (98.5 ± 2.5 pmol min^{-1} mg^{-1} protein, basal: 21.2 ± 0.9) was mediated through β_1-adrenoceptors. Protein content per assay tube was 34.0 ± 0.3 μg. (b) For (−)-adrenaline $pEC_{50.1} = pEC_{50.2} = 6.25 \pm 0.16$; for (−)-atenolol $pK_{B1} = 5.91 \pm 0.22$, $pK_{B2} = 4.71 \pm 0.33$; for ICI 118,551 $pK_{B1} = 7.21 \pm 0.34$, $pK_{B2} = 9.08 \pm 0.24$; $50 \pm 8\%$ of maximum cyclase activity (95.5 ± 2.1 pmol min^{-1} mg^{-1} protein, basal: 21.5 ± 0.5) was mediated through β_1-adrenoceptors. Protein content per assay tube was 19.4 ± 0.2 μg. Membranes for both experiments were derived from 5 patients with coronary heart disease. Symbols represent mean, vertical lines indicate s.e.mean (not shown if smaller than the size of the symbol), of duplicate determinations.

therefore likely that (−)-atenolol antagonizes the effects of low (−)-noradrenaline concentrations mainly by blocking β_1-adrenoceptors. On the other hand, (−)-adrenaline has approximately the same affinity for myocardial β_1- and β_2-adrenoceptors (Kaumann & Lemoine, 1985) and it is possible that both receptor subtypes contribute to the effects of (−)-adrenaline. Because (−)-atenolol has a lower affinity for β_2- than for β_1-adrenoceptors it is expected that the β_2-component of the effects of (−)-adrenaline is more resistant to blockade than the

β_1-component. As a result the blocking potency of (−)-atenolol would be greater against (−)-noradrenaline (mostly acts through β_1-adrenoceptors) than against (−)-adrenaline (acts through both β_1- and β_2-adrenoceptors); this was indeed observed. Evidence with physiological catecholamines (Gille *et al.*, 1985; Kaumann & Lobnig, 1986; Kaumann & Lemoine, 1987) and non-physiological agonists (Mügge *et al.*, 1985; Bristow *et al.*, 1986; Zerkowski *et al.*, 1986) supports the interpretation that not only β_1-adrenoceptors but also β_2-adrenoceptors contribute to the increased contractile force of human myocardium.

(−)-Atenolol revealed a fundamental difference between atrial and ventricular preparations in their responses to (−)-adrenaline. The parallel shift of the concentration-effect curves for (−)-adrenaline by (−)-atenolol suggests that not only β_1-adrenoceptors but also β_2-adrenoceptors could mediate maximum positive inotropic effects in atria from patients not treated with β-adrenoceptor blocking drugs. The contribution of β_2-adrenoceptors may even become functionally predominant in atria obtained from patients treated chronically with (−)-atenolol until 24 h before the operation as found recently by Hall *et al.* (1988). They observed that (−)-adrenaline (but not (−)-noradrenaline) was a more potent inotropic stimulant on atria from (−)-atenolol-treated than on those from untreated patients and that the enhanced responses were not affected by selective blockade of β_1-adrenoceptors but eliminated by selective blockade of β_2-adrenoceptors.

In ventricular preparations, in contrast to atrial tissues, concentration-effect curves for (−)-adrenaline (but not for (−)-noradrenaline) became considerably flatter in the presence of increasing (−)-atenolol concentrations. These results suggest that the submaximal effects of low (−)-adrenaline concentrations, which are blocked less by (−)-atenolol than those of high concentrations, are mediated through β_2-adrenoceptors. By using the β_1-selective antagonist CGP 20,712 A we also uncovered a submaximal β_2-component (i.e. inhibitable by the β_2-selective antagonist ICI 118,551) of the positive inotropic effects of (−)-adrenaline in human ventricle (Kaumann & Lemoine, 1987), which agrees with the present findings with (−)-atenolol.

Relationships between receptor binding, adenylate cyclase stimulation and inotropic effects

From both binding and blockade of positive inotropic effects of catecholamines we estimated an approximately 20 fold greater affinity of (−)-atenolol for β_1-adrenoceptors compared to β_2-adrenoceptors. The average β_1-selectivity of (−)-atenolol estimated from blockade of cyclase stimulation was somewhat more variable (3 to 15 fold). Although the β_1-selectivity of (−)-atenolol was similar in intact tissues and membrane particles, the absolute affinities were smaller in the latter than in the former. Similar observations, made with other β_1- and β_2-selective ligands suggest an affinity drop inherent to the procedure of membrane preparation (Kaumann & Lemoine, 1985; 1987). It might be argued that the affinity of (−)-atenolol for β_1- and β_2-adrenoceptors was actually overestimated in intact tissues of human heart. However, this is unlikely because from unpublished experiments we have also estimated similar absolute affinities and β_1-selectivity of (−)-atenolol for guinea-pig β_1-(heart) compared to β_2-adrenoceptors (trachea).

The partial affinity loss of β_1- and β_2-adrenoceptors detected by us in membrane particles precludes precise comparisons with inotropic events studied in intact tissues. Nevertheless, some parameters from experiments in membranes, such as selectivities of agonists and antagonists for receptor subtypes or relative densities of β_1- and β_2-adrenoceptors may help us to understand the positive inotropic effects of catecholamines. Robberecht *et al.* (1983) and Hedberg *et al.* (1985) obtained, from binding studies in human atrial membranes, a 50 : 50 distribution of β_1- and β_2-adrenoceptors, whereas in the ventricle a $75(\beta_1) : 25(\beta_2)$ distribution has been found (Stiles *et al.*, 1983; Kaumann & Lemoine, 1987). This relative predominance of β_2-adrenoceptors in atrial over ventricular membranes is in line with our finding that atrial but not ventricular β_2-adrenoceptors can mediate maximal inotropic effects. This hypothesis should, however, be interpreted with caution because others have detected only a $25(\beta_2) : 75(\beta_1)$ distribution in human atria (Stiles *et al.*, 1983; Brodde *et al.*, 1983). The discrepancies between the data may be related to technical problems with the binding assays.

Another rough analogy between the results on membranes and tissues is that (−)-noradrenaline has higher potencies both for adenylate cyclase stimulation and positive inotropic effects mediated through β_1-adrenoceptors than through β_2-adrenoceptors. However, there are also major discrepancies. For instance, although the ventricular cyclase is stimulated with equal potency through β_1- and β_2-adrenoceptors by (−)-adrenaline, its inotropic effects are mediated predominantly through β_1-adrenoceptors. The discrepancy is even more puzzling because 2/3 of the ventricular cyclase is stimulated through β_2-adrenoceptors and only 1/3 through β_1-adrenoceptors by (−)-adrenaline. Also a discrepancy exists between the relatively low density of ventricular β_2-adrenoceptors ($25[\beta_2] : 75[\beta_1]$, Kaumann & Lemoine, 1987) and the predominant

β_2-component of cyclase stimulation by catecholamines. The latter discrepancy appears to be related to the more efficient coupling of β_2- than β_1-adrenoceptors to the cyclase in human ventricle (Waelbroeck *et al.*, 1983; Gille *et al.*, 1985; Kaumann & Lemoine, 1987). This preferential coupling of ventricular β_2-adrenoceptors to the cyclase (see also this paper) appears to be a peculiarity of human ventricle because it has not been observed in feline ventricle (Kaumann & Lemoine, 1985).

We conclude that the rôle of myocardial β_2-adrenoceptors is more important in man (this paper, Gille *et al.*, 1985; Kaumann & Lemoine, 1987; Hall *et al.*, 1988) than in several other mammals (rat, Kaumann, 1986; guinea-pig, Lemoine *et al.*, 1985; cat, Kaumann *et al.*, 1983; Kaumann & Lemoine, 1985).

Relationship between clinical effects, plasma levels and receptor affinities of (−)-atenolol

Our *in vitro* observations with (−)-atenolol are consistent with those obtained *in vivo*. Rehling *et al.* (1986) infused (−)-adrenaline in volunteers treated with equipotent doses of (−)-atenolol, (−)-propranolol and (−)-pindolol with respect to reduction of exercise-induced tachycardia (as an indication of blockade of the interaction of endogenous (−)-noradrenaline with sinoatrial β_1-adrenoceptors). They found that (−)-adrenaline increased heart rate, cardiac output and systolic volume in volunteers treated with (−)-atenolol but not in volunteers treated with (−)-propranolol or (−)-pindolol. This finding can be understood taking into account the 2–3 fold β_2-selectivity of both (−)-propranolol (Gille *et al.*, 1985) and (−)-pindolol (Kaumann & Lobnig, 1986) detected in human cardiac tissues. (−)-Adrenaline probably caused the cardiostimulant effects in the (−)-atenolol-treated volunteers through activation of myocardial β_2-adrenoceptors, which were left unoccupied by (−)-atenolol due to its 20 fold lower affinity for β_2- than for β_1-adrenoceptors. On the other hand, due to their slight β_2-selectivity both (−)-propranolol and (−)-pindolol completely block the cardiostimulant effects of (−)-adrenaline.

The plasma level of racemic (−)-atenolol of approximately 600 nM associated with a 20% reduction of exercise-induced tachycardia (McAinsh, 1977) would cause 88% β_1-adrenoceptor occupancy (K_B = 40 nM for β_1) but only 23% β_2-adrenoceptor occupancy (K_B = 1000 nM for β_2), which is in line with blockade of β_1-adrenoceptors but negligible blockade of β_2-adrenoceptors.

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