Selective $\beta_1$-Adrenoceptor Blockade Enhances Positive Inotropic Responses to Endogenous Catecholamines Mediated Through $\beta_2$-Adrenoceptors in Human Atrial Myocardium

J.A. Hall, A.J. Kaumann, and M.J. Brown

We determined the relative contribution of $\beta_1$- and $\beta_2$-adrenoceptor stimulation to the positive inotropic responses of human atrial myocardium to catecholamines. ($\alpha$)Norepinephrine produced stimulation predominantly through $\beta_1$-receptors and ($\alpha$)epinephrine through both $\beta_1$- and $\beta_2$-receptors. However, there were marked differences in the responses of tissues from patients treated with the $\beta_1$-selective antagonist atenolol compared with non-$\beta$-blocker-treated patients; surprisingly, $\beta_1$-mediated responses were enhanced, and $\beta_2$-mediated responses were unaltered. There was an enhanced responsiveness to ($\alpha$)epinephrine (atenolol treated: $-\log M EC_{50} 7.57\pm0.07$; non-$\beta$-blocker treated: $-\log M EC_{50} 6.77\pm0.17$; $p<0.001$), and the relative importance of $\beta_2$-adrenoceptor stimulation was increased for both ($\alpha$)norepinephrine and ($\alpha$)epinephrine. In tissues from atenolol-treated patients, salbutamol, a $\beta_2$-selective partial agonist, had an enhanced potency and a greater intrinsic activity (atenolol treated: $-\log M EC_{50} 7.13\pm0.09$; intrinsic activity, 0.86$\pm0.04$; non-$\beta$-blocker treated: $-\log M EC_{50} 5.76\pm0.44$; intrinsic activity, 0.39$\pm0.13$). We investigated possible mechanisms underlying the enhanced responsiveness to $\beta_2$-adrenoceptor stimulation. Determination of $\beta_2$-adrenoceptor affinity for salbutamol showed no change of affinity in atenolol-treated patients. Responses of the tissues to the cyclic AMP analogue dibutryl cyclic AMP were not different between atenolol-treated and non-$\beta$-blocker-treated patients. The results suggest that chronic blockade of $\beta_1$-adrenoceptors causes enhanced coupling of $\beta_2$-adrenoceptors to adenylate cyclase or to other mechanisms leading to increased contractile force. (Circulation Research 1990;66:1610–1623)

Lands et al\textsuperscript{1} were the first to differentiate $\beta_1$ and $\beta_2$ subtypes of $\beta$-adrenoceptor (BAR). They showed that rabbit cardiac tissue contains only the $\beta_1$ subtype, suggesting an organ specificity for the distribution of BAR subtypes. However, it has now been shown in most mammalian hearts that $\beta_2$-adrenoceptors ($\beta_2$ARs) coexist and participate in the mediation of the effects of catecholamines.\textsuperscript{2–6} Radioligand binding measurements of BARs reveal a higher proportion of $\beta_2$ARs in human heart than in other mammals; $\beta_2$ARs represent from 25% to 60% of total cardiac BARs.\textsuperscript{7–11} Autoradiography shows that both $\beta_1$-adrenoceptors ($\beta_1$ARs) and $\beta_2$ARs are situated on myocardial cells.\textsuperscript{12} In isolated strips of human myocardium, both atrium and ventricle, $\beta_2$AR stimulation causes positive inotropic effects.\textsuperscript{11–16} In vivo, stimulation of sinoatrial $\beta_2$ARs causes a positive chronotrophic effect.\textsuperscript{17}

The fact that myocardial $\beta_2$ARs have functional roles in humans calls into question the appropriate use of $\beta_1$-selective and nonselective $\beta$-blockers in the treatment of cardiovascular diseases. To address this question we need to know the relative importance of $\beta_1$AR and $\beta_2$AR stimulation in the heart. It has already been shown that in isolated strips of ventricle (from patients without severe heart failure) $\beta_2$AR stimulation by endogenous catecholamines can achieve only 50% of the maximum BAR-mediated inotropic response.\textsuperscript{11} Our study was primarily designed to determine the relative contribution of $\beta_1$AR and $\beta_2$AR stimulation to the effects of endogenous catecholamines on human atrium.

Initial results suggested that the inotropic $\beta_2$ responsiveness is enhanced in tissues from patients treated with atenolol (a $\beta_1$-selective antagonist),
while $\beta_1$ responsiveness is unchanged.\textsuperscript{15} This was surprising because radioligand binding studies, using membrane preparations of right atrial myocardium, had shown an increased $\beta_2$AR density and an unchanged $\beta_2$AR density in atenolol-treated patients compared with non-$\beta$-blocker-treated patients.\textsuperscript{18} One would therefore expect, in tissues from atenolol-treated patients, an increased $\beta_1$ responsiveness to inotropic stimulation by catecholamines rather than an enhanced $\beta_2$ responsiveness.

Therefore, the second objective of our study was to investigate possible mechanisms underlying this unexpected increase in tissue sensitivity to $\beta_2$AR stimulation. We examined two hypotheses: 1) We tested whether the enhanced $\beta_1$ responsiveness was due to a selective increase in the affinity of $\beta_2$ARs, which we measured using salbutamol.\textsuperscript{19} 2) We investigated whether the tissues from atenolol-treated patients had an enhanced responsiveness to positive inotropic stimuli that act distal to the $\beta$ARs and adenylate cyclase, by using the cyclic AMP (cAMP) analogue dibutyryl cAMP (dbcAMP).\textsuperscript{20}

**Subjects and Methods**

**Patients**

Myocardial tissue was obtained from patients undergoing routine cardiac surgery at Papworth Hospital. Patients were having coronary artery bypass grafts, valve surgery, or both. Their degree of preoperative heart failure was classified according to New York Heart Association (NYHA) criteria, NYHA classes I, II, and III. NYHA class I heart failure is no dyspnea during ordinary physical activity, NYHA class II is dyspnea during ordinary physical activity, and NYHA class III is dyspnea during less than ordinary physical activity. None had been receiving $\beta$AR stimulants preoperatively. Patients receiving atenolol were taking 50 or 100 mg daily for more than 2 months, up to and including the day of operation.

Patients included in the initial experiments to determine the receptor subtype involvement in the responses to (-)epinephrine and (-)norepinephrine were divided into subgroups for further analysis according to prior drug treatment, NYHA classification, and disease state. For subsequent experiments patients were prospectively divided into two groups, those treated with atenolol and those not treated with $\beta$-blockers. Patients from these two groups were matched as closely as possible for age, gender, and NYHA status.

**Preparation of Strips of Atrial Myocardium**

The right atrial appendage was excised immediately before the institution of cardiopulmonary bypass. Anesthesia was induced with midazolam and thiopental, maintained with trichloethyline and fentanyl with atracurium used as a muscle relaxant. Tissues were immediately placed in an oxygenated modified Krebs' solution of the following composition (mM):

- $Na^+$ 125, $K^+$ 5, $Ca^{2+}$ 2.25, $Mg^{2+}$ 0.5, $Cl^-$ 98.5, $SO_4^{2-}$ 0.5, $HCO_3^-$ 32, $HPO_4^{2-}$ 1, and EDTA 0.04.

Dissection and setting up of the tissues was started within 45 minutes of surgical removal. Strips with a thickness <1 mm were prepared to facilitate diffusion of oxygen and drugs. Each atrial appendage yielded two to six strips. The tissues were mounted in a 50-ml organ bath\textsuperscript{21,22} at 37°C, containing a modified Krebs' solution as above supplemented with (mM) Na 15, fumarate 5, pyruvate 5, $l$-glutamate 5, and glucose 10 and constantly bubbled with 95% $O_2$, 5% $CO_2$. Water for the solution was deionized and double glass distilled.

Atrial muscle strips were attached to strain-gauge transducers and driven at 2-second intervals with square-wave pulses of 5-msec duration and of just over threshold voltage. A length-force curve was determined, and the length was set at 40% of the length associated with maximum developed force ($L_{max}$). Determination of the length-force curve for individual strips allowed us to use a standardized resting tension that takes account of the elasticity of each strip. A length of 40% $L_{max}$ was used rather than $L_{max}$ because setting the length at $L_{max}$ would be associated with a continuous decay of resting tension throughout the experiment due to the elasticity of atrial strips. Also, the increases in force caused by catecholamines are larger at 40% $L_{max}$ than at $L_{max}$ and the greater signal allows better quantitative analysis. Developed tension was recorded on a chart recorder; recordings at 50 mm/sec of single contractions allow measurement of time to peak tension.

The tissues were incubated for 2 hours with 5 $\mu$M phenoxybenzamine to inhibit uptake of catecholamines and to block irreversibly myocardial $\alpha$-adrenoceptors. This treatment causes irreversible potentiation of the effects of endogenous catecholamines in atrial tissues of humans.\textsuperscript{14}

**Responses to Endogenous Catecholamines**

**Experiment 1: Determination of $\beta$-adrenoceptor subtype involvement in responses to catecholamines.** The design of these experiments was as in Reference 11. After the phenoxybenzamine was washed out and the tissues were allowed to stabilize, cumulative concentration-effect curves were determined for (-)epinephrine and (-)norepinephrine in the presence and absence of antagonists. The selective $\beta_2$ antagonist CGP 20,712A (300 nM)\textsuperscript{5,11,23} and the selective $\beta_3$-antagonist ICI 118,551 (50 nM)\textsuperscript{24,25} were used. The chosen concentrations of CGP 20,712A and ICI 118,551 were calculated to occupy greater than 99.5% of $\beta_2$ARs and $\beta_3$ARs, respectively, without causing significant blockade of the $\beta$AR subtype for which these antagonists have low affinity.\textsuperscript{5,11,23} The tissues were preincubated for 2 hours to achieve equilibrium blockade with the antagonists. The concentration-effect curves were terminated with 0.2 mM (-)-isoproterenol to measure the maximal $\beta$AR-mediated response. This concentration of (-)-isoproterenol was calculated to
overcome completely blockade by the two antagonists, administered alone or in combination. Because atrial appendages usually produced four strips (range, 2–6), responses were determined for either (−)epinephrine or (−)-norepinephrine (only both when there were more than four strips). If there were fewer than four strips, the responses were determined in the following priority: with no blocker (+0), with either blocker (+ICI 118,551 or +CGP 20,712A), and then with both blockers (+ICI 118,551 + CGP 20,712A).

**Experiment 2: Onset and offset of blockade by atenolol.** To determine the time of onset and offset of β-blockade by (±)atenolol in our experimental tissues, we exposed atrial tissues of non-β-blocker-treated patients to 600 nM (±)atenolol. We chose 600 nM because this is the expected plasma concentration in patients taking oral atenolol and because it is expected to cause a (−)norepinephrine concentration ratio of 15. Assuming that the slope of the concentration-effect curve for (−)-norepinephrine is unchanged in the presence of atenolol, a concentration ratio of 15 would allow us to observe a residual stimulant effect of (−)-norepinephrine in the presence of atenolol.

The experimental design was as in Reference 27. First, a concentration-effect curve to (−)-norepinephrine was determined. From this a concentration of (−)-norepinephrine was selected that produced 70% of the maximum response. The tissue was repeatedly exposed to this concentration of (−)-norepinephrine, with washout of (−)-norepinephrine between each exposure. The tissue was then exposed to 600 nM (±)atenolol, and the responses to (−)-norepinephrine were repeated until equilibrium blockade was seen. The atenolol was then washed out with three changes of the bathing medium, and responses to (−)norepinephrine were again repeatedly determined until the responses had returned to a new equilibrium. The responses of a control strip not exposed to atenolol were also determined. This allowed us to measure the decline of the response seen with repeated exposure to (−)-norepinephrine.

**Investigation of Possible Mechanisms of Increased Sensitivity to β2-AR Stimulation**

**Experiment 3: Affinity estimates of salbutamol for the β2ARs.** An initial experiment was performed to determine whether the positive inotropic effects of salbutamol were selective through β2-AR stimulation in human atrial myocardium. Concentration-effect curves for salbutamol in the presence and absence of antagonists were determined. The antagonists were 300 nM CGP 20,712A and 50 nM ICI 118,551, used separately and together. After determining the β2-AR selectivity of salbutamol’s positive inotropic effects, we were able to use it to estimate the affinity of β2ARs. Tissues were preincubated with 300 nM CGP 20,712A for 2 hours to inhibit the β2 effects of (−)epinephrine. First, a concentration-effect curve for (−)epinephrine was determined, and then the (−)epinephrine was washed out, with a return of contractile force to baseline. Next, a concentration-effect curve to salbutamol was determined, and in the presence of salbutamol a second concentration-effect curve to (−)epinephrine was determined.

The equilibrium dissociation constant, $K_S$, for the salbutamol-β2AR complex was estimated from the comparison of the stimulant effects of salbutamol and (−)epinephrine and independently from the antagonism by salbutamol of the stimulant effects of (−)epinephrine. We compared the effects of (−)epinephrine (E) and salbutamol (S) by determining concentration-effect curves for the two drugs on the same tissue. $K_S$ was estimated from linear plots $\log \frac{S}{E}$ against $1/S$: $K_S$ was estimated from $30$:

$$K_S = \frac{m}{i} \tag{1}$$

where $m$ is the slope and $i$ is the intercept. To estimate slope and intercept of the double reciprocal plot we used the weight $30$:

$$\frac{([S]+EC_{50} \text{ of } S)}{[S]}^4 \tag{2}$$

where the $EC_{50}$ is the concentration of salbutamol that causes half maximum inotropic effects. To estimate $K_S$ from the antagonism by salbutamol of the effects of (−)epinephrine we first obtained the receptor occupancy $Y_S$ from the linear plot $31$:

$$E_2 = i + m E_3 \tag{3}$$

where $E_2$ and $E_3$ are equieffective concentrations of (−)epinephrine in the presence and absence of the used concentration of salbutamol, $i$ is the intercept, and $m$ is the slope. The slope $m$ of the regression of Equation 3 is related to $Y_S$ by:

$$Y_S = \frac{1}{1-m} \tag{4}$$

For the estimation of the slope $m$ of the regression the fitting weights $([E_3]+EC_{50} \text{ of } E_3)^4$ were used. $K_S$ was calculated from $32$:

$$\log \left( \frac{1}{m-1} \right) = \log [S] - \log K_S \tag{5}$$

For the case of a single class of drug receptor complex the slope of Equation 5 should be unity. $E_2$ concentrations were corrected for desensitization as $E_2(\text{corrected}) = E_2 \times 10^d$, where $d$ is the desensitization factor. Desensitization factors were obtained from two successive concentration-effect curves for (−)epinephrine in the absence of salbutamol estimated at the $EC_{50}$ level. The factor was $0.32\pm0.03$ log (mean±SEM, $n=22$), for atenolol-treated and non-β-blocker-treated patients.

In some atria obtained from patients not treated with β-blockers, salbutamol was devoid of stimulant effects but blocked the effects of (−)epinephrine. To estimate $K_S$ for the case of salbutamol as pure antagonist we used the following equation $33$:

$$\log (CR-1) = \log [S] - \log K_S \tag{6}$$

where CR is the concentration ratio of (−)epinephrine in the presence and absence of salbutamol, corrected for desensitization.
The efficacy of salbutamol as an agonist, $e_s$, was estimated from matching effects of (-)epinephrine and salbutamol:\(^34\)

$$e_s = (Y_E/Y_S) \times e_E$$  \( (7) \)

where the efficacy of (-)epinephrine, $e_E$, is taken as 1. $Y_E$ is the receptor occupancy by (-)epinephrine

$$Y_E = [E]/(K_E + [E])$$  \( (8) \)

$Y_S$ is the receptor occupancy by salbutamol

$$Y_S = [S]/(K_S + [S])$$  \( (9) \)

$[E]$ and $[S]$ are concentrations of (-)epinephrine and salbutamol with matching inotropic effects. $K_E$ is the equilibrium dissociation constant for (-)epinephrine, and $K_S$ is the equilibrium dissociation constant of salbutamol.

Both methods use the assumption that there is a large $\beta_2$AR reserve for (-)epinephrine, that is, that the maximum response to (-)epinephrine is produced by occupying only a small fraction of the receptor pool.\(^34\)

**Experiment 4: Inotropic responses to dibutyryl cyclic AMP.** To determine whether tissues from atenolol-treated patients were more sensitive to inotropic stimuli acting at a level beyond the $\beta$ARs and adenylcyclase we examined the responses of tissues to dbcAMP. Cumulative concentration-effect curves were determined for dbcAMP for tissues from atenolol-treated patients and non-$\beta$-blocker-treated patients. The concentration-effect curves were followed by the addition of (-)isoproterenol (0.2 mM) to determine if maximum cAMP-mediated effects had been achieved. Then calcium chloride was added to raise the calcium concentration to 6.75 mM to determine if there was residual tissue responsiveness to calcium.

**Statistics**

All data were expressed as mean$\pm$SEM. Concentration-effect curves were constructed for individual strips for developed force expressed as a percent of isoproterenol, epinephrine, or salbutamol maximum. To determine agonist potencies and concentration ratios from the concentration-effect curves, concentrations were determined that produced 10%, 30%, 50%, 70%, and 90% of maximum responses (EC_{10}, EC_{20}, EC_{50}, EC_{70} and EC_{90}) by linear interpolation. Such values are logarithmically normally distributed.\(^35\)

Comparisons of EC_{50}s, intrinsic activities, and dissociation constants were by Student's $t$ test. Comparisons of times to peak tension, basal and maximal, were by paired Student's $t$ test.

**Materials**

(-)Epinephrine bitartrate, (-)norepinephrine bitartrate, (-)isoproterenol bitartrate, salbutamol hemisulfate, (+)-atenolol, and N\(_2\),2'-O-dibutyryladenosine 3':5'-cyclic monophosphate sodium were from Sigma Chemical, Poole, UK. ICI 118,551-HCl (erythro[±]-1-[7-methylindan-4-olxy]-3-isopropylamino-butanol) was from ICI Pharmaceuticals, Macclesfield, UK. CGP 20,712A (1-[2-[3-carbamoyl-4-hydroxyphenoxy]-ethylamino]-3-[4-[1-methyl-4-trifluoromethyl 2-imidazolyl]phenoxy]-2-propanol methane sulfonate) was from CIBA-Geigy Pharmaceuticals, Horsham, UK. Phenoxybenzamine-HCl was from Smith, Kline & French, Welwyn, UK.

**Results**

**Responses to Endogenous Catecholamines**

**Experiment 1:** $\beta$-Adrenoceptor subtypes involved in the responses to endogenous catecholamines. A representative experiment is shown in Figure 1. Concentration-effect curves for the positive inotropic effects of (-)epinephrine and (-)norepinephrine are shown in Figures 2 and 3. Inotropic potencies of the catecholamines are shown in Table 1.

The resting tension of the atrial strips in non-$\beta$-blocker-treated patients was 4.0$\pm$0.6 mN (mean$\pm$SEM, $n=22$). Tissues from atenolol-treated patients had resting tensions of 3.3$\pm$0.4 mN (mean$\pm$SEM, $n=30$) (not significantly different). Developed force was also not significantly different between non-$\beta$-blocker-treated patients and atenolol-treated patients. For non-$\beta$-blocker-treated patients, $n=22$, basal force was 1.8$\pm$0.5 mN (mean$\pm$SEM) and maximal force (after isoproterenol) was 9.7$\pm$1.4 mN (mean$\pm$SEM). For atenolol-treated patients, $n=30$, basal force was 1.6$\pm$0.5 mN (mean$\pm$SEM) and maximal force was 8.0$\pm$1.5 mN (mean$\pm$SEM).

CGP 20,712A and ICI 118,551 when producing blockade, whether given alone or together, caused parallel shifts of the concentration-effect curves to (-)epinephrine and (-)norepinephrine (Figures 1–3). Either antagonist given alone produced less blockade than when both were used together.

**Experiment 1:** $\beta$-Adrenoceptor subtype involvement in catecholamine responses of non-$\beta$-blocker-treated patients. There were 20 non-$\beta$-blocker-treated patients, 17 males and 3 females, aged 61$\pm$3 years (mean$\pm$SEM). Their NYHA status was: NYHA I, 9 patients; NYHA II or III, 11 patients.

In these patients the response to (-)epinephrine was not significantly antagonized by either $\beta$AR blockade with CGP 20,712A or $\beta_2$AR blockade with ICI 118,551 (Figure 2). However, when both antagonists were used together they produced marked blockade ($p<0.001$) (Table 1). The response to (-)norepinephrine was antagonized by $\beta_1$AR blockade with CGP 20,712A ($p<0.001$) and was not significantly antagonized by $\beta_2$AR blockade with ICI 118,551 (Table 1, Figure 2).

**Experiment 1:** $\beta$-Adrenoceptor subtype involvement in catecholamine responses of atenolol-treated patients. There were 25 atenolol-treated patients, 22 males and 3 females, aged 61$\pm$2 years (mean$\pm$SEM). Their NYHA status was: NYHA I, 18 patients; NYHA II or III, 7 patients.

Tissues from patients treated with atenolol were sixfold more sensitive to the positive inotropic effects of (-)epinephrine (i.e., EC_{50} 0.80 log M lower,
p<0.001) but only slightly more sensitive to
(−)-norepinephrine (i.e., EC50 0.28 log M lower,
\( p<\text{NS} \)) (Table 1). This suggests an enhanced
responsiveness to \( \beta_2\text{AR} \) stimulation.

In tissues from patients treated with atenolol
the responses to (−)-epinephrine were antagonized by
\( \beta_2\text{AR} \) blockade with ICI 118,551 (\( p<0.01 \)) but not by
\( \beta_1\text{AR} \) blockade with CGP 20,712A (Figures 1 and 3).
The responses to (−)-norepinephrine were antag-
onized by both \( \beta_1\text{AR} \) blockade with CGP 20,712A
(\( p<0.01 \)) and \( \beta_2\text{AR} \) blockade with ICI 118,551
(\( p<0.01 \)) (Figure 3).

**Experiment 1:** Effect of \( \beta_1\text{AR} \) and \( \beta_2\text{AR} \) stimulation
on times to peak tension. Times to peak tension are
shown in Table 2. Both \( \beta_1\text{AR} \) and \( \beta_2\text{AR} \) stimulation
cause a decrease in times to peak tension, \( p<0.001 \).
The decrease in time to peak tension is similar for both
\( \beta_1\text{AR} \) and \( \beta_2\text{AR} \) stimulation and is not influenced by
prior atenolol therapy (no significant differences).

**Experiment 1:** Effect of other drug therapies
and disease state on responsiveness to (−)-epinephrine.
Comparison of patients with NYHA I heart failure
with those with NYHA II or III heart failure shows that
responses to (−)-epinephrine were not significa-
cantly affected by heart failure (Table 3). The
enhanced responsiveness to (−)-epinephrine in
atenolol-treated patients was still seen in patients
grouped as having NYHA I heart failure alone or
NYHA II or III heart failure alone.

Patients studied were on a range of medications.
Calcium channel blockers have previously been
reported to increase \( \beta_1\text{AR} \) density.9 When our
patients were divided into two groups according to
whether or not they received calcium channel antag-
onists (nifedipine, verapamil, diltiazem, or nicar-
dipine), no effect of these drugs was apparent on the
responsiveness to (−)-epinephrine. The same was
true when non-\( \beta \)-blocker-treated patients were ana-
lyzed separately.

Other medications included nitrates (\( n=12 \)), diure-
tics (\( n=12 \)), digoxin (\( n=7 \)), warfarin (\( n=5 \)), oral hypo-
glycemic agents (\( n=3 \)), captopril (\( n=2 \)), lipid-lowering
agents (\( n=2 \)), and nonsteroidal anti-inflammatory
drugs (\( n=2 \)).

Patients had coronary artery disease, valvular
heart disease, or a mixture. When the comparisons of
the response to (−)-epinephrine between atenolol
treated and non-\( \beta \)-blocker treated were confined to
the subgroup of patients with coronary artery disease,
the increased sensitivity to (−)-epinephrine
stimulation was still found.

**Experiment 2:** Onset and offset of blockade by
(±)-atenolol. All tissues had achieved equilibrium
blockade by 10 minutes after exposure to 600 nM
(±)-atenolol (Figure 4). This blockade was rapidly
reversed when the tissues were washed with
atenolol-free Krebs' solution; all tissues were free
of blockade by 20 minutes.

**Investigations of Possible Mechanisms of Increased
\( \beta_2\text{AR} \) Sensitivity**

**Experiment 3:** Salbutamol responses. Concentration-
effect curves for the \( \beta_2\text{AR} \)-selective partial agonist
salbutamol showed that it exerts its positive inotropic
effects entirely through \( \beta_2\text{AR} \)s. Thus, \( \beta_2\text{AR} \) blockade
with CGP 20,712A did not block the effects of
salbutamol, whereas \( \beta_2\text{AR} \) blockade with ICI 118,551
produced marked blockade (\( p<0.001 \)) (Figure 5).
After \( \beta_2\text{AR} \) blockade with ICI 118,551, CGP 20,712A
still did not produce significant additional blockade.
This was found in both atenolol-treated and non-
\( \beta \)-blocker-treated patients.

Relative to isoproterenol, salbutamol was a partial
agonist. The intrinsic activity was greater in atenolol-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{\( \beta_2\text{-Adrenoceptor-mediated effects of (−)-epinephrine (E).} \) Contractile responses to \( E \) of three strips of atrium from a 59-year-old male atenolol-
treated patient undergoing coronary artery bypass grafting. A cumulative concentration-effect curve for \( E \) was determined for each strip. Top tracing: No antagonists, resting tension 5.8 mN. Middle tracing: In the presence of \( \beta_2\text{-adrenoceptor-selective 300 nM CGP 20,712A (CGP), resting tension 3.7 mN. Bottom tracing: In the presence of \( \beta_2\text{-adrenoceptor-selective 50 nM ICI 118,551 (ICI), resting tension 2.1 mN. On reaching a plateau response, 0.2 mM isoproterenol (ISO) is added} \) to determine maximal \( \beta_2\text{-adrenoceptor} \) response.}
\end{figure}
treated patients (0.86±0.04 of isoproterenol maximum) than in non-β-blocker-treated patients (0.39±0.13 of isoproterenol maximum) (p<0.001), and the EC50 was also lower in atenolol-treated patients (p<0.001) (Figures 5 and 6).

Experiment 3: Affinity estimates of salbutamol for the β2ARs. There were 12 atenolol-treated patients, 10 males and 2 females, aged 61±4 years (mean±SEM). Their NYHA status was NYHA I, 8 patients; NYHA II or III, 4 patients. There were 10 non-β-blocker-treated patients, 7 males and 3 females, aged 62±6 years (mean±SEM). Their NYHA status was NYHA I, 6 patients; NYHA 2 or 3, 4 patients.

A representative experiment is shown in Figure 7. The estimated affinity of salbutamol for β2ARs, Ks, was similar for the two methods of determination and did not differ significantly between the two groups of patients (Table 4).

In this group of experiments there were tissues from four non-β-blocker-treated patients in which salbutamol produced no positive inotropic effects and therefore acted purely as a β-blocker, with Ks values (Equation 6), 5.98±0.13–log M (not significantly different from Ks determined above).

Experiment 3: Sensitivity of tissues to stimulation by salbutamol and (−)epinephrine. Salbutamol was a partial agonist relative to isoproterenol. Compared with non-β-blocker-treated patients, the inotropic potency and intrinsic activity of salbutamol was higher in atenolol-treated patients (Table 4). The inotropic potency of salbutamol (−log M EC50) correlated with the intrinsic activity of salbutamol (r=0.89, p<0.001) (Figure 8). There was also a close correlation between salbutamol’s intrinsic activity and the inotropic potency of (−)epinephrine (−log M EC50) (r=0.91, p<0.001) (Figure 8).

Experiment 4: Inotropic responses to dibutyryl cyclic AMP. The inotropic responses to dbcAMP were not significantly different in atenolol-treated (−log M EC50, 3.66±0.05, n=5) and in non-β-blocker-treated

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**FIGURE 2.** Concentration-effect curves for (−)epinephrine (E) and (−)norepinephrine (NE) on tissues from non-β-blocker-treated patients. Responses are developed contractile force expressed as a percent of the maximum response of the tissue to 0.2 mM isoproterenol (iso). ○, Control (n=11 [E], n=11 [NE]); ■, +300 nM CGP (n=9 [E], n=8 [NE]); □, +50 nM ICI (n=9 [E], n=9 [NE]); ▲, +50 nM ICI+300 nM CGP (n=6 [E], n=8 [NE]).

**FIGURE 3.** Concentration-effect curves for (−)epinephrine (E) and (−)norepinephrine (NE) on tissues from atenolol-treated patients. Responses are developed contractile force expressed as a percent of the maximum response of the tissue to 0.2 mM isoproterenol (iso). ○, Control (n=16 [E], n=14 [NE]); ■, +300 nM CGP (n=16 [E], n=10 [NE]); □, +50 nM ICI (n=7 [E], n=7 [NE]); ▲, +50 nM ICI+300 nM CGP (n=7 [E], n=8 [NE]).
TABLE 1. Inotropic Potencies of (-)Epinephrine and (-)Norepinephrine

<table>
<thead>
<tr>
<th>Patients</th>
<th>E</th>
<th>E+CGP</th>
<th>E+ICI</th>
<th>E+ICI+CGP</th>
<th>NE</th>
<th>NE+CGP</th>
<th>NE+ICI</th>
<th>NE+ICI+CGP</th>
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<tbody>
<tr>
<td>Non-β-blocker treated</td>
<td>6.77±0.17</td>
<td>6.37±0.23*</td>
<td>6.41±0.19*</td>
<td>4.48±0.29†</td>
<td>6.52±0.16</td>
<td>5.01±0.15†</td>
<td>6.49±0.13*</td>
<td>4.25±0.16†</td>
</tr>
<tr>
<td>Atenolol treated</td>
<td>7.57±0.07‡</td>
<td>7.40±0.08*‡</td>
<td>6.23±0.17$</td>
<td>5.11±0.11†</td>
<td>6.80±0.09$</td>
<td>6.50±0.13†</td>
<td>6.42±0.15§¶</td>
<td>4.56±0.09§¶</td>
</tr>
</tbody>
</table>

Results are -log M EC50, mean±SEM. Numbers of patients in each set are between 6 and 16 (see legends to Figures 2 and 3). Comparisons are between log EC50 in the absence of antagonists and log EC50 with antagonists and between atenolol-treated and non-β-blocker-treated patients. E, (-)epinephrine; CGP, 300 nM CGP 20,712A; ICI, 50 nM ICI 118,551; NE, (-)norepinephrine.

*p=NS vs. no antagonist.
†p<0.001 vs. no antagonist.
‡p<0.01 vs. non-β-blocker-treated patients.
||p<0.01 vs. non-β-blocker-treated patients.
%p<0.05 vs. no antagonist.

patients (-log M EC50, 3.50±0.18, n=5) (Figure 9). The response to dbcAMP appears to represent maximal cAMP-mediated effects since the addition of (-)isoproterenol (0.2 mM) produced no further increase in contractile force in any of the tissues. The addition of calcium chloride was able to increase force further to a similar, not significantly different, extent in both atenolol-treated (28±12% of dbcAMP maximum) and non-β-blocker-treated patients (34±15% of dbcAMP maximum).

**Discussion**

We have found that stimulation of β2ARs in human atrial myocardium can produce maximal βAR-mediated inotropic responses. We have also found that myocardium from patients taking the β1-selective antagonist atenolol is selectively more sensitive to the positive inotropic effects of β2AR stimulation than tissue from non-β-blocker-treated patients. Consequently, β2AR stimulation plays a greater part in the responses to both (-)epinephrine and (-)norepinephrine in atenolol-treated patients compared with non-β-blocker-treated patients.

The analysis of our experiments is critically dependent on the high selectivity of the antagonists CGP 20,712A and ICI 118,551 for β2ARs and β2ARs, respectively. The blockade of β1- and β2-mediated effects by CGP 20,712A and ICI 118,551 can be predicted from their affinities for β1ARs and β2ARs. For CGP 20,712A the equilibrium dissociation constant, Kd, for β1ARs is 9.5–log M and the Kd for β2ARs is 5.2–log M. For ICI 118,551 the Kd for β2ARs is 7.1–log M and the Kd for β1ARs is 9.6–log M. Therefore, CGP 20,712A 300 nM and ICI 118,551 50 nM would be predicted to produce a 3.0 log shift of the concentration-effect curve for β2AR effects and a 2.4 log shift of the curve for β1AR effects, respectively, with no significant blockade of the β2ARs for which the antagonist has a low affinity.

In tissues from all patients, CGP 20,712A or ICI 118,551 alone caused much less than the expected blockade of the effects of (-)epinephrine and (-)norepinephrine with parallel shifts of the concentration-effect curves. When given together, the drugs produced approximately the expected degree of blockade of both (-)epinephrine and (-)norepinephrine. These findings indicate that the positive inotropic effects of both (-)epinephrine and (-)norepinephrine are produced through stimulation of β2ARs and β2ARs and also that the maximum positive inotropic effects can be achieved through stimulation of either β2AR or β2AR subtypes with the second receptor being stimulated when the predominant receptor is blocked.

Within this overall pattern, the point of great interest was the different behavior of tissues from non-β-blocker-treated patients and atenolol-treated patients. In non-β-blocker-treated patients the response to (-)epinephrine is not blocked by either CGP 20,712A or ICI 118,551 alone but blocked by hinge point.

**Table 2. Shortening of Times to Peak Tension Produced by Selective β1- and β2-Adrenoceptor Stimulation**

<table>
<thead>
<tr>
<th>Patients</th>
<th>E+CGP</th>
<th>NE+ICI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Maximal</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>Maximal</td>
</tr>
<tr>
<td>Atenolol treated</td>
<td>97.8±15.5</td>
<td>85.2±16.8</td>
</tr>
<tr>
<td></td>
<td>86.5±6.6</td>
<td>76.4±7.1</td>
</tr>
<tr>
<td>Non-β-blocker</td>
<td>92.7±17.1</td>
<td>83.3±17.5</td>
</tr>
<tr>
<td></td>
<td>107.5±16.4</td>
<td>95.0±17.5</td>
</tr>
</tbody>
</table>

Results are time to peak tension in milliseconds, mean±SEM. E+CGP, (-)epinephrine with 300 nM CGP 20,712A (atenolol treated, n=11; non-β-blocker treated, n=11); NE+ICI, (-)norepinephrine with 50 nM ICI 118,551 (atenolol treated, n=10; non-β-blocker treated, n=6); basal, before exposure to catecholamine; maximal, after exposure to catecholamine has produced the maximal increase in contractile force; decrease, difference in time to peak tension between basal and maximal.

*p<0.001 (differences between basal and maximal). Decrease in time to peak tension is not significantly different between NE+ICI (β1-adrenoceptors) and E+CGP (β2-adrenoceptors) nor between atenolol-treated and non-β-blocker-treated patients.
both antagonists given together. Hence the effects are due to a mixture of β₁AR and β₂AR stimulation, with neither receptor predominating (Figure 2). On the other hand, the response to (-)norepinephrine is predominantly due to β₁AR stimulation being blocked by CGP 20,712A but not by ICI 118,551. However, (-)norepinephrine was capable of stimulating β₂ARs at high concentrations since 1) the blockade produced by CGP 20,712A alone was less than would be predicted if (-)norepinephrine could stimulate through only β₁ARs and 2) ICI 118,551 produced additional blockade in the presence of CGP 20,712A (Figure 2).

In atenolol-treated patients, the response to (-)epinephrine was blocked by ICI 118,551 but not by CGP 20,712A and hence was predominantly due to β₂AR stimulation (Figures 1 and 3). Compared with the responses of non-β-blocker-treated patients, this shows that the relative contribution of β₂AR stimulation to (-)epinephrine’s effects is increased. The response to (-)norepinephrine was blocked by both CGP 20,712A and ICI 118,551 alone and so was due to a mixture of β₁AR and β₂AR stimulation (Figure 3). Hence, the contribution of β₂AR stimulation to (-)norepinephrine’s effect was also increased in tissues from patients treated with atenolol compared with tissues from non-β-blocker-treated patients.

Such selective enhancement of responsiveness of tissues to β₂AR stimulation after treatment with a β₁AR-selective antagonist was unexpected. Could the atenolol be masking an enhanced β₂AR responsiveness? Patients received atenolol up to and including the day of operation so they would still have appreciable plasma levels of (±)atenolol, up to 600 nM. Atenolol is a highly hydrophilic β-blocker, and one would predict that the several washing steps involved in the preparation of tissues before the determination of concentration-effect curves would remove all atenolol. The experiment determining the kinetics of the onset and offset rates of (±)atenolol supports this conclusion with rapid onset and offset of blockade. Therefore, the responses of tissues from patients treated with atenolol are probably free from the influence of residual atenolol, and the selective increase in β₂AR sensitivity is unlikely to be artifactual.

Could the differences in the responses to (-)epinephrine be due to other differences between the two

---

**TABLE 3. Effect of Heart Failure, Treatment With Calcium Channel Blockers, and Disease State on Inotropic Potencies of (-)Epinephrine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NYHA I</th>
<th>NYHA II or III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol and non-β-blocker treated</td>
<td>7.40±0.10 (n=14)</td>
<td>7.09±0.20 (n=13)*</td>
</tr>
<tr>
<td>NYHA I only</td>
<td>7.55±0.07 (n=11)</td>
<td>6.93±0.24 (n=3)†</td>
</tr>
<tr>
<td>NYHA II or III only</td>
<td>7.59±0.14 (n=5)</td>
<td>6.72±0.22 (n=8)†</td>
</tr>
<tr>
<td>Atenolol and non-β-blocker treated</td>
<td>Calcium blocker</td>
<td>No calcium blocker</td>
</tr>
<tr>
<td>Atenolol and non-β-blocker treated</td>
<td>7.35±0.15 (n=11)</td>
<td>7.18±0.15 (n=16)*</td>
</tr>
<tr>
<td>Non-β-blocker treated only</td>
<td>6.76±0.23 (n=3)</td>
<td>6.78±0.22 (n=8)*</td>
</tr>
<tr>
<td>Coronary artery disease only</td>
<td>7.58±0.07 (n=15)</td>
<td>6.63±0.13 (n=5)‡</td>
</tr>
</tbody>
</table>

Results are −log M EC₅₀ (mean±SEM). NYHA is New York Heart Association classification of heart failure. Calcium blocker is calcium channel antagonist (nifedipine, verapamil, nicardipine, or diltiazem).

* p<0.001.
† p<0.01.
‡ p=NS.

---

**FIGURE 4. Kinetics of the onset and offset of β-blockade with (±)atenolol. Left panel: ○, Concentration-effect curve for norepinephrine (NE) (n=9). Right panel: ○, Responses to repeated challenges with NE (n=5); ■, responses to repeated challenges with NE with 600 nM (±)atenolol present between 21 and 50 minutes (filled bar) (n=4).**
FIGURE 5. Inotropic responses to salbutamol (S) of tissues from atenolol-treated (A, n=6) (upper panel) and non-β-blocker-treated (NBB, n=4) (lower panel) patients. Responses are developed contractile force expressed as a percent of the maximum response of the tissue to S. Tissues from NBB patients exposed to ICI 118,551 (ICI) did not reach a maximum response at a concentration of 100 μM; therefore, the interpolated responses were calculated assuming that the intrinsic activity was the same as in the paired control tissue. Comparisons are between control response and response in the presence of blockers: ○, Control (EC₅₀: 7.13±0.09 [A], 5.76±0.44 [NBB]); ■, +300 nM CGP 20,712A (CGP) (EC₅₀: 7.11±0.06 [A, p=NS], 5.77±0.61 [NBB, p=NS]); □, +50 nM ICI (EC₅₀: 5.39±0.05 [A, p<0.001], 4.37±0.12 [NBB, p<0.001]); ▲, +50 nM ICI+300 nM CGP (EC₅₀: 5.07±0.14 [A, p<0.001], 4.36±0.10 [NBB, p<0.001]).

FIGURE 6. Inotropic responses to salbutamol (S). Responses are developed contractile force expressed as a percent of the maximum response of the tissue to 0.2 mM isoproterenol (iso). Receptor occupancy by S, Ys, is calculated from Ys=|S|/([S]+Kᵦ), where Kᵦ is the equilibrium dissociation constant. ○, Atenolol-treated patients (n=12); ○, non-β-blocker-treated patients (n=10); line without symbols, calculated receptor occupancy; bars, SEM.

groups of patients independent of the atenolol treatment? Previous studies have indicated that disease state, heart failure, and treatment with calcium channel blocking drugs can alter βAR density and function.9,37–40 We examined our results taking these factors into account and found that they could not explain the increased responsiveness to (-)-epinephrine in our patients (Table 3). We also examined the effects of other drug treatment, age, and gender and found that these did not account for the differences seen. Patients not taking β-blockers were not doing so for a variety of reasons, for example, peripheral vascular disease, dia-

betes mellitus, chronic obstructive airways disease, aortic valve disease, heart failure, Raynaud’s phenomenon, conduction tissue disease, intolerance of the medication, or physician preference. Such contraindications for β-blocker therapy were not absolute so that patients with these conditions were also represented in the atenolol-treated group. Obviously, a placebo-controlled, prospective, randomized trial of the effects of atenolol on βAR responsiveness would be needed to fully exclude all confounding variables. Unfortunately, such a study is not feasible in patients undergoing cardiac surgery, and therefore, it is probably impossible to examine so rigorously the effects of β-blocker treatment on the responses of isolated human myocardium. We feel it is unlikely that such extraneous factors can account for the differences consistently found between atenolol-treated and non-β-blocker-treated patients. Our conclusion is that the differences in the responsiveness of the tissues to β₂AR stimulation are due to a chronic effect of atenolol treatment.

What is the mechanism underlying this effect of atenolol? It would be unlikely that changes in receptor density alone could explain the increased sensitivity of tissues since the magnitude of the increased sensitivity to β₂AR stimulation, almost 10-fold, would require a similar 10-fold increase in receptor density. Such extreme changes have never been previously reported. It is also unlikely that a change in receptor density even contributes to the increased sensitivity since radioligand binding studies of right atrial myocardium comparing tissue from atenolol-treated patients with tissue from non-β-blocker-treated patients have shown an increased β₁AR density and an unchanged β₂AR density in atenolol-treated patients.18 These considerations led us to concentrate our experimental effort on alternative explanations. The selectively increased sensitivity to β₂AR stimulation could be due to either increased
affinity of the β2-ARs or increased coupling of the β2-ARs to effector mechanisms.

The salbutamol experiments were designed both to confirm the effect of atenolol on responsiveness to β2AR stimulation and to determine β2AR affinity. In human atrium, salbutamol's inotropic effect is due purely to β2AR stimulation (Figures 8 and 9, Table 4). This is in contrast to the findings in other species since in cat right ventricular papillary muscle salbutamol is capable of inotropic stimulation through β1-ARs. A possible explanation for this species discrepancy is that feline cardiac β1ARs are better coupled to inotropic effects than human cardiac β2ARs.

The affinity of salbutamol for β2-ARs was determined by two different methods and found to be unaffected by prior atenolol treatment. The first method is dependent on salbutamol's stimulant action, and the second on salbutamol's ability to block the effect of (-)epinephrine on β2-ARs. Both methods make the assumption that there are spare receptors for (-)epinephrine to produce positive inotropic effects, that is, that responses are maximal when only a small fraction of the receptors is occupied by epinephrine. Support for this assumption comes from membrane radioligand binding studies in which the affinity of (-)epinephrine is estimated to be 5.7–log M, whereas the EC50 for (-)epinephrine is 7.57–log M in atenolol-treated patients and 6.77–log M in non-β-blocker-treated patients.

From this series of experiments we were also able to determine that the responses to salbutamol were enhanced in tissues from atenolol-treated patients compared with non-β-blocker-treated patients. This part of the study was as close as practicable to a prospective case-control study, and again there was no discernible systematic bias in the physicians' pre-

![Figure 7](https://i.imgur.com/3H5X5.png)

**Figure 7.** Salbutamol (S) acting as agonist and antagonist in single tissues from an atenolol-treated patient. Left panel: All responses are in the presence of 300 nM CGP 20,712A. ○, First response to (-)epinephrine (E); ×, first response corrected for desensitization, determined from the successive responses of a paired tissue; □, response to S; ■, response to E in the presence of 20μM S; line without symbols, calculated receptor occupancy (Ys) curve. Responses are developed contractile force expressed as a percent of the maximum response of the tissue to E. Ys is calculated from Ys=[S]/([S]+Ks), where Ks is the equilibrium dissociation constant. Upper right panel: Determination of Ks from blockade by S of the responses to E. E2, control response; E3, response in the presence of S according to Equations 3 and 4. Lower right panel: Determination of Ks from equieffective responses to E and S according to Equations 1 and 2.

### Table 4. Receptor Affinity of Salbutamol for β2-Adrenoceptors

<table>
<thead>
<tr>
<th>Patients</th>
<th>Salbutamol</th>
<th>(-)Epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ks*</td>
<td>Ks†</td>
</tr>
<tr>
<td>Atenolol treated</td>
<td>6.08±0.11</td>
<td>6.13±0.10‡</td>
</tr>
<tr>
<td>(n=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-β-blocker</td>
<td>5.92±0.07§</td>
<td>6.00±0.20§</td>
</tr>
<tr>
<td>treated</td>
<td>(n=10)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. Comparisons are between atenolol-treated and non-β-blocker-treated patients and between methods of estimating Ks. ISA, intrinsic activity with reference to 0.2 mM (-)isoproterenol.

*Ks estimated by Equations 1 and 2.

†Ks estimated by Equations 3–5.

‡p=NS vs. other method of estimating Ks.

§p=NS vs. atenolol-treated patients.

||p<0.001 vs. atenolol-treated patients.
Figure 8. Correlations between the intrinsic activity (ISA) of salbutamol (S) and the EC50s of S (upper panel) and (-)epinephrine (E) (lower panel). Points represent responses of individual patients. ISA is the maximum response to salbutamol as a percent of the response to 0.2 mM isoproterenol. ●, Atenolol-treated patients; ○, non-β-blocker-treated patients.

vious decision to use or avoid β-blockade to explain the enhanced responsiveness of atenolol-treated patients. In the atenolol group salbutamol had a significantly higher intrinsic activity and lower EC50 (Table 4) with a close correlation between these two parameters (Figure 8). There was also a close correlation between the intrinsic activity of salbutamol and sensitivity to (-)epinephrine (EC50) (Figure 8). These correlations suggest that there is a common mechanism involved in the enhanced tissue respon-
siveness to (-)epinephrine stimulation and the enhanced responsiveness to salbutamol.

Since the affinity of salbutamol for the β2-ARs was unaltered in atenolol-treated patients, we suggest that the enhanced inotropic responses are due to an increased coupling of β2-ARs to intermediary biochemical pathways. Support for this conclusion comes from analysis of the responses to salbutamol and (-)epinephrine in terms of the degree of receptor occupancy required to produce a given inotropic response. For atenolol-treated patients, the calculated receptor occupancy curve against agonist concentration is situated to the right of the curve for the inotropic effect against agonist concentration (Figure 6). For non-β-blocker-treated patients the curves for receptor occupancy and inotropic response are superimposed (Figure 6).

Calculation of stimulant effect against receptor occupancy curves for both (-)epinephrine and salbutamol (Figure 10) shows that the responses to both agonists are greater for a given degree of receptor occupancy in atenolol-treated patients than in non-β-blocker-treated patients. The implication from this is that the signal resulting from occupation of each receptor by (-)epinephrine or salbutamol is greater in atenolol-treated patients. The relative efficacies of (-)epinephrine, εE, and salbutamol, εS, can be estimated from comparisons of the receptor occupancies required to produce equal responses (Equation 7). Taking the efficacy of (-)epinephrine, εE, to be 1 (for both atenolol-treated and non-β-blocker-treated patients) the mean (±SEM) calculated εS from the curves in Figure 10 is 0.15 (±0.004) for atenolol-treated patients and 0.14 (±0.006) for non-β-blocker-treated patients (not significantly different). The similarity of these two estimates of the relative efficacy of salbutamol compared with (-)epinephrine shows that the responses to the two agonists are enhanced to the same extent in atenolol-treated patients. This sustains the argument that the enhanced responsiveness of tissues, from atenolol-treated patients, to stimulation by salbutamol and (-)epinephrine is due to the same mechanisms.

At which level between receptor occupancy and increased contractile force could this amplification occur? There is evidence, in human myocardium, that both β1AR and β2AR stimulation produce rises in cellular cAMP leading to activation of cAMP-dependent protein kinase.35,43 dbcAMP is a stimulant that acts after the BARs and adenylate cyclase and before cAMP-dependent protein kinase activation. The tissues from atenolol-treated patients were shown not to be more sensitive to dbcAMP. This suggests that the alteration in sensitivity induced by atenolol treatment occurs at a point, in the chain of events linking occupation of a receptor by an agonist with increased contractile force, earlier than cAMP-dependent protein kinase activation.

The cAMP produced by cardiac βAR stimulation is hydrolyzed by phosphodiesterase IV, thus limiting its activity. Enoximone, a selective phosphodiesterase
IV inhibitor enhances the responses to selective \( \beta_1 \)-AR and \( \beta_2 \)-AR stimulation to a similar extent. Therefore, it is unlikely that decreased phosphodiesterase IV activity could explain the selectively enhanced response to \( \beta_2 \)-AR stimulation seen in atenolol-treated patients. Therefore, we must consider that the enhanced coupling of the \( \beta_2 \)-ARs to inotropic effects could be occurring at the level of the stimulatory G protein, Gs, linking the receptor to adenylate cyclase. Another intriguing possibility is that \( \beta_2 \)-AR-activated Gs bypasses the adenylate cyclase and directly opens calcium channels. This mechanism could perhaps increase contractile force, and its relevance to the enhanced responsiveness of tissues from atenolol-treated patients to \( \beta_2 \)-AR stimulation is uncertain. Direct activation of calcium channels would not be expected to abbreviate time to peak tension, while cAMP-dependent protein kinase activation would. Analysis of fast speed recordings from our experiments suggests that the shortening of time to peak tension seen with \( \beta_2 \)-AR activation is similar for \( \beta_1 \)-AR and \( \beta_2 \)-AR stimulation and is not altered by prior atenolol therapy (Table 2). Therefore, these results are consistent with the hypothesis that both \( \beta_1 \)-AR and \( \beta_2 \)-AR stimulation lead to cAMP-dependent phosphorylation of the contractile proteins involved in hastened relaxation but do not indicate a role for direct activation of calcium channels by Gs.

Clinical Implications

It is important to consider the relevance of responses observed in isolated strips of atrial myocardium in an organ bath to responses seen in vivo. The behavior of \( \beta \)-ARs in right atrial myocardium may parallel the behavior of \( \beta \)-ARs in the sinoatrial node, but right atrial appendage does not have reliable pacemaker activity (sinoatrial node tissue is not removed) and so it is impossible to measure chronotropic responses of human atrium in vitro. However, we have recently reported a study of the chronotropic responses to intracoronary salbutamol in patients undergoing coronary angiography for the assessment of ischemic heart disease, most of whom were taking atenolol. We found that salbutamol produced a sinus tachycardia by stimulating sinoatrial \( \beta_2 \)-ARs. In one patient not taking atenolol there was a suggestion of a reduced responsiveness to salbutamol that would be in keeping with our in vitro findings. We are now seeking to confirm this finding in further clinical studies.

Are the concentrations of agonists used in our experiments physiologically relevant? Normal resting levels of \((-\)\text{e}pinephrine are 0.25–1 nM, and at these concentrations threshold responses were observed in tissues from atenolol-treated patients. During stress much higher levels of \((-\)\text{e}pinephrine have been found, for example, 30 nM in patients with myocardial infarction, which would produce responses in all our tissues in vitro. Plasma levels of salbutamol in patients may be up to 400 nM after oral therapy. Such a level is within our concentration-effect curves in vitro. Hence, the tachycardia seen in patients treated with salbutamol probably contains a component that is due to direct stimulation of myocardial \( \beta_2 \)-ARs.

These considerations permit us, finally, to consider which therapeutic implications follow from our finding that atenolol treatment leads to increased \( \beta_2 \)-AR coupling to inotropic effects. In patients with myocardial infarction the increased sensitivity to \( \beta_2 \)-AR stimulation may prove to be either beneficial or harmful. If one speculates that the same phenomenon that is occurring in atrium is also occurring in ventricle, then beneficial effects may include an increased inotropic effect to support the compromised pumping action of the heart. Deleterious effects could be due to a greater increase in heart rate and oxygen consumption of the heart thereby increasing the risks of arrhythmia and increasing the extent of infarction. It has been noted that oral salbutamol used to treat patients with heart failure has beneficial hemodynamic effects but has limited usefulness because of its adverse effect of provoking arrhythmia.

If \( \beta_2 \)-AR stimulation is generally deleterious in situations of high \((-\)\text{e}pinephrine levels, then this suggests that patients with myocardial infarction or patients who are at risk of myocardial infarction would be better treated by nonselective \( \beta \)-blockers rather than selective \( \beta_2 \)-AR blockade.

Another setting in which our findings may be of clinical relevance is in the treatment of patients with heart failure with \( \beta \)-blockers. In patients with heart failure due to congestive cardiomyopathy, treatment with the \( \beta_1 \)-selective antagonist metoprolol has been reported to be associated with beneficial hemodynamic effects. The mechanism underlying this has been assumed to be upregulation of \( \beta_2 \)-ARs. However, a problem with accepting this assumption is that such upregulation would have to be to such an extent as to overcome the \( \beta_1 \)-AR blocking effects of continu-
ing metoprolol treatment. But, if the mechanism is analogous to our finding of increased sensitivity to β₂AR stimulation after atenolol treatment, then the continued presence of β₁AR blockade would not antagonize the increased responses to β₂AR stimulation. Therefore, it is this increase in β₂AR sensitivity and activity that may account for the beneficial hemodynamic effects of β₁AR blockade in patients with heart failure. We have studied the responses to (−)epinephrine in four patients treated with metoprolol and found a −log M EC₅₀ of 7.52±0.31 (not significantly different from our atenolol-treated group).

**Conclusion**

The human heart contains a higher proportion of β₁ARs than other mammals. We have demonstrated that endogenous catecholamines can produce maximal effects through stimulation of β₁ARs in human atrial myocardium and that the effect of (−)epinephrine is mediated through β₁AR and β₂AR stimulation. After treatment with the β₁-selective antagonist atenolol the β₂ARs are coupled better to production of inotropic effects and consequently have a more important role in the mediation of the effects of endogenous catecholamines.

**Acknowledgments**

We would like to thank the cardiac surgeons at Papworth Hospital, Mr. T.A.H. English, Mr. J. Wallwork, and Mr. F.C. Wells, for providing us with tissues from their patients. We would like to thank Mrs. L. Sanders and Mr. M. Latimer for technical assistance.

**References**

5. Kaumann AJ: The β₁ adrenoceptor antagonist CGP 20,712A unmasks β₂ adrenoceptors activated by (−)adrenaline in the rat sino-atrial node. *Naunyn Schmiedebergs Arch Pharmacol* 1986;333:73–76
30. Lemoine H, Bilski A, Kaumann AJ: Xamoterol activates β₁, but not β₂ adrenoceptors in mammalian myocardium: Comparison of its affinity for β₁ and β₂ adrenoceptors coupled to

KEY WORDS • β₁-adrenoceptors • human myocardium • receptor regulation
Selective beta 1-adrenoceptor blockade enhances positive inotropic responses to endogenous catecholamines mediated through beta 2-adrenoceptors in human atrial myocardium.

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