Beta3-adrenoceptors are involved in metabolism, gut relaxation, and vascular vasodilation. However, their existence and role in the human heart have not been documented. We investigated the effects of several beta-adrenoceptor agonists and antagonists on the mechanical properties of ventricular endomyocardial biopsies. In the presence of nadolol, a beta1- and beta2-adrenoceptor antagonist, isoprenaline produced consistent negative inotropic effects. Similar negative inotropic effects also resulted from the action of beta3-adrenoceptor agonists with an order of potency: BRL 37344 > SR 58611 approximately CL 316243 > CGP 12177. The dose-response curve to BRL 37344-decreasing myocardial contractility was not modified by pretreatment with nadolol, but was shifted to the right by bupranolol, a nonselective beta-adrenoceptor antagonist. Beta3-adrenoceptor agonists also induced a reduction in the amplitude and an acceleration in the repolarization phase of the human action potential. Beta3-adrenoceptor transcripts were detected in human ventricle by a polymerase chain reaction assay. These results indicate that: (a) beta3-adrenoceptors are present and functional in the human heart; and (b) these receptors are responsible for the unexpected negative inotropic effects of catecholamines and may be involved in pathophysiological mechanisms leading to heart failure.
Functional $\beta_3$-Adrenoceptor in the Human Heart

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Abstract

$\beta_3$-adrenoceptors are involved in metabolism, gut relaxation, and vascular vasodilatation. However, their existence and role in the human heart have not been documented. We investigated the effects of several $\beta$-adrenoceptor agonists and antagonists on the mechanical properties of ventricular endomycocardial biopsies. In the presence of nadolol, a $\beta_1$- and $\beta_3$-adrenoceptor antagonist, isoprenaline produced consistent negative inotropic effects. Similar negative inotropic effects also resulted from the action of $\beta_3$-adrenoceptor agonists with an order of potency: BRL 37344 and SR 58611, which have little effect on $\beta_1$- and $\beta_2$-adrenoceptors. The biological effects mediated by $\beta_3$-adrenoceptor stimulation have been identified in a variety of tissues. As $\beta_3$-adrenoceptors mediate lipolysis in white adipose tissues and thermogenesis in brown adipose tissues (4–7), they constitute a target for antiobesity and antidiabetic drugs (8, 9). They also inhibit the contractile activity of ileum and colon (10–12). $\beta_3$-adrenoceptors modulate neural bronchomotor control, inducing relaxation of airway smooth muscle (13) and producing sustained peripheral vasodilatation that is predominant in skin and fat (14, 15).

Although considerable information is available on $\beta_1$-adrenoceptor physiology in fat and the gastrointestinal tract, it is still unclear whether or not $\beta_3$-adrenoceptors exist in the human heart. In animal models, the use of partial agonists inducing chronotropic and inotropic effects resistant to blockade by conventional $\beta_1$- and $\beta_2$-adrenoceptor antagonists has suggested the existence of a third cardiac $\beta$-adrenoceptor, designated as atypical $\beta$-adrenoceptor (16). However, these partial agonists have no effect on inotropy in the human heart (17). In vivo studies have recently demonstrated that positive $\beta_3$-adrenoceptor–related chronotropic effects were prevented by $\beta_1$- or $\beta_2$-adrenoceptor antagonists and are likely due to baroreflex activation in response to $\beta_3$-adrenoceptor agonist–induced vasodilatation (18–21). Thus, the existence of a putative $\beta_3$-adrenoceptor in the heart has not been clearly demonstrated. The purpose of the present study was to investigate a combination of preferential $\beta_3$-adrenoceptor agonists and $\beta$-adrenoceptor antagonists in human myocardial fragments obtained from endomyocardial biopsies. We found that $\beta_3$-adrenoceptor stimulation of the human cardiac muscle, in stark contrast with $\beta_1$- and $\beta_2$-adrenoceptor stimulation, resulted in a profound dose-dependent negative inotropic effect. This unexpected finding suggests that $\beta_3$-adrenoceptors may participate in the pathogenesis of cardiac failure, during which modification of $\beta_1$- and $\beta_2$-adrenoceptor expression occurs (22).

Introduction

$\beta_3$-Adrenoceptor differs from classical $\beta_1$- and $\beta_2$-adrenoceptor subtypes by its molecular structure and pharmacological profile (for review, see reference 1). $\beta_3$-adrenoceptor shares only 40–50% amino acid sequence identity with $\beta_1$- and $\beta_2$-adrenoceptors, possesses an intron whereas $\beta_1$- and $\beta_2$-adrenoceptors are intronless (2), and lacks recognition sites for the cAMP-dependent and $\beta$-adrenoceptor kinases implicated in the desensitization of $\beta_2$-adrenoceptors (3). $\beta_3$-adrenoceptor is activated by preferential pharmacological agonists (e.g., BRL 37344 and SR 58611), which have little effect on $\beta_1$- and $\beta_2$-adrenoceptors. The biological effects mediated by $\beta_3$-adrenoceptor stimulation have been identified in a variety of tissues. As $\beta_3$-adrenoceptors mediate lipolysis in white adipose tissues and thermogenesis in brown adipose tissues (4–7), they constitute a target for antiobesity and antidiabetic drugs (8, 9). They also inhibit the contractile activity of ileum and colon (10–12). $\beta_3$-adrenoceptors modulate neural bronchomotor control, inducing relaxation of airway smooth muscle (13) and producing sustained peripheral vasodilatation that is predominant in skin and fat (14, 15).

Methods

Human ventricular biopsies. All protocols were approved by the Ethics Committee of the Centre National de la Recherche Scientifique (France). 45 human endomyocardial biopsies were obtained from the right interventricular septum of cardiac transplant patients (40 men and 5 women, mean age 55.9±1.3 years) during right jugular vein catheterization performed routinely to detect possible rejection. None of the patients had evidence of cardiac rejection. All received immunosuppressive therapy (azathioprine, prednisolone and cyclosporine). In addition, 11 were given a calcium antagonist, 2 were given an $\alpha$-adrenoceptor antagonist, and 6 were given a diuretic. 27 had no treatment known to possess cardiovascular effects. The effects of $\beta$-agonists obtained in biopsies from patients treated with calcium antagonists were similar to those obtained in biopsies from patients not receiving these drugs. Biopsies were also performed in two pa-
Patients undergoing open-heart surgery and receiving no cardioactive or immunosuppressive drugs. In these two patients, the effects of β-adrenoceptor agonists were similar to those obtained in biopsies from transplanted patients.

**Experimental protocol.** Tissues were placed in a transport solution containing (in mM): 120 NaCl, 5 KCl, 1 CaCl₂, 1.1 MgCl₂, 0.33 NaH₂PO₄, 5 glucose, and 10 Hepes (pH adjusted to 7.4 with NaOH) and transported to the laboratory in less than 5 min. Preparations were then placed in an experimental chamber and superfused at a flow rate of 5 ml/min with oxygenated (95% O₂, 5% CO₂) Tyrode’s solution (37±0.5°C) containing (in mM): 120 NaCl, 5 KCl, 2.7 CaCl₂, 1.1 MgCl₂, 0.33 NaH₂PO₄, 5 glucose, and 20 NaHCO₃. Tissues were equilibrated for 60 min and then subjected to field stimulation at a pacing cycle length of 1,700 ms. Stimulus pulse width was set to 1 ms and amplitude was twice the diastolic threshold.

Tension and action potentials were measured as previously described (23). Tension was recorded using a mechatronic force transducer (Akers, AE 801; SensNor, Norway). Endomyocardial biopsies were stripped stepwise (10-μm increments) to a length at which contraction force was maximal. Studies were then performed at 90% of maximal tension. Action potentials were recorded using conventional 3 M KCl-filled microelectrodes (resistance, 10 to 25 MΩ). Electrodes were coupled to an Ag–AgCl electrode connected to an amplifier (VF 102; Biologic, Claix, France). The tissue chamber was grounded through an Ag–AgCl electrode.

After equilibration, the cumulative dose-response curves of β-adrenoceptor agonists were determined by superfusion with successively increasing concentrations of drugs. For all concentrations, tension and action potentials were recorded at steady state.

**Data analysis.** Tension and action potentials were recorded on a digital storage oscilloscope (400; Gould Inc., Les Ulis, France), a strip chart recorder (8188; Gould Inc.) and a digital tape recorder (DTR-1200: Biologic). Twitch and action potential parameters were analyzed using DATAPAC software (Caen University, France).

The results are expressed as mean±SEM of n number of experiments. The statistical significance of the drug effect was assessed using one-way analysis of variance, followed by a Bonferroni test. To determine agonist potencies from the dose-response curves, the concentrations producing 50% of maximum effect (EC₅₀) were determined by fitting curves with the Bolzmann equation. pD₂ values were then calculated according to the equation pD₂ = - log(EC₅₀) and compared using Student’s t test (P < 0.05 being considered significant). Apparent pA₂ values were calculated according to pA₂ = - log ([agonist]/DR-1), where DR was the dose ratio between the EC₅₀ value for an agonist in the presence of a given antagonist concentration and the EC₅₀ value in the absence of the antagonist.

**mRNA preparation.** Tissue samples weighing between 4 and 10 mg were homogenized in liquid nitrogen in 1.5-ml Eppendorf tubes. The samples were selected visually for absence of fat. The powder was resuspended in 0.8 ml RNA-PLUS™ extraction solution (Bioprobe Systems, Montreuil, France), to which 0.1 ml chloroform was added. The suspension was then shaken vigorously and kept on ice for 5 min before being centrifuged at 13,000 rpm (4°C) for 15 min. The aqueous phase was transferred to a fresh tube and an equal volume of isopropanol was added. The sample was then kept on ice for 45 min and centrifuged at 13,000 rpm (4°C) for 15 min. The supernatant was removed and the pellet washed once with 0.8 ml 75% ethanol. The RNA pellet was then dried and resuspended with an appropriate volume of diethylpyrocarbonate-treated water.

To avoid contamination with genomic DNA, total RNA was treated with amplification-grade DNase I (GIBCO BRL, Cergy Pontoise, France). Digestion was carried out at room temperature for 15 min and stopped by addition of 20 mM EDTA, pH 8.0, and incubation at 65°C for 10 min.

PolyA+ RNA was directly prepared from DNase I-treated RNA, using the Dynabeads mRNA purification kit (Biosys, Compiègne, France). Briefly, total RNA was heated at 65°C for 5 min, and then hybridized to oligo (dT)₂₅-linked magnetic beads at room temperature for 10 min, cleaned twice with washing solution (10 mM Tris-HCl, pH 7.5, 0.15 M LiCl, and 1 mM EDTA) and eluted with 24 μl of 2 mM EDTA, pH 8.0, at 65°C. PolyA+ RNA was used immediately or stored at −20°C for subsequent analysis.

**PCR analysis.** PolyA+ RNA was treated with 200 U Superscript TM II RNase H⁻ reverse transcriptase (GIBCO BRL) in 20 μl reverse transcriptase buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, and 3 mM MgCl₂) containing 5 mM DTT and 0.5 mM each dNTP, 100 ng oligo (dT)₁₂-18 and 2 U/ml RNase Inhibitor (Pharmacia, St. Quentin en Yvelines, France). A control without reverse transcriptase was performed to verify that amplification did not proceed from residual genomic DNA. cDNA was heated 5 min at 92°C, and then amplified by 30 cycles (92°C, 1 min; 55°C, 1.5 min; 72°C, 1.5 min), followed by a 5-min extension at 72°C in a temperature cycler (Biotium Biomed, Analytik GmbH, Göttingen, Germany) in 100 μl of PCR buffer containing 2.5 U of Thermus aquaticus polymerase (Promega Corp., Madison, WI), 2.5 mM MgCl₂, 50 μM each dNTP, 125 nM each sense and antisense oligonucleotide primer, 2.5% (vol/vol) formamide, and 10% (vol/vol) DMSO. For β-adrenoceptor gene, the sequences of sense and antisense oligonucleotide primers were 5’-GCATGCTC-CGTTGCCCTACAGAGA-3’ and 5’-CTGGCTCATGATGG-GCGC-3’, respectively. The expected fragment length was 525 bp. PCR products were visualized by electrophoresis through 2% agarose ethidium bromide–stained gels. Gels were blotted onto nylon membranes (Hybond N; Amersham International, Little Chalfont, UK) that were hybridized at 65°C to the human β-adrenoceptor cDNA (24). Final washing conditions were 15 mM NaCl, 1.5 mM sodium citrate, and 0.1% SDS at 65°C. Membranes were then subjected to autoradiography.

**Drugs.** (-)-Isoprenaline, norepinephrine, nadolol, adenosine, and pertussis toxin were obtained from Sigma Chemical Co. (St. Louis, MO). Metoprolol and CGP 12177 (4-[3-(3-butoxy-2-hydroxy-prooxy)benzimidazol-2-yl] was gifts from Ciba Geigy (Basel, Switzerland), bupranolol from Schwarz Pharma (Monheim, Germany), BRL 37344 (4-[2-hydroxy-(3-chlorophenyl)ethyl-amino]propyl) (R)-[2-hydroxyethyl]aminopyrrolidinone from Smith Kline-Beecham Pharmaceuticals (Surrey, UK) and CL 316243 (5-[2-[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl)-1-benz oxioxole-2,2-dicarboxylate from American Cyanamid (Pearl River, NY) and SR58611 ((RS)-(N-(25)-7-ethoxy carbonylmethoxy-1,2,3,4-tetrahydronaphth-2-yl)-(2R)-2-(3-chlorophenyl)-2-hydroxyethanamine hydrochloride) from Sanofi Recherche (Montpellier, France).

**Results**

**Effects of isoprenaline on mechanical responses.** We explored the effects of cumulative concentrations of isoprenaline on the mechanical properties of human endomyocardial biopsies (Fig. 1, A and B). As expected, isoprenaline alone (n = 5) induced a dose-dependent increase in peak tension and produced an acceleration of twitch, the maximal effect being obtained with a 1-μM concentration. At this concentration, isoprenaline increased peak tension by 268±74% (P < 0.05) and accelerated twitch, decreasing total duration from 496±9 to 429±16 ms (P < 0.05), time-to-peak from 186±5 to 145±4 ms (P < 0.05), half-contraction time from 82±4 to 65±2 ms (P < 0.05) and half-relaxation time from 142±9 to 110±9 ms (P < 0.05). In another set of experiments (n = 4), we explored the effects of isoprenaline in the presence of 10 μM nadolol, a potent β₁- and β₂-adrenoceptor antagonist (25). Under these conditions, isoprenaline induced a dose-dependent decrease in peak tension at concentrations ranging from 0.7 to 10 μM. 10 μM isoprenaline decreased peak tension by 27.2±4.5% (P < 0.05). Total twitch duration was also significantly decreased from 530±35 to 513±33 ms (P < 0.05), but other twitch parameters were not significantly modified. At higher concentrations (e.g.,
catecholamines exerted opposite effects on the mechanical properties of the human ventricle.

Effects of \( \beta_R \)-adrenoceptor agonists on mechanical and electrical responses. The first set of experiments suggested that the stimulation of a third cardiac \( \beta \)-adrenoceptor, possibly \( \beta_3 \), was responsible for the unexpected negative inotropic effect produced by isoprenaline. To further investigate this possibility, a series of preferential \( \beta_R \)-adrenoceptor agonists was used on human endomyocardial biopsies. As illustrated in Fig. 2, BRL 37344 induced a dose-dependent negative inotropic effect at concentrations ranging from 0.1 nM to 1 mM. pD values for this effect are presented in Table I. The maximum effect, obtained at a concentration of 1 \( \mu \)M, decreased peak tension by 59.4\( \pm \)2.8\% (\( P < 0.05 \)) as compared to the control. BRL 37344 accelerated twitch at the highest concentrations tested (0.7 to 3 \( \mu \)M). At 1 \( \mu \)M, it decreased total duration from 512\( \pm \)47 to 452\( \pm \)42 ms (\( P < 0.05 \)), time-to-peak from 186\( \pm \)19 to 169\( \pm \)19 ms (\( P < 0.05 \)), half-contraction time from 79\( \pm \)7 to 72\( \pm \)8 ms (\( P < 0.05 \)) and half-relaxation time from 145\( \pm \)12 to 119\( \pm \)12 ms (\( P < 0.05 \)). Since BRL 37344 possesses low affinity for \( \beta_R \) and \( \beta_2 \)-adrenoceptors (26), dose-response curves for this agonist were also plotted in the presence of several \( \beta \)-adrenoceptor antagonists. The dose-response curve to BRL 37344 was not modified by pretreatment with 1 \( \mu \)M metoprolol, a \( \beta_1 \)-adrenoceptor antagonist (Fig. 3 A) or with 10 \( \mu \)M nadolol, a \( \beta_1 \) and \( \beta_2 \)-adrenoceptor antagonist (Fig. 3 B). We also used bupranolol, which combines \( \beta_1 \), \( \beta_2 \) and \( \beta_3 \)-adrenoceptor antagonist properties (6, 16, 27). In the presence of 1 \( \mu \)M bupranolol, the dose-response curve to BRL 37344 on peak tension was shifted about tenfold to the right (apparent pA\( _1 \) value for bupranolol was 6.88; Fig. 3 C). Acceleration in the twitch, as observed at the highest concentrations of BRL 37344, was prevented in the presence of 1 \( \mu \)M bupranolol (data not shown).

Figure 1. Effects of isoprenaline on the twitch tension of human endomyocardial biopsies alone and in the presence of nadolol. (A) Time course of the effects of cumulative concentrations of isoprenaline alone (left) and in the presence of 10 \( \mu \)M nadolol, a \( \beta_1 \) and \( \beta_2 \)-adrenoceptor antagonist (right), on the twitch tension. In both cases, each concentration of isoprenaline was perfused for 6 min. When nadolol was used, it was first perfused alone and defined as control (C) when steady state was reached (15–20 min). Then, cumulative concentrations of isoprenaline were also perfused in the presence of nadolol. This figure is representative of data obtained in four other experiments. (B) Superimposed twitches obtained from the experiments illustrated in A.

Effects of isoprenaline on the twitch tension of human endomyocardial biopsies alone and in the presence of nadolol. (A) Time course of the effects of cumulative concentrations of isoprenaline alone (left) and in the presence of 10 \( \mu \)M nadolol, a \( \beta_1 \) and \( \beta_2 \)-adrenoceptor antagonist (right), on the twitch tension. In both cases, each concentration of isoprenaline was perfused for 6 min. When nadolol was used, it was first perfused alone and defined as control (C) when steady state was reached (15–20 min). Then, cumulative concentrations of isoprenaline were also perfused in the presence of nadolol. This figure is representative of data obtained in four other experiments. (B) Superimposed twitches obtained from the experiments illustrated in A.

Figure 2. Effects of BRL 37344 and SR 58611 on the twitch tension of human endomyocardial biopsies. (A) Time course of the effects of cumulative concentrations of BRL 37344 (left) and SR 58611 (right) on twitch tension. After control (C), the \( \beta_R \)-adrenoceptor agonists were perfused for 10 min for each concentration to obtain a steady state effect. This figure is representative of the data obtained in six other experiments for BRL 37344 and five other experiments for SR 58611. (B) Superimposed twitches obtained from the experiments illustrated in A. For the clarity of A and B, data obtained at 1 and 3 \( \mu \)M are not shown. (C) Dose-response curves for the negative inotropic effect of BRL 37344 (left) and SR 58611 (right) on peak tension. Values are the means\( \pm \)SEM of seven experiments for BRL 37344 (C) and six experiments for SR 58611 (C). The response is expressed as a percentage of decrease in peak tension compared to the control. The continuous line was obtained by curve fitting using the Bolzmann equation.

*Significant statistical difference (\( P < 0.05 \)) from basal peak tension.
Dose-dependent negative inotropic effects were also observed with SR 58611 at concentrations ranging from 0.3 nM to 3 μM (Fig. 2). The maximum effect was observed with 3 μM, which decreased peak tension by 64.3±6.1% (P < 0.05). As observed with BRL 37344, an acceleration in the twitch occurred at the highest doses: at 3 μM, total duration decreased from 558±7 to 479±17 ms (P < 0.05), time-to-peak from 202±4 to 173±11 ms (P < 0.05) and half-relaxation time from 164±4 to 128±10 ms (P < 0.05). Two other β₁-adrenoceptor agonists also induced dose-dependent negative inotropic effects: CL 316243 reduced peak tension for concentrations ranging from 1 nM to 10 μM (at 10 μM, peak tension decreased by 49.9±4.8%, P < 0.05) and CGP 12177 decreased peak tension for concentrations ranging from 0.01 to 100 μM (at 100 μM, peak tension decreased by 34.8±5.7%, P < 0.05). pD₂ values for SR 58611, CL 316243, and CGP 12177 are shown in Table I.

The effects of β₁-adrenoceptor stimulation were also studied on the electrophysiological characteristics of human endomyocardial biopsies (Fig. 4). BRL 37344 modified action potential parameters in the same range of concentrations in which it altered mechanical response (Fig. 4, A and B). At a 1-μM concentration (n = 4), BRL 37344 induced a reduction in the action potential duration measured at 30% of repolarization (APD₉₀) from 203±15 to 164±9 ms (P < 0.05), APD₅₀ from 256±17 to 218±17 ms (P < 0.05) and APD₀ from 336±30 to 294±31 ms (P < 0.05). Action potential amplitude was also reduced from 111.3±2.1 to 107.5±1.6 mV (P < 0.05). Comparable effects were produced by SR 58611 (Fig. 4 C). It is noteworthy that a similar reduction in action potential duration was observed with isoproterenol in the presence of 10 μM nadolol (data not shown).

Effects of β₁-adrenoceptor stimulation in the presence of pertussis toxin. In an attempt to define the involvement of Gᵢ proteins in the negative inotropic effects produced by β₁-adrenoceptor agonists, the effects of BRL 37344 were tested on human endomyocardial biopsies pretreated with pertussis toxin (PTX, Fig. 5). To determine PTX concentrations that would block Gᵢ proteins, adenosine stimulation was used as an assay because adenosine is generally thought to negatively modulate β-adrenoceptor stimulation by activating a PTX-sensitive G-protein pathway. Norepinephrine was used to stimulate adenylate cyclase in the presence of 1 μM prazosin to block α-adrenoceptors. In non-PTX-pretreated tissues, 1 μM norepinephrine alone produced a positive inotropic effect (peak tension increased by 61.7±19.3%, n = 6). When a steady state was reached in the presence of norepinephrine, 10 μM adenosine was added. Under these conditions, adenosine blunted the positive inotropic response to norepinephrine (peak tension decreased by 31.4±5.24%, n = 6). The same experiments were repeated in endomyocardial biopsies pretreated with 0.5 μg/ml PTX for 2 h at 30°C. In the presence of PTX, 1 μM norepinephrine increased peak tension by 42.1±13.2% (n = 5). But in this case, adenosine did not induce negative inotropic effects, demonstrating that activation of Gᵢ was effectively blocked. The effects of 10 nM BRL 37344 were then studied in control and PTX-pretreated endomyocardial biopsies. In con-

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**Table I. Negative Inotropic Effects of Several β₁-Adrenoceptor Agonists Alone or in the Presence of Antagonists on Human Endomyocardial Biopsies**

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Antagonists</th>
<th>pD₂</th>
<th>Maximal decrease (μM)</th>
</tr>
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<tbody>
<tr>
<td>BRL 37344</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRL 37344</td>
<td>10 Nadolol</td>
<td>8.65</td>
<td>59.4±2.8 (1)</td>
</tr>
<tr>
<td>BRL 37344</td>
<td>1 Metoprolol</td>
<td>8.34</td>
<td>60.0±2.5 (1)</td>
</tr>
<tr>
<td>BRL 37344</td>
<td>1 Bupranol</td>
<td>7.64</td>
<td>60.2±3.3 (3)</td>
</tr>
<tr>
<td>SR 58611</td>
<td></td>
<td>8.16</td>
<td>64.3±6.1 (3)</td>
</tr>
<tr>
<td>CL 316 243</td>
<td></td>
<td>8.24</td>
<td>49.9±4.8 (10)</td>
</tr>
<tr>
<td>CGP 12177</td>
<td></td>
<td>5.15</td>
<td>34.8±5.7 (100)</td>
</tr>
</tbody>
</table>

The pD₂ values of the agonists (-log EC₅₀) were estimated from each concentration-response curve. The pD₂ values are means±SEM of n experiments. The percentage of maximal decrease of peak tension is reported for each agonist. The results are mean±SEM of n experiments. The value of concentration in brackets is the concentration giving the maximal effect.

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1. Abbreviations used in this paper: APD, action potential duration; PTX, pertussis toxin.
control tissues, BRL 37344 induced a decrease in peak tension of 40.5±5.1% (n = 7). In PTX-pretreated tissues, the negative inotropic effects of BRL 37344 were markedly reduced (peak tension only decreased by 14.1±8.3%, n = 3).

**β₂-adrenoceptor mRNA in human myocardium.** To confirm the identity of the receptor involved in β₂-adrenoceptor agonist effects, mRNA expression of β₂-adrenoceptors was studied in human endomyocardial biopsies. A reverse transcription PCR protocol on PolyA⁺ RNA was developed to avoid contamination by genomic DNA (Fig. 6). When human β₂-adrenoceptor gene primers were used, the length of PCR-amplified product corresponded to the gene structure. Hybridization to human β₂-adrenoceptor cDNA confirmed the identity of the fragment. With this technique, β₂-adrenoceptor mRNA expression was detected in the myocardium of 5/5 subjects. We checked for possible contamination of the biopsies with fat cells expressing β₂-adrenoceptors, using hormone-sensitive lipase as a marker of brown and white fat cells. When primers specific for the human hormone-sensitive lipase gene were used (28), no specific products were amplified from human adipocytes (data not shown).

**Discussion**

Various studies have shown that β₁- and β₂-adrenoceptors coexist in the human heart and that their stimulation produces positive inotropic effects in in vitro human atrial and ventricular preparations (29–33). To our knowledge, there have been no previous reports as to a direct cardiac effect through β₁-adrenoceptor stimulation. Our study conclusively demonstrates the presence of functional β₂-adrenoceptors in the human heart ventricle and indicates that their stimulation induces surprisingly negative inotropic effects.

In stark contrast with the effects of isoprenaline alone, we observed that isoprenaline induced a negative inotropic effect when applied in the presence of nadolol, a potent β₁- and β₂-adrenoceptor antagonist possessing no β₂-adrenoceptor antagonist properties (27, 34). From this result, the existence of another adrenoceptor distinct from β₁ and β₂ was suspected. The existence of β₂-adrenoceptors in the human myocardium was further assumed when we observed that β₂-adrenoceptor ago-
nists also induced negative inotropic effects with an order of potency: BRL 37344 > SR 58611 ≈ CL 316243 > CGP 12177 similar to that observed in Chinese hamster ovary cells transfected with human β2-adrenoceptors (8, 26). The most convincing pharmacologic evidence for the presence of β2-adrenoceptors in the human heart was supported by the use of β2-adrenoceptor antagonists. The mechanical effects of BRL 37344 were not modified by pretreatment with metoprolol (a β1-adrenoceptor antagonist) or nadolol indicating that this effect was not mediated by β1- or β2-adrenoceptors. By contrast, bupranolol, which possesses β2-adrenoceptor antagonist properties (6, 27, 35), antagonized the negative inotropic effects of BRL 37344 with a pA2 value similar to that determined in adipocytes (6, 27). Finally, pharmacological evidence for myocardial β2-adrenoceptor was strengthened by detection of β2-adrenoceptor transcripts in the human ventricle. β2-adrenoceptor mRNA expression has already been reported in human heart biopsies, particularly at the atrial level (36, 37). However, these reports did not exclude an adipose origin for β2-adrenoceptor transcripts since a significant amount of adipose tissue was found in the samples (36). Adipocytes are rare in endomyocardial biopsies, it is not surprising that no specific products corresponding to the human hormone-sensitive lipase gene were detected in our own samples. Yet this would indicate that expression of β2-adrenoceptors in the human ventricle was not due to the presence of adipocytes.

Previous in vivo studies have evaluated whether β2-adrenoceptors exist in the cardiovascular system. In dogs, intravenously administered β2-adrenoceptor agonists induced a positive chronotropic effect (18). Because positive chronotropic effects were not observed in denervated animals, it was concluded that tachycardia resulted from a baroreceptor-mediated reflex in response to a drop in blood pressure caused by the vasodilating action of β2-adrenoceptor agonists (14, 15). Positive chronotropic and inotropic effects were also reported in isolated dog atrium perfused with blood from a donor dog injected with β2-adrenoceptor agonists. These effects were attributed to β2-adrenoceptor stimulation (19) and could be interpreted as resulting from baroreflex activation in the donor dog. In the clinical setting, β2-adrenoceptor agonists were shown to increase heart rate and blood pressure in man, an effect that was prevented by β2-adrenoceptor antagonists (21). In vitro studies are more suitable to analyzing the cardiac effects of β2-adrenoceptors. A typical example of the masking effects of baroreflex activation lies with 1,4-dihydropyridines, which induce a negative inotropic effect in vitro, but a positive chronotropic and inotropic effect in vivo as a consequence of vasodilation (38). In mammalian hearts, purported “atypical β-adrenoceptors” were suggested to be responsible for the positive inotropic and chronotropic effects of partial β-adrenoceptor agonists such as pindolol and alprenolol (16). However, human β2-adrenoceptors are distinct from atypical β-adrenoceptors of the mammalian heart inasmuch as the positive inotropic and chronotropic effects of partial agonists are not observed in isolated human tissues (17), and β2-adrenoceptor agonists induced a negative inotropic effect opposite the reported effects of “atypical β-adrenoceptors” stimulation in animals.

As the β2-adrenoceptor stimulation induced a marked negative inotropic effect, it could be hypothesized that this receptor is not coupled to Gs protein. It has been previously shown that in adipocytes β2-adrenoceptors could be linked to Gi proteins (39, 40). Furthermore, the β2-adrenoceptor has recently been shown to activate both Gi and Gq proteins (41). In our study, in PTX-pretreated tissues, the negative inotropic effects of BRL 37344 were markedly reduced, suggesting the involvement of Gi proteins in the β2-adrenoceptor signaling pathway. However, the negative inotropic effects of BRL 37344 are not fully suppressed, either because Gi proteins are not completely blocked by PTX or because another mechanism coexists with the Gi pathway. Therefore, a more complete study will be necessary to determine the receptor-effector pathways.

The present study raises the question as to the role of β2-adrenoceptors in cardiac diseases. In heart failure, increased activity of the sympathetic nervous system leads to downregulation of cardiac β1- and β2-adrenoceptors (22) resulting from their phosphorylation by cAMP-dependent protein kinase or β-adrenoceptor kinase. Reduced β1- and β2-adrenoceptors lead to a decrease in the contractile response to β-adrenoceptor agonists. Contrary to β1- and β2-adrenoceptors, β2-adrenoceptors lack phosphorylation sites for cAMP-dependent protein kinase or β-adrenoceptor kinase (3), and thus may not be downregulated in heart failure. According to this hypothesis, the high adrenoceptor tone during heart failure may alter the cardiac contractile activity as a result of unmasked β2-adrenoceptor stimulation in the presence of reduced β1- and β2-adrenoceptors. Finally, the present study also has implication in clinical pharmacology. Indeed, the development of β2-adrenoceptor agonists has led to the elaboration of prom-

![Image](https://example.com/image.png)

**Figure 6.** β2-adrenoceptor mRNA expression in human myocardium. (A) The negative of an agarose gel electrophoresis photo of β2-adrenoceptor cDNA amplified by PCR. PolyA+ RNAs were subjected (+) or not (–) to reverse transcription. Size markers (M) are the 100-bp DNA ladder. (B) Southern blot hybridized to a human β2-adrenoceptor cDNA probe. The membrane was autoradiographed for 14 h.
ising new drugs. The predicted therapeutic indications for these drugs are obesity and obesity-linked diabetes. However, we have demonstrated that β3-adrenoceptors are also involved in regulation of the mechanical and electrophysiological activities of the human heart and thus may produce cardiac side effects. Clearly, further studies are needed to address the precise role of cardiac β3-adrenoceptor in human cardiac physiology and physiopathology.

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