Bucindolol Exerts Agonistic Activity on the Propranolol-Insensitive State of β₁-Adrenoceptors in Human Myocardium

ANDREAS BUNDKIRCHEN, KLARA BRIXIUS, BIRGIT BÖLCK, and ROBERT H. G. SCHWINGER

Labor für Herzmuskelphysiologie und Molekulare Kardiologie, Klinik III für Innere Medizin der Universität zu Köln, Köln, Germany

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ABSTRACT

In congestive heart failure patients, treatment with β-adrenoceptor antagonists improves symptoms and decreases mortality. However, intrinsic sympathomimetic activity of β-adrenoceptor antagonists might be disadvantageous in chronic heart failure. The nonselective β₁- and β₂-adrenoceptor antagonist bucindolol has failed to decrease mortality in clinical trials. A putative β₂-adrenoceptor, which mediates positive inotropic effects by activation of the adenylate cyclase has been identified to be a propranolol-insensitive state of the β₂-adrenoceptor. The present study aimed to characterize whether bucindolol exhibits agonistic activity on this atypical β₂-adrenoceptor state as one possible reason for clinical inefficacy. For comparison, stimulation of atypical state β₂-adrenoceptor with forskolin-stimulated, left ventricular papillary muscle strips (donor hearts, nonfailing; n = 5) and in right auricular trabeculae (bypass operation; n = 4). Functional studies on the propranolol-insensitive state of β₂-adrenoceptors were performed in isolated muscle preparations after β₁- and β₂-adrenoceptor antagonism (propranolol, 1 μM), inhibition of β₂-mediated inotropic effects (L-nitro-arginine, 100 μM) and forskolin treatment (0.3 μM). Positive inotropic response to stimulation of atypical state β₂-adrenoceptors could be demonstrated in right auricular as well as left ventricular human myocardium (CGP 12177 treatment, 10 μM). Under these conditions, also bucindolol, but not metoprolol and nebivolol, significantly increased contractility (all 10 μM). In conclusion, bucindolol but not metoprolol or nebivolol mediate positive inotropic effects in human myocardium due to activation of atypical state β₂-adrenoceptors. Thus, the agonistic activity of bucindolol may influence outcome in heart failure patients.

Impressive evidence has been given for a beneficial effect on mortality mediated by β-adrenoceptor antagonist therapy in chronic heart failure patients (CIBIS-II Investigators and Committees, 1999; MERIT-HF Study Group, 1999). In a recent study the nonselective β-adrenoceptor antagonist carvedilol was associated with a decrease in mortality even in New York Heart Association (NYHA) class IV patients (Packer et al., 2001). However, beneficial effects could not be shown for all β-adrenoceptor antagonists. Xamoterol, a β₁-selective β-adrenoceptor antagonist with intrinsic sympathomimetic activity (ISA) (Schwinger et al., 1990; Böhm et al., 1990), increased mortality in heart failure patients (Xamoterol in Severe Heart Failure Study Group, 1990). It was assumed that the ISA of xamoterol was responsible for this negative outcome. Celiprolol, a β-adrenoceptor antagonist with β₂-agonistic properties (Thulesius et al., 1982; Böhm et al., 1992), had no beneficial effect in chronic heart failure (Wichtitz et al., 2000). These data suggest an unfavorable role of any agonistic property in heart failure. In contrast, metoprolol and carvedilol have been shown to exert no ISA in human myocardium (Brixius et al., 2001). However, in β₁-adrenoceptor transgenic mice, a slight, but not statistically significant stimulation of spontaneous beating frequency of isolated right atria was detected after carvedilol treatment (Engelhardt et al., 2001).

Recently, the Beta-Blocker Evaluation of Survival Trial (2001) using the nonselective β-adrenoceptor antagonist bucindolol was terminated because no benefit in overall mortality reduction was observed. Subgroup analysis of this study suggests that the nonefficacy of bucindolol was mainly due to a rather enhanced mortality in the black population and in NYHA class IV patients, whereas a survival benefit in nonblack NYHA class III patients was observed. However, in contrast to bucindolol, carvedilol was also effective in the treatment of black patients suffering from chronic heart failure (Yancy et al., 2001). This also holds true for a relatively small trial (a cohort of 54 patients) of metoprolol (Freudenberg et al., 1997). Thus, the lack of overall survival benefit of bucindolol in heart failure patients still remains unclear.

ABBREVIATIONS: NYHA, New York Heart Association; ISA, intrinsic sympathomimetic activity; CGP 12177, (S)-4-((3′-t-butyramino-1′-hydroxypropoxy)-benzimidazole-2; L-NMA, L-nitro-arginine.
There is ongoing discussion regarding whether bucindolol may have intrinsic sympathomimetic activity. In the rat, bucindolol has clearly shown ISA (Willette et al., 1999); in human myocardium, the situation is controversial (Hershberger et al., 1990; Maack et al., 2000; Sederberg et al., 2000).

Only recently, the presence of additional β-adrenoceptors was described in human myocardium. The β2-adrenoceptor is reported to mediate negative inotropic effects in human myocardium by activation of nitric oxide synthetase involving inhibitory G-proteins (Gauthier et al., 1998) and seems to be up-regulated in human heart failure (Gauthier et al., 1998; Moniotte et al., 2001). However, the physiological role of the β2-adrenoceptor still remains unclear in the human heart.

The existence of a new "putative β2-adrenoceptor" was postulated because several β-adrenoceptor antagonists (so-called nonconventional partial agonists) cause cardiotimulant effects at concentrations that exceed their affinity for β1- and β2-adrenoceptors. CGP 12177, a hydrophilic β-adrenergic ligand (Staehelin et al., 1983), which was supposed to be a β1- and β2-adrenoceptor antagonist with β2-partial agonistic properties (Mohell and Dicker, 1989) is the most frequently investigated nonconventional partial agonist. The cardiotimulant effects of these nonconventional partial agonists are resistant to blockade by propranolol.

This putative β2-adrenoceptor has been functionally demonstrated in several cardiac tissues, e.g., atrial myocardium of β2-knockout mice (Kaumann et al., 1998), rat myocytes (Malinowska and Schlöcker, 1996; Sarsro et al., 1999), and human myocardium (Sarsero et al., 1996; Kaumann and Molenaar, 1997). However, recent studies have demonstrated that the putative β2-adrenoceptor does not exist as a distinct entity. It was reported, that the putative β2-adrenoceptor response no longer existed in brown adipose tissue of β1-knockout mice (Konkar et al., 2000). This holds true for both wild-type and β2-knockout mice the effect was present (Kaumann et al., 2001). In addition, it could be demonstrated in a rat model of cardiac failure that the pharmacology of the putative β2-adrenoceptor parallels that of the β1-adrenoceptor (Kompa and Summers, 1999). Thus, the putative β2-adrenoceptor has to be defined as a propranolol-insensitive state of the β1-adrenoceptor. However, it cannot be excluded that this rarely described state of the β2-adrenoceptor has an impact on inotropic or chronotropic cardiac response due to β-agonistic stimulation.

Previous studies have shown that increases in cAMP worsen the prognosis of heart failure patients (Xamoterol in Severe Heart Failure Study Group, 1990; Cruickshank, 1993). Thus, besides detrimental effects due to ISA mediated by typical β1- and β2-adrenoceptors, it may be possible that β-adrenoceptor antagonists induce detrimental effects on heart failure patients by interaction with additional atypical β1-adrenoceptors.

Therefore, besides determination of ISA on β1- and β2-adrenoceptors, the present study investigated the interaction of bucindolol with the atypical state of the β1-adrenoceptor in human myocardium. For comparison, the "typical" nonconventional partial agonist CGP 12177 as well as the frequently administrated metoprolol and the newly developed highly β1-selective adrenoceptor antagonist nebivolol were studied.

Experimental Procedures

Preparation of Isolated Auricular Trabeculae

Right atrial tissue was taken from patients undergoing aortocoronary bypass operation (n = 10, seven males, three females; age 59 ± 2 years) who were without clinical signs of cardiac failure as measured by cardiac catheterization (normal ejection fraction, end systolic volume, and stroke volume) and echocardiography. None of the patients had received Ca2+ channel antagonists or Ca2+ channel agonists within 7 days of surgery or β-adrenoceptor agonists 48 h before surgery. All of the patients received β-adrenoceptor antagonists. Drugs used for general anesthesia were propofol, fentanyl, and pancuronium bromide. The tissue was delivered within 5 min into the laboratory in ice-cold pre-aerated Bretschneider solution of the following composition: 15 mM NaCl, 10 mM KCl, 4 mM MgCl2, 100 mM histidine, 2 mM tryptophan, 30 mM mannitol, and 1 mM potassium dihydrogen oxoglutarate. From each native myocardial tissue sample, auricular trabeculae were selected of 0.4 to 0.6 mm thickness and 6 to 8 mm length under microscopic control (Axiovert 100; Carl Zeiss, Oberkochen, Germany).

Preparation of Left Ventricular Papillary Muscle Strips

Nonfailing human myocardium was obtained from five donors with brain death caused by traumatic injury (n = 5, four males, one female; age 52 ± 6 years). The nonfailing hearts could not be transplanted because of technical reasons. Failing myocardium was obtained during cardiac transplantation due to dilated cardiomyopathy (n = 3, two males, one female; age 61.3 ± 3.5 years; ejection fraction, 22.7 ± 1.8; cardiac index, 2.2 ± 0.1 l/min x m² x min). Patients suffered from heart failure clinically classified as NYHA class IV on the basis of clinical symptoms and signs as judged by the attending cardiologist shortly before operation. Medical therapy consisted of diuretics, nitrates, angiotensin-converting enzyme inhibitors, and cardiac glycosides. Patients receiving catecholamines, β-adrenoceptor- or Ca2+ -agonists were withdrawn from the study.

Immediately after excision, the papillary muscles were placed in ice-cold pre-aerated Bretschneider solution and delivered to the laboratory within 5 min. Muscle strips 0.6 to 0.8 mm thick and 6 to 8 mm long with muscle fibers running approximately parallel to the length of the strips were carefully dissected under microscopic control in aerated bathing solution at room temperature. Connective tissue, if visibly present, was carefully trimmed away.

The experiments were performed on isolated electrically driven muscle preparations. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 75-ml glass tissue chambers to record the isometric contractions. The bathing solution used was modified Tyrode's solution that contained 119.8 mM NaCl, 5.4 mM KCl, 1.05 mM MgCl2, 1.8 mM CaCl2, 22.6 mM NaHCO3, 0.42 mM NaH2PO4, 0.05 mM Na2EDTA, and 5.5 mM glucose. It was continuously gassed with 95% O2 and 5% CO2 and maintained at 37°C; the pH was 7.4. The isometric force of contraction was measured with an inductive force transducer (W. Fleck, Freiburg, Germany) attached to a Hellige Helco Scriptor (Hellige, Mainz, Germany) or Gould recorder (Cleveland, OH). The muscles were electrically stimulated at 1 Hz with rectangular pulses of 5-ms duration (Grass stimulator SD 9, Quincy, MA), and the voltage was 20% above threshold. All preparations were allowed to equilibrate at least 90 min in drug-free bathing solution until complete stabilization. After 45 min, the solution was changed. The duration of stimulation for a given concentration was constant until there was complete stabilization of force development. Control strips of native myocardium showed no change in baseline isometric tension during the time necessary to complete pharmacological testing. In all experiments, the final concentration of solvent (dimethyl sulfoxide) was 0.1%. Compound-dependent changes in force of contraction were determined. None of the substances changed pH or temperature.

Determination of ISA. To investigate ISA of the different β-adrenoceptor antagonists, left ventricular muscle preparations from...
nonfailing myocardium were preincubated with forskolin (0.3 μM) (Jasper et al., 1988). Forskolin facilitates the coupling of the stimulatory G-protein with the catalytic subunit of the adenylate cyclase. Dose-response curves for bucindolol, metoprolol, and nebivolol were measured (0.1–10 μM). In addition, the influence of low and high concentrations of bucindolol and CGP 12177 on right auricular trabeculae under baseline conditions was measured to determine possible partial agonistic activity in right atrial myocardium.

**Investigations on Propranolol-Insensitive State β1-Adrenoceptors.** Effects mediated by propranolol-insensitive state β1-adrenoceptors were investigated after successive administration of 1) propranolol (1 μM) to block cardiac β1- and β2-adrenoceptors (Wellstein et al., 1986); 2) N-nitro-l-arginine (l-NMA; 100 μM), to inhibit the nitric-oxide synthase (Griffith and Kilbourn, 1996) and thus the suggested key enzyme of the β1-adrenoceptor pathway (Gauthier et al., 1998; Moniotte et al., 2001); 3) forskolin (0.3 μM); and 4) CGP 12177, bucindolol, metoprolol, or nebivolol (all 10 μM). In each experiment CGP 12177 (10 μM) was added at the end of the experiment. Under these conditions, the inotropic effects of the β1-adrenoceptor antagonists should be mediated by stimulation of the atypical state of the β1-adrenoceptor.

**Materials**

Bucindolol was generously provided by Knoll AG (Mannheim, Germany), metoprolol by Astra GmbH (Wedel, Germany), and nebivolol by Berlin Chemie (Berlin, Germany). CGP 12177A was obtained from BioTrend (Köln, Germany). Bupranolol was provided by Schwarz Pharma (Zwickau, Germany). All other chemicals were of analytical grade or the best grade commercially available.

**Statistics**

All values are means ± S.E.M. Statistical significance was analyzed with Student’s t test for paired observations. Significance was imparted at a p value of <0.05.

**Results**

**Investigation of CGP 12177.** The influence of the β1-adrenoceptor agonist CGP 12177 on basal force of contraction was investigated using electrically driven (1 Hz, 37°C) right auricular trabeculae. Basal force of contraction of right auricular trabeculae was 10.3 ± 1.1 mN/mm². CGP 12177-treatment mediated a biphasic inotropic response. In a low concentration (10 nM), CGP 12177 decreased force of contraction (−9.4 ± 2.9%), whereas an increase in force of contraction could be observed in a high concentration (10 μM) (+12.8 ± 2.5%). Figure 1 shows one representative trace recording (A) as well as the means ± S.E.M. of four experiments (B).

Functional studies on the propranolol-insensitive state of β1-adrenoceptors were performed in isolated muscle preparations after β1- and β2-adrenoceptor antagonism (propranolol, 1 μM), inhibition of β1-mediated inotropic effects by blockade of the nitric oxide synthase (l-NMA; 100 μM), and forskolin treatment (0.3 μM) to sensitize adenylate cyclase. Under these conditions, CGP 12177 (10 μM) increased force of contraction significantly. This increase in force of contraction is suggested to be mediated by the atypical state of the β1-adrenoceptor (Kaumann et al., 2001). Figure 2A shows one representative original recording, demonstrating the positive inotropic effect of CGP 12177. Average values (means of four experiments) are given in Table 1 and are presented in Fig. 6.

The positive inotropic response to CGP 12177 mediated by the propranolol-insensitive state of the β1-adrenoceptor could be observed in right auricular trabeculae (n = 4) as well as in left ventricular papillary muscle strips from failing myocardium (n = 4) (Fig. 3). The increase in force of contraction was more pronounced in right auricular trabeculae compared with left ventricular papillary muscle strips as judged by comparison of the percentage of increase in force of contraction (178.7 ± 11% versus 144.6 ± 14%, p < 0.05). Thus, examinations of the influence of other β-adrenoceptor antagonists on the atypical state of the β1-adrenoceptor were performed in right auricular trabeculae.

**Investigation of Bucindolol.** The influence of bucin dolol on force of contraction was investigated under basal conditions in right auricular trabeculae and after forskolin stimulation in left ventricular papillary muscle strips. In contrast to CGP 12177, bucindolol concentration dependently decreased force of contraction in right auricular trabeculae. Basal force of contraction of right auricular trabeculae was 10.4 ± 0.8 mN/mm² (n = 4). Bucindolol (10 nM) significantly decreased force of contraction by 13.8 ± 5.9%, whereas 10 μM further decreased force of contraction by 40.1 ± 1.5% (Fig. 4A).

Investigation of left ventricular papillary muscle strips from nonfailing myocardium after pretreatment with forskolin (0.3 μM) showed similar results (Fig. 4B). Basal force of contraction of left ventricular papillary muscle strips was 7.0 ± 0.9 mN/mm² (n = 5). Forskolin (0.3 μM) increased force of contraction significantly to 12.3 ± 2.1 mN/mm². Bucindolol (10 nM) significantly decreased force of contraction by 10.9 ± 2.3%, whereas 10 μM further decreased force of contraction by 37.0 ± 0.1%. Taken together, bucin dolol did not mediate
agonistic activity in right auricular trabeculae and left ventricular papillary muscle strips in a condition where all β-adrenoceptors are present.

According to the investigation of CGP 12177, the influence of bucindolol on the propranolol-insensitive state of the β₁-adrenoceptor was investigated in isolated muscle preparations after β₁- and β₂-adrenoceptor antagonism (propranolol, 1 μM), inhibition of β₃-mediated inotropic effects (L-NMA, 100 μM), and forskolin treatment (0.3 μM).

Similar to CGP 12177, bucindolol (10 μM) increased force of contraction significantly under the condition studied. Figure 2B shows an original recording demonstrating the positive inotropic effect of bucindolol mediated by the atypical state of the β₁-adrenoceptor. Average values of five experiments are given in Table 1 and are presented in Fig. 6.

However, in the presence of bupranolol, which has been shown to antagonize β₁-, β₂-, β₃- and even putative β₄-adrenoceptors (Kaumann and Molenaar, 1997), the positive inotropic effect of bucindolol mediated by atypical state β₁-adrenoceptors was absent. One representative recording for investigations of the influence of bucindolol on the propranolol-insensitive state of the β₁-adrenoceptor in the presence of bupranolol is shown in Fig. 5. The finding that bucindolol is unable to increase force of contraction in the presence of bupranolol is indicative that the observed positive inotropic effect was due to β₁-adrenoceptor stimulation and not stimulation/inhibition of non-β-adrenergic receptors.

Investigation of Nebivolol and Metoprolol. The influence of nebivolol and metoprolol on the propranolol-insensitive state of the β₁-adrenoceptor was investigated according to CGP 12177 and bucindolol. Neither nebivolol nor metoprolol mediated positive inotropic response to atypical state β₁-adrenoceptors as demonstrated in Fig. 2, C and D, respectively. Both lead to a decrease in force of contraction after

![Fig. 2. Original force recordings of electrically driven isolated right auricular trabeculae illustrating the inotropic response of CGP 12177, bucindolol, nebivolol, and metoprolol to propranolol-insensitive state β₁-adrenoceptors. β₁- and β₂-adrenoceptors were blocked by propranolol (1 μM). L-NMA (100 μM) inhibits the key enzyme of the β₂-adrenoceptor pathway, whereas forskolin (0.3 μM) sensitizes adenylate cyclase.](image)

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<th>TABLE 1</th>
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<td>Data of functional studies on the propranolol-insensitive state of the β₁-adrenoceptor in right auricular trabeculae</td>
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<td>Substances were cumulatively administered as presented. Force of contraction is given in millinewtons per millimeter squared.</td>
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FOC, force of contraction; n, quantity of experiments; β-AR ant., β-adrenoceptor antagonist.

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**Fig. 2.** Original force recordings of electrically driven isolated right auricular trabeculae illustrating the inotropic response of CGP 12177, bucindolol, nebivolol, and metoprolol to propranolol-insensitive state β₁-adrenoceptors. β₁- and β₂-adrenoceptors were blocked by propranolol (1 μM). L-NMA (100 μM) inhibits the key enzyme of the β₂-adrenoceptor pathway, whereas forskolin (0.3 μM) sensitizes adenylate cyclase.
pretreatment with propranolol, L-NMA, and forskolin (26.0 ± 0.1% and 18.0 ± 0.1%, respectively) (Fig. 6 and Table 1) and thus seem to be antagonists.

Figure 7 shows representative original tracings after stimulation of atypical state β₁-adrenoceptors demonstrating the influence on the time course of the contractile twitch of right auricular trabeculae. No change in time to peak contraction and time to half-peak relaxation could be demonstrated by stimulation of the propranolol-insensitive state of the β₁-adrenoceptor with CGP 12177 or bucindolol or by inhibition with metoprolol or nebivolol.

Discussion

Recently, a putative β₁-adrenoceptor has been described that mediates positive inotropic effects in myocardial tissue by activation of adenylyl cyclase. However, it has been demonstrated that this is not a distinct β-adrenoceptor entity but an atypical, propranolol-insensitive state of the β₁-adrenoceptor (Kauermann et al., 2001). Agonistic activation on this state of the β₁-adrenoceptor may be one candidate leading to the neutral effects on mortality in the Beta-Blocker Evaluation of Survival Trial (2001) using bucindolol. Thus, we studied whether bucindolol exhibits agonistic activity to this atypical state of the β₁-adrenoceptor.

In heart failure β₁-adrenoceptors are down-regulated (Bristow et al., 1982; Brodde, 1991; Schwinger et al., 1991). Therefore, it might be possible that the functional importance of other pathways, such as β₂-adrenoceptors or atypical state β₁-adrenoceptors may increase. Since increase in cAMP worsens the prognosis of heart failure patients (Xamoterol in Severe Heart Failure Study Group, 1990; Cruickshank, 1993), it may be possible that the different effects of β-adrenoceptor antagonists on mortality are due to their agonistic activity on the propranolol-insensitive state of the β₁-adrenoceptor. Furthermore, it has been demonstrated that stimulation of this β-adrenoceptor mediates arrhythmic Ca²⁺-transients in mice ventricular myocytes (Freestone et al., 1999), which might also be of importance for treating heart failure patients.
In this study, bucindolol did not possess ISA in a multicellular system containing all receptor types that might impact cardiac contractility. Since net increase/decrease in force of contraction can still be negative although ISA is present, the experiments do not provide final evidence for a lack of ISA of bucindolol.

We investigated the influence of bucindolol, nebivolol, and metoprolol on the putative \( \beta_1 \)-adrenoceptor in human myocardium on a functional level. Functional studies on this propranolol-insensitive state of \( \beta_1 \)-adrenoceptors were performed in isolated muscle preparations after \( \beta_1 \)- and \( \beta_2 \)-adrenoceptor antagonism (propranolol, 1 \( \mu \)M), inhibition of \( \beta_3 \)-mediated inotropic effects (L-NMA, 100 \( \mu \)M), and forskolin treatment (0.3 \( \mu \)M). The present study indicates that bucindolol, like CGP 12177, exhibits positive inotropic effects mediated by the atypical state of the \( \beta_1 \)-adrenoceptor (of the same magnitude as the forskolin-mediated increase in force of contraction), whereas nebivolol and metoprolol appear to be antagonists in human hearts.

Another explanation for the increase in force of contraction induced by bucindolol might be a reversal of the negative inotropic effect of propranolol due to the lower inverse agonistic activity of bucindolol compared with propranolol. However, one would expect a similar effect after nebivolol treatment because nebivolol has low inverse agonistic activity comparable with bucindolol (Brixius et al., 2001; Maack et al., 2001). In addition, the positive inotropic effect of bucindolol was absent in the presence of bupranolol.

**Fig. 5.** Influence of bupranolol (10 \( \mu \)M) on the agonistic activity of bucindolol on the propranolol-insensitive state of the \( \beta_1 \)-adrenoceptor. Note that in the presence of bupranolol, the positive inotropic effect of bucindolol was no longer evident.

**Fig. 6.** Bar graphs give the means of all experiments in percentage of basal force of contraction (FOC). The first bar represents basal force of contraction, the second bar force of contraction after cumulative addition of propranolol (prop, 1 \( \mu \)M), L-NMA (100 \( \mu \)M), and forskolin (Fors, 0.3 \( \mu \)M). Effects of CGP 12177 (CGP, panel A), bucindolol (Buc, panel B), nebivolol (Neb, panel C) and metoprolol (Met, panel D) (all 10 \( \mu \)M) on force of contraction are demonstrated in the third bar. The last bar represents further addition of CGP 12177 (CGP, 10 \( \mu \)M). Note that the additional increase in force of contraction by CGP 12177 was less pronounced when bucindolol was added before and more pronounced after nebivolol and metoprolol. \# \( p < 0.05 \) versus \( \beta_1 \)-adrenoceptor antagonist; *, \( p < 0.05 \) versus +Prop, +L-NMA, +Fors.
mortality in heart failure patients due to activation of the molecular levels of action of the different ever, this has to be further clarified in studies focusing on the molecular level are necessary. In this study, the β-adrenoceptor antagonists were investigated at concentrations that exceed their affinity for β1- and β2-adrenoceptors, respectively. In addition, the investigated concentrations exceed the plasma concentrations of the investigated β-adrenoceptor antagonists. The positive inotropic effect of bucindolol has been observed in nonfailing myocardium from patients undergoing aortocoronary bypass operation. Thus, it cannot be excluded that the results may differ in heart failure patients.

Whether a positive inotropic effect of the atypical state of the β1-adrenoceptors, as has been shown for bucindolol in the present study, may have consequences for its clinical use has to be further investigated and remains at the moment speculative. However, the β1-selective β-adrenoceptor antagonists metoprolol (studied by the MERIT-HF Study Group, 1999) and nebivolol (now investigated in the Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalisation in Seniors with Heart Failure (SENIOR) trial) are not capable of any agonistic activation.

Acknowledgments

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References


Fig. 7. Representative original tracings after stimulation of atypical state β1-adrenoceptors demonstrating the influence on the time course of the contractile twitch of right auricular trabeculae. The solid twitch was recorded after cumulative application of propranolol (1 μM), t-NAME (100 μM), and forskolin (0.3 μM); the dotted twitch after further addition of CGP 12177 (panel A), bucindolol (panel B), nebivolol (panel C), or metoprolol (panel D) (all 10 μM).

There is an ongoing discussion whether bucindolol may have ISA in human myocardium. Despite investigations that did not reveal ISA (Hershberger et al., 1990; Sederberg et al., 2000), Maack et al. (2000) detected ISA in three of eight specimens from human left ventricular myocardium, suggesting that ISA of bucindolol may depend on the examined tissue. In a recent study we did not reveal any agonistic activity (Brixius et al., 2001). The present study provides new information about an “ISA-like” intrinsic activity that appears at high concentrations and is unmasked only if the classic β1- and β2-pathway is blocked.

It may be suggested that bucindolol has failed to decrease mortality in heart failure patients due to activation of the cAMP-dependent atypical β1-adrenoceptor stimulation. However, this has to be further clarified in studies focusing on the molecular levels of action of the different β-adrenoceptors. The presented agonistic activity on atypical β1-adrenoceptor may influence regulation of intracellular ion homeostasis and/or cell regulatory processes. This has to be addressed in further studies as well.

In our studies, no change in time to half-peak relaxation could be demonstrated by activation of the propranolol-insensitive state of the β1-adrenoceptor. An agonistic activation of the β-adrenoceptor pathway results in a decrease in time to half-peak relaxation (Brixius et al., 1997). Thus, the typical feature for a cAMP-mediated mechanism is not shown. One speculative explanation for this is that cAMP formation by the atypical state of the β1-adrenoceptor is preliminarily located in a subsarcolemmal microdomain and thus force development is increased due to agonistic activation without changes in relaxation parameters. This feature has also been described for the β2-adrenoceptor (Xiao et al., 1999). Additionally, forskolin stimulation, which acts via activation of the catalyst itself, may lead to shortened relaxation, which may not be further enhanced by the agents studied.

The present study was performed under in vitro conditions, and experiments were either performed on isolated muscle preparations or crude membrane preparations of human hearts. All of the patients received β-blocking agents. It cannot be excluded that in vivo effects may differ from those observed in vitro. Furthermore, the interaction on the propranolol-insensitive state of the β1-adrenoceptors cannot be studied with a more direct approach. Additional studies focusing on the molecular level are necessary. In this study, the β-adrenoceptor antagonists were investigated at concentrations that exceed their affinity for β1- and β2-adrenoceptors, respectively. In addition, the investigated concentrations exceed the plasma concentrations of the investigated β-adrenoceptor antagonists. The positive inotropic effect of bucindolol has been observed in nonfailing myocardium from patients undergoing aortocoronary bypass operation. Thus, it cannot be excluded that the results may differ in heart failure patients.

Whether a positive inotropic effect of the atypical state of the β1-adrenoceptors, as has been shown for bucindolol in the present study, may have consequences for its clinical use has to be further investigated and remains at the moment speculative. However, the β1-selective β-adrenoceptor antagonists metoprolol (studied by the MERIT-HF Study Group, 1999) and nebivolol (now investigated in the Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalisation in Seniors with Heart Failure (SENIOR) trial) are not capable of any agonistic activation.