Differential Effects of Bucindolol and Carvedilol on Noradrenaline-Induced Hypertrophic Response in Ventricular Cardiomyocytes of Adult Rats

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ABSTRACT

In adult rat ventricular cardiomyocytes, noradrenaline exerts dual effects on protein synthesis: increases via $\alpha_1$-adrenoceptors and decreases via $\beta_1$-adrenoceptors. Carvedilol and bucindolol are $\beta$-blockers with additional $\alpha_1$-adrenoceptor blocking activities. We studied the effects of carvedilol and bucindolol on noradrenaline-induced protein synthesis (assessed by $[^3H]$phenylalanine incorporation) in adult rat ventricular cardiomyocytes. Radioligand binding studies with $[^125]$iodocyanopindolol and $[^3H]$prazosin revealed that carvedilol had a much higher affinity to $\alpha_1$-adrenoceptors than bucindolol ($\beta_1$/-$\alpha_1$-adrenoceptor ratio for carvedilol, 1:2.7; for bucindolol, 1:43). Noradrenaline-evoked increases in protein synthesis were enhanced by propranolol (1 $\mu$M) and $\beta_1$-adrenoceptor-selective antagonists bisoprolol (1 $\mu$M) and CGP 20712A [1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethyl-amino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxo]-2-propranol methanesulfonate] (300 nM). Carvedilol (100 pM–10 $\mu$M) inhibited 1 $\mu$M noradrenaline-induced increase in protein synthesis with monophasic concentration-inhibition curves independent of whether CGP 20712A was present or not; $K_i$ values for carvedilol were 5 to 6 nM. In contrast, bucindolol (100 pM–10 $\mu$M) inhibited 1 $\mu$M noradrenaline-induced increase in protein synthesis with a bell-shaped concentration-inhibition curve; it increased noradrenaline-induced protein synthesis at 10 nM, although at concentrations >100 nM it was inhibited. In the presence of 300 nM CGP 20712A or 1 $\mu$M propranolol, however, bucindolol inhibited 1 $\mu$M noradrenaline-induced increase in protein synthesis with monophasic concentration-inhibition curves; $K_i$ values were 40 to 75 nM. On the other hand, both carvedilol and bucindolol inhibited 1 $\mu$M phenylephrine-induced protein synthesis with monophasic concentration-inhibition curves; $K_i$ values were 4 (carvedilol) and 45 nM (bucindolol). These results indicate that, at low ($\beta_1$-adrenoceptor blocking) concentrations, bucindolol can enhance noradrenaline-induced protein synthesis whereas it is inhibited by carvedilol.

$\beta$-Adrenoceptor antagonists are now commonly used in the treatment of chronic heart failure patients (for review, see Bristow, 2000). Beneficial effects including reduction of mortality have been demonstrated not only for $\beta_1$-adrenoceptor-selective antagonists such as metoprolol (MERIT-HF Study Group, 1999; Hjalmarson and Fagerberg, 2000) or bisoprolol (CIBIS-II Investigators and Committees, 1999) but also for the nonselective $\beta_1$-adrenoceptor antagonist carvedilol (CAPRICORN Investigators, 2001; Packer et al., 2001; Tendler and Ochala, 2001). On the other hand, a recent large trial with bucindolol, another nonselective $\beta_1$-adrenoceptor antagonist, failed to produce a significant reduction in mortality in patients with chronic heart failure (Bristow, 2000). Bucindolol (Hershberger et al., 1990) and carvedilol (Bristow et al., 1992) are $\beta$-adrenoceptor antagonists with a similar affinity at $\beta_1$- and $\beta_2$-adrenoceptors but differ in their affinities at $\alpha_1$-adrenoceptors. Carvedilol exhibited about 2- to 3-fold selectivity for $\beta_1$- versus $\alpha_1$-adrenoceptors whereas bucindolol was found to have about 60 to 70 times higher affinity at $\beta_1$- versus $\alpha_1$-adrenoceptors (Sponer and Feuerstein, 1999; Bristow, 2000). We have recently shown that, in adult rat ventricular cardiomyocytes, the noradrenaline-induced increase in rate of protein synthesis (a marker of the development of a hypertrophic phenotype) is composed of two components: an $\alpha_1$-adrenoceptor mediated increase in rate of protein synthesis and a $\beta_1$-adrenoceptor mediated reduction in rate of protein synthesis (Schäfer et al., 2001). The aim of this study was to find out whether the differences in affinities to $\alpha_1$-adrenoceptors of carvedilol and bucindolol might lead to differences in their interaction with noradrenaline-induced increase in rate of protein synthesis (assessed by...
Materials and Methods

Preparation of Cardiomyocyte Culture of Adult Rats. Left ventricular cardiomyocytes were isolated from 12-week-old male Wistar rats exactly as recently described (Pönice et al., 2000). Freshly isolated cardiomyocytes were gently diluted in sterile culture medium M199, pH 7.4, supplemented with 10% newborn calf serum. To study [3H]phenylalanine incorporation, the cardiomyocyte suspension was seeded into 12-well plates (16,000 cells per well), which had been coated with 4% fetal calf serum in medium M199 for 24 h at 37 °C (in a humidified incubator at 95% air/5% CO2) and incubated for 16 h at 37 °C. Thereafter, the cultures were rinsed with serum-free Hanks balanced salt solution to remove damaged, rounded, and nonattached cells, and the rod-shaped cells were cultured in serum-free medium M199 supplemented with 2 mM L-carnitine, 5 mM taurine, 5 mM creatine, and antibiotics (100 U ml−1 penicillin and 100 μg ml−1 streptomycin). To prevent growth of nonmyocytes the culture medium was supplemented with 10 μM cytosine-β-D-arabinofuranoside.

[3H]Phenylalanine Incorporation. Protein synthesis by cardiomyocytes was assayed as incorporation of [3H]phenylalanine into cells as recently described (Pönice et al., 2000). Briefly, after addition of [3H]phenylalanine (0.5 μCi ml−1) at 37 °C and the addition of various concentrations of noradrenaline or phenylephrine in the presence or absence of the various antagonists, cells were cultured for 16 h at 37 °C in 95% air/5% CO2. Ascorbate (100 μM) was always present in the medium during this incubation period as an antioxidant. Thereafter cells were washed with ice-cold 0.9% NaCl solution to remove attached radioactivity and incubated for 24 h at 4 °C with 10% trichloroacetic acid. Acid-insoluble precipitates were washed again with 10% trichloroacetic acid and twice with 0.9% NaCl. The remaining precipitate on the culture dishes was solubilized in 1 N NaOH supplemented with 0.1% sodium dodecyl sulfate at room temperature for 24 h, and incorporation of radioactivity into acid-insoluble cell mass was determined by the use of a liquid scintillation counter (Beckman LS 6000; Beckman Coulter, Inc., Fullerton, CA). We have recently shown that under these experimental conditions [3H]phenylalanine incorporation was paralleled by increases in protein mass, cell volume, and cross-sectional area of the cells indicating hypertrophic growth of the cardiomyocytes (Schafer et al., 2001).

Radioligand Binding Studies. Affinities of carvedilol and bucindol to β1- and β2-adrenoceptors were determined by (–)-[125I]iodocyanopindolol binding to membranes from rat heart left ventricle (in the presence of 50 nM ICI 118,551 = homogeneous population of β1-adrenoceptors) and rat lung [in the presence of 300 nM CGP 20712A (1-[2-(3-carbamoyl-4-hydroxyphenoxyl)-ethyl-amino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxyl]-2-propanol methanesulfonate] = homogeneous population of β2-adrenoceptors; Brown et al., 1992]. Crude membrane fractions were prepared by standard homogenization and centrifugation, and (–)-[125I]iodocyanopindolol binding was performed in 10 mM Tris, 154 mM NaCl buffer, pH 7.4, in a total volume of 250 μl for 90 min at 37 °C; nonspecific binding was defined as binding in the presence of 1 μg M CGP 12177 [4-(3’-tert-butylamino-2’-hydroxypropoxy)-benzimidazole-2-hydrochloride] (Michel et al., 1987). Affinities of carvedilol and bucindol for α1-adrenoceptors were assessed by [3H]prazosin binding to rat liver membranes. [3H]Prazosin binding was performed in 50 mM Tris, 1 mM EDTA buffer, pH 7.4, in a total volume of 1 ml for 45 min at 25 °C; nonspecific binding was defined by 10 μM phentolamine (Michel et al., 1993).

Affinities of Carvedilol and Bucindol at α1- and β-Adrenoceptors. Carvedilol (10 pM−10 μM) and bucindol (10 pM−10 μM) inhibited [125I]iodocyanopindolol binding (100 pM) to membranes from rat left ventricle (in the presence of 50 nM ICI 118,551 = homogeneous population of β1-adrenoceptors) and from rat lung (in the presence of 300 nM CGP 20712A = homogeneous population of β2-adrenoceptors) with concentration-inhibition curves that were best described by a one-site fit. From these curves, Kᵢ values of 0.7 to 1.0 nM for carvedilol and 1.2 to 1.8 nM for bucindol were calculated (see Table 1). Studies with human cardiac membranes had suggested that binding of carvedilol and bucindol to β-adrenoceptors can be modulated by guanine nucleotides (Hershberger et al., 1990; Bristow et al., 1992; Maack et al., 2000). In the present study, however, addition of 100 μM GTP to the incubation medium did not affect the concentration-inhibition curves of carvedilol or bucindol, neither at rat β1- nor at rat β2-adrenoceptors (Table 1), in accordance with previous findings (Pönice et al., 2000).

### Table 1

<table>
<thead>
<tr>
<th>β₁-Adrenoceptors</th>
<th>0 + GTP</th>
<th>β₂-Adrenoceptors</th>
<th>0 + GTP</th>
<th>α₁-Adrenoceptors</th>
<th>0 + GTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvedilol</td>
<td>0.81 ± 0.06 (4)</td>
<td>0.67 ± 0.09</td>
<td>0.96 ± 0.21 (6)</td>
<td>0.88 ± 0.11</td>
<td>2.2 ± 0.2 (4)</td>
</tr>
<tr>
<td>Bucindol</td>
<td>1.61 ± 0.21 (7)</td>
<td>1.75 ± 0.17</td>
<td>1.20 ± 0.31 (7)</td>
<td>1.44 ± 0.26</td>
<td>68.9 ± 8.9 (4)</td>
</tr>
<tr>
<td>Propranolol</td>
<td>4.12 ± 0.61 (4)</td>
<td></td>
<td>2.93 ± 0.41 (4)</td>
<td></td>
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</tr>
</tbody>
</table>
with recently published data from Willette et al. (1999). Carvedilol (100 μM–10 μM) and bucindolol (100 pM–10 μM) inhibited [3H]prazosin binding (1 nM) to rat liver membranes with monophasic concentration-inhibition curves; affinity of carvedilol to rat α₁-adrenoceptors was, however, about 30 times higher than that of bucindolol (Table 1).

Effect of Carvedilol and Bucindolol on Noradrenaline-Induced Increase in Rate of Protein Synthesis in Cardiomyocytes of Adult Rats. We have recently shown that noradrenaline (NA, 1 nM–10 μM) in adult rat ventricular cardiomyocytes concentration-dependently increases the rate of protein synthesis (assessed as [3H]phenylalanine incorporation; this NA effect can be enhanced by the β-adrenoceptor antagonists propranolol (nonselective), CGP 20712A and atenolol (β₁-adrenoceptor-selective; Schäfer et al., 2001). In the present study, NA (1 nM–10 μM) concentration-dependently increased [3H]phenylalanine incorporation into cardiomyocytes (Fig. 1), maximal increase amounted to 130 ± 1% of control, pEC_{50} value was 6.0 ± 0.2. The β₁-adrenoceptor antagonist bisoprolol (1 μM) led to a significant increase in rate of protein synthesis at each NA concentration (NA ≥ 100 nM), maximal increase was 151 ± 5% of control. In addition, the concentration-effect curve for NA was significantly shifted to the left; pEC_{50} value in the presence of bisoprolol was 7.2 ± 0.2 (p < 0.05, Fig. 1). Bucindolol (100 pM–10 μM) influenced 1 μM NA-induced increase in rate of protein synthesis with a bell-shaped concentration-inhibition curve (Fig. 2A); at concentrations up to 10 nM, it increased NA-induced protein synthesis whereas at concentrations >10 nM, NA-induced protein synthesis was inhibited. Addition of 300 nM CGP 20712A to the incubation medium significantly (p < 0.05) increased 1 μM NA-induced protein synthesis from 131 ± 4% to 154 ± 5%; in the presence of CGP 20712A, bucindolol (100 μM–10 μM) inhibited NA-induced protein synthesis with a monophasic concentration-inhibition curve and a K_{i} value of 38.4 ± 8.0 nM (n = 9, Fig. 2A). A monophasic concentration-inhibition curve for bucindol (K_{i} value 72.5 ± 16 nM, n = 4) was also observed when

![Fig. 1. Effect of the β₁-selective adrenoceptor antagonist bisoprolol (1 μM) on NA-induced [3H]phenylalanine incorporation in ventricular cardiomyocytes of 12-week-old male Wistar rats. Ordinate scale, [3H]phenylalanine incorporation as percentage of basal incorporation; abscissa scale, molar concentrations of noradrenaline. Basal [3H]phenylalanine incorporation amounted to 1062 ± 83 cpm in control cells (n = 5). *, p < 0.05 versus NA-stimulated control cells. Values are means and vertical lines show S.E.M.](image1)

![Fig. 2. A, effect of bucindolol in the presence or absence of the β₁-selective adrenoceptor antagonist CGP 20712A (300 nM) on 1 μM NA-induced [3H]phenylalanine incorporation in ventricular cardiomyocytes of 12-week-old male Wistar rats (n = 9). Ordinate scale, [3H]phenylalanine incorporation as percentage of basal incorporation; abscissa scale, molar concentrations of bucindolol. Basal [3H]phenylalanine incorporation amounted to 874 ± 73 cpm in control cells (n = 9). #, p < 0.05 versus NA control (without bucindolol); #, p < 0.05 versus without CGP 20712A. B, effect of bucindolol in the presence or absence of the nonselective β-adrenoceptor antagonist propranolol (1 μM) on 1 μM NA-induced [3H]phenylalanine incorporation in ventricular cardiomyocytes (n = 4). Ordinate scale, [3H]phenylalanine incorporation as percentage of basal incorporation; abscissa scale, molar concentrations of bucindolol. Basal [3H]phenylalanine incorporation amounted to 912 ± 118 cpm in control cells (n = 4). #, p < 0.05 versus without propranolol. In panels A and B, values are means and vertical lines show S.E.M; the dotted lines represent a mathematical fit of the data to a theoretical one-site model. Control = effects of the different bucindolol concentrations on basal [3H]phenylalanine incorporation.](image2)
protein synthesis was significantly reduced from 133.9 ± 7.9% to 116.0 ± 6.3% (n = 5, p < 0.05; Fig. 3).

In contrast to bucindolol, carvedilol (100 pM–10 μM) inhibited 1 μM NA-induced increase in rate of protein synthesis with a monophasic concentration-inhibition curve (Fig. 4A) independent of whether or not β₁-adrenoceptors were blocked with CGP 20712A (300 nM); Kᵢ values for carvedilol were 5.0 ± 1.3 nM (n = 7) in the absence and 5.7 ± 1.4 nM (n = 7) in the presence of 300 nM CGP 20712A. Accordingly, carvedilol did not exert a dual effect on the concentration-response curve for NA-induced protein synthesis; the low (10 nM) concentration of carvedilol was without effect whereas the higher (100 nM and 1 μM) concentrations of carvedilol shifted the concentration-response curve for NA to the right to higher concentrations (Fig. 4B).

Effects of Carvedilol and Bucindolol on Phenylephrine-Induced Increase in Rate of Protein Synthesis. In a final set of experiments, we studied the effects of carvedilol and bucindolol on phenylephrine (PE)-induced increase in rate of protein synthesis because PE is an α₁-adrenoceptor agonist that exerts β-adrenoceptor agonist activity only in concentrations >1 μM. PE (1 μM) increased protein synthesis in the adult rat cardiomyocytes to 150 ± 3% (Fig. 5, A and B, n = 20), i.e., a value in a range similar to that induced by 1 μM NA in the presence of CGP 20712A in the same cardiomyocyte preparations (cf. Figs. 2A and 4A); 300 nM CGP 20712A did not affect the increase in protein synthesis induced by 1 μM PE [maximal increase in the presence of CGP 20712A was 148 ± 10% (n = 4, Fig. 5A), respectively, 153 ± 6% (n = 4, Fig. 5B)]. Both bucindolol and carvedilol inhibited 1 μM PE-induced protein synthesis with monophasic concentration-inhibition curves; Kᵢ values were 44.7 ± 6.3 nM (n = 10) for bucindolol and 3.5 ± 0.4 nM (n = 10) for carvedilol (Fig. 5B).

Discussion

Adult rat cardiomyocytes contain α₁- (Brown et al., 1985; Buxton and Brunton, 1986) and β₁- and β₂-adrenoceptors (Im et al., 1984; Xiao and Lakatta, 1993; for a recent review, see Steinberg, 1999). We have recently shown that noradrenaline, an α₁, α₂, and β₁-adrenoceptor agonist, exerts dual growth-promoting effects on the cardiomyocytes. It increases rate of protein synthesis via α₁-adrenoceptor stimulation and decreases rate of protein synthesis via β₁-adrenoceptor stimulation in a cyclic AMP-dependent fashion (Schäfer et al., 2001). Accordingly, β-adrenoceptor antagonists such as propranolol, CGP 20712A, and atenolol increased noradrenaline-induced protein synthesis whereas β-adrenoceptor agonists such as isoprenaline, dobutamine, or xamoterol decreased noradrenaline-induced protein synthesis (Brodde et al., 2001; Schäfer et al., 2001). Moreover, forskolin and
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dibutyryl cyclic AMP inhibited noradrenaline-induced protein synthesis whereas Rp-cAMPS, an inhibitor of protein kinase A, could enhance it (Schäfer et al., 2001; Brodde et al., 2001).

On the other hand, the increases in rate of protein synthesis evoked by phenylephrine, a "pure" \( \alpha_1 \)-adrenoceptor agonist (at least in concentrations up to 1 \( \mu \)M), were not affected by \( \beta \)-adrenoceptor antagonists but were inhibited by the \( \beta \)-adrenoceptor agonists isoprenaline, dobutamine, and xamoterol (Brodde et al., 2001).

Carvedilol and bucindolol are nonselective third-generation \( \beta \)-blockers (Bristow, 2000) with additional \( \alpha_1 \)-adrenoceptor blocking activity. Our radioligand binding data confirm the selective affinity for \( \alpha_1 \)-adrenoceptors than bucindolol. We found a \( \beta_1 \)/\( \alpha_1 \)-adrenoceptor ratio for carvedilol of 1:2.7 and for bucindolol of 1:43 (cf. Table 1).

In the present study, in adult rat ventricular cardiomyocytes, bucindolol—in contrast to carvedilol—exerted a dual effect on noradrenaline-induced increase in rate of protein synthesis. At low, rather selective, \( \beta \)-adrenoceptor blocking concentrations (\( \leq 10 \) nM), bucindolol caused an increase in noradrenaline-induced protein synthesis (similar to what is observed with other \( \beta \)-adrenoceptor antagonists; see above) while at higher (now \( \alpha_1 \)-adrenoceptor blocking) concentrations, it inhibited noradrenaline-induced increase in rate of protein synthesis. The increase in noradrenaline-induced protein synthesis is due to the \( \beta \)-adrenoceptor blocking activity of bucindolol because it completely disappeared when noradrenaline effects were assessed in the presence of 300 nM CGP 20712A or 1 \( \mu \)M propranolol; on the other hand, the inhibitory effects of bucindolol on noradrenaline-induced protein synthesis is due to its \( \alpha_1 \)-adrenoceptor blocking activity since nearly identical concentration-inhibition curves for bucindolol were obtained inhibiting increase in rate of protein synthesis induced by noradrenaline in the presence of 300 nM CGP 20712A (now acting solely at \( \alpha_1 \)-adrenoceptors) or by phenylephrine (a pure \( \alpha_1 \)-adrenoceptor agonist).

In contrast to bucindolol, carvedilol did not exert dual effects on noradrenaline-induced increase in rate of protein synthesis; it inhibited noradrenaline-induced increases in rate of protein synthesis in the presence as well as in the absence of 300 nM CGP 20712A with monophasic concentration-inhibition curves and nearly identical \( K_i \) values (Fig. 4A). In addition, similar \( K_i \) values were also obtained for carvedilol-evoked inhibition of phenylephrine-induced increase in rate of protein synthesis; this supports the view that the inhibitory effect of carvedilol is due to its \( \alpha_1 \)-adrenoceptor blocking activity.

It should be noted, however, that bucindolol (1 \( \mu \)M) and carvedilol (100 nM and 1 \( \mu \)M) caused shifts to the right of the concentration-response curve for noradrenaline-induced increase in rate of protein synthesis that were by far less than could be expected from their affinities for \( \alpha_1 \)-adrenoceptors. Thus, at a concentration of 1 \( \mu \)M, carvedilol should cause about a 200-fold shift to the right, but the shift observed was only about 20-fold; similarly, bucindolol at a concentration of 1 \( \mu \)M should cause about a 25- to 30-fold shift to the right, but the shift observed was only about 10-fold. However, at these rather high concentrations, both carvedilol and bucindolol will inhibit also \( \beta \)-adrenoceptors and, by this, induce a shift to the left of the concentration-response curve for noradrenaline. As a consequence, these \( \beta \)-adrenoceptor blocking activities will partly counteract the \( \alpha_1 \)-adrenoceptor blocking effects of both \( \beta \)-adrenoceptor antagonists; this is very likely the reason why the shifts to the right induced by bucindolol and carvedilol are less than could be expected from their \( \alpha_1 \)-adrenoceptor blocking activities.

In conclusion, carvedilol and bucindolol differentially affect noradrenaline-induced increase in rate of protein synthesis in adult rat ventricular cardiomyocytes. Bucindolol exerts a dual effect with low (pure \( \beta \)-adrenoceptor blocking) concentrations increasing noradrenaline-induced protein synthesis and high (now \( \alpha_1 \)-adrenoceptor blocking) concentrations inhibiting noradrenaline-induced increase in rate of protein synthesis. In contrast, carvedilol does not exert such dual effects. It causes no increase but only inhibition of noradrenaline-induced increase in rate of protein synthesis. However, how and/or whether these differential effects of carvedilol and bucindolol on noradrenaline-induced development of a hypertrophic phenotype might contribute to their different...
effects on mortality in long-term treatment of patients with chronic heart failure (see Introduction) remains to be elucidated.

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References
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