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In Vitro and In Vivo Characterization of Intrinsic Sympathomimetic Activity in Normal and Heart Failure Rats

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ABSTRACT

Clinical studies conducted with carvedilol suggest that β-adrenoceptor antagonism is an effective therapeutic approach to the treatment of heart failure. However, many β-adrenoceptor antagonists are weak partial agonists and possess significant intrinsic sympathomimetic activity (ISA), which may be problematic in the treatment of heart failure. In the present study, the ISAs of bucindolol, xamoterol, bisoprolol, and carvedilol were evaluated and compared in normal rats [Sprague-Dawley (SD)], in rats with confirmed heart failure [spontaneously hypertensive heart failure (SHHF)], and in isolated neonatal rat cardiomyocytes. At equieffective β_1-adrenolytic doses, the administration of xamoterol and bucindolol produced a prolonged, equieffective, and dose-related increase in heart rate in both pithed SD rats (ED_{50} = 5 and 40 μg/kg, respectively) and SHHF rats (ED_{50} = 6 and 30 μg/kg, respectively). The maximum effect of both compounds in SHHF rats was approximately 50% of that observed in SD rats. In contrast, carvedilol and bisoprolol had no significant effect on resting heart rate in the pithed SD or SHHF rat. The maximum increase in heart rate elicited by xamoterol and bucindolol was inhibited by treatment with propranolol, carvedilol, and betaxolol (β_1-adrenoceptor antagonist) but not by ICI 118551 (β_2-adrenoceptor antagonist) in neonatal rat. When the β-adrenoceptor-mediated cAMP response was examined in cardiomyocytes, an identical partial agonist/antagonist response profile was observed for all compounds, demonstrating a strong correlation with the in vivo results. In contrast, GTP-sensitive ligand binding and tissue adenylate cyclase activity were not sensitive methods for detecting β-adrenoceptor partial agonist activity in the heart. In summary, xamoterol and bucindolol, but not carvedilol and bisoprolol, exhibited direct β_1-adrenoceptor-mediated ISA in normal and heart failure rats.

The use of β-adrenoceptor antagonists in the treatment of congestive heart failure is gaining acceptance (Doughty and Sharpe, 1997; Hash and Prisant, 1997). Recently, carvedilol, a potent nonselective β-adrenoceptor antagonist with vasodilator and antioxidant actions, has been approved for the treatment of congestive heart failure in the United States and approximately 20 other countries. In prospective, randomized, double-blind, placebo-controlled clinical studies in patients with congestive heart failure, carvedilol, when added to conventional therapy consisting of diuretics, digoxin, and an angiotensin-converting enzyme inhibitor, reduced mortality rates by 65% and decreased the rate of hospitalization by approximately 30% (Packer et al., 1996). Bucindolol, another nonselective β-adrenoceptor antagonist, and bisoprolol, a selective β_1-adrenoceptor antagonist, are being evaluated for their effects on mortality in a large congestive heart failure trials (The BEST Steering Committee, 1995; The CIBIS II Scientific Committee, 1997).

Many β-adrenoceptor-blocking agents are not pure competitive antagonists but rather have weak to moderate agonist activity resulting in stimulation of cardiac β-adrenoceptors, which increases heart rate; this activity is referred to as intrinsic sympathomimetic activity (ISA) (Panfilov et al., 1995). Despite the initial optimism regarding the clinical efficacy of β-adrenoceptor antagonists with ISA (Northcote, 1987), evidence now indicates that ISA offers no clear advantage over pure competitive antagonists (which lack ISA) in the treatment of hypertension, and the presence of ISA may actually be detrimental in the treatment of congestive heart failure and myocardial infarction. Thus, there were no differences in the effects of xamoterol, a β_1-adrenoceptor partial agonist (or alternatively, a β_1-adrenoceptor antagonist with a significant degree of ISA), and metoprolol, a β_1-adrenoceptor antagonist with no ISA, on exercise tolerance in patients with mild to moderate heart failure (Persson et al., 1995). Furthermore, in a longer clinical trial in congestive heart failure, treatment with xamoterol was associated with a significant increase in mortality rates (The Xamoterol Study Group, 1990). In essential hypertensive patients, β-adrenoceptor blockers with ISA are also associated with significant

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ABBREVIATIONS: ISA, intrinsic sympathomimetic activity; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; SD, Sprague-Dawley; SHHF, spontaneously hypertensive heart failure.

increases in serum and myocardial creatinine phosphokinase, suggestive of myocardial damage (Imai et al., 1995). In addition, a recent meta-analysis indicates that β-adrenoceptor blockers with ISA are less likely to reduce mortality rates after myocardial infarction (for reviews, see Packer, 1988; Soriano et al., 1997). Thus, the evaluation of ISA has again become an important issue, especially when evaluating β-adrenoceptor antagonists for use in the treatment of congestive heart failure.

Because carvedilol has recently been approved for the treatment of congestive heart failure and bucindolol and bisoprolol are currently in clinical trials for heart failure, we initiated this investigation to determine whether these drugs possess ISA. All compounds were evaluated and compared with xamoterol (a known partial β-adrenoceptor agonist) in a standard in vivo model for identifying ISA, the pithed rat. Further evaluations and mechanism of action studies were also performed in aged spontaneously hypertensive heart failure (SHHF) rats and in neonatal rat cardiomyocytes, respectively.

Materials and Methods

Surgical Procedure

Male Sprague-Dawley (SD) rats weighing 270 to 300 g were housed in an accredited laboratory animal facility, and all procedures were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (US Department of Health, Education, and Welfare; Department of Health and Human Services publication no. NIH 85-23). All procedures were approved by the Animal Care and Use Committee at SmithKline Beecham Pharmaceuticals.

The surgical preparation of the pithed rat was similar to that described previously (Willette et al., 1990). All animals were anesthetized with isoflurane (4% in O_2). A tracheal cannula was inserted, and the vagus nerve was transected bilaterally. A stainless steel pithing rod was inserted into the spinal canal via the orbit, and the animal was ventilated mechanically with room air. Cannulas were placed in the left femoral artery and vein for the measurement of arterial blood pressure and the i.v. administration of drugs, respectively. Heart rate was derived electronically from the blood pressure pulse and was expressed as beats/min.

In Vivo Determination of Myocardial β-Adrenoceptor Agonist Activity

The heart rate responses to cumulative doses of isoproterenol (1–3000 ng/kg i.v.), bucindolol (10–1000 μg/kg i.v.), and carvedilol (10–1000 μg/kg i.v.) were determined in separate groups of animals (n = 4–6/group). The cumulative dose-response relationships for increases in heart were also obtained 10 min after treatment with propranolol (1 mg/kg i.v.), carvedilol (1 mg/kg i.v.), betaxolol (0.1 mg/kg i.v.), and ICI 118551 (0.1 mg/kg i.v.).

Primary Neonatal Rat Cardiomyocyte Cultures and Determination of cAMP

Primary cultures of neonatal rat cardiomyocytes were prepared according to the method of Iwaki et al. (1990) with only minor modifications. Briefly, the hearts were isolated from 1- to 2-day-old SD rats. The myocardial cells were dispersed by digestion with collagenase (type II, 80 U/ml)/pancreatin (0.6 mg/ml). Cardiomyocytes were purified on a discontinuous Percoll gradient (1.052:1.062:1.086). The buffer used for digestion and washing was 1× Ads (116 mM NaCl, 20 mM HEPES, 1 mM Na_2HPO_4, 5.5 mM dextrose, 5.4 mM KCl, 0.8 mM $MgSO_4$, pH 7.15). The cardiomyocyte viability, as determined by trypan blue exclusion, was ≥95%. The cardiomyocytes were then placed into plating media containing Dulbecco's modified Eagle's medium (68%), medium 199 (17%), FBS (5%), horse serum (10%), penicillin (200 U/ml), and streptomycin (0.2 mg/ml). The cellular suspension (1.5×10^6/well) was placed onto laminin-coated plates (6-well tissue culture plates) and cultured for 24 h at 37°C in a CO_2 incubator. The medium was then replaced with a maintenance medium containing Dulbecco's modified Eagle's medium (80%), medium 199 (20%), penicillin (200 U/ml), and streptomycin (0.2 mg/ml) and incubated for an additional 72 h before use. The purity of the resultant cardiomyocyte culture, as assessed by immunohistochemical analysis of sarcomeric α-actin, was 94.5 ± 0.15% (n = 39).

On day 4, the maintenance medium was aspirated and the cells were washed twice with Dulbecco's PBS. 3-Isobutyl-1-methylxanthine (0.5 mM) was then added to the buffer, and the cells were incubated for 10 min at room temperature. Various β-adrenoceptor agonists and/or antagonists were added and incubated at 37°C for 15 min. The reaction was stopped by the addition of 100 μl of 100% ice-cold trichloroacetic acid to each well. After centrifugation (5000g for 15 min), the supernatant was then extracted three times with water-saturated ether. cAMP levels in each well were determined by radioimmunoassay according to the manufacturer's protocol (Perseptive Diagnostics, Cambridge, MA). Assays were performed in triplicate, and each experiment was performed at least three times. Values were derived from a standard curve, and all results were expressed as the percent change in control (untreated cardiomyocytes).

Radioligand Binding

Membrane Preparation. The right and left ventricles were removed and placed into ice-cold 10 mM Tris/1 mM EGTA buffer, pH 8.0. Fibrous tissue was dissected free, and the sample was weighed, placed into 20 volumes of fresh buffer, and minced before homogenization with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY), at setting 6, with three 10-s bursts. Contractile protein was extracted by the addition of 2.5 M KCl (1 ml/40 ml of homogenate), followed by stirring at 4°C for 15 min (Bristow et al., 1986) The suspension was centrifuged at 48,000g for 15 min. The pellet was resuspended in fresh buffer with several quick Polytron bursts and resuspended. After the third centrifugation and resuspension, protein concentrations were determined using a modification of the Bradford method (Bio-Rad, Hercules, CA), with BSA as the standard. Aliquots were frozen in liquid nitrogen and stored at (−70°C) until needed.

Ligand-Binding Assays. Membranes, [^{125}I]cyanopindolol (2000 Ci/mmol; Amersham), and competing drugs were incubated in 60 mM Tris (pH 7.4 at 25°C) and 10 mM $MgCl_2$ for 60 min, in a total volume of 300 μl. The concentration of radioligand used for competition assays was 50 pM. Competition assays, with or without guanosine-5′-*O*-(3-thio)triphosphate (GTPγS), were run in parallel. The radioligand concentration in the saturation assays was varied between 2 and 250 pM. Protein concentrations were adjusted so that specific binding was >10% of the total radioactive counts added. Nonspecific binding was defined by the presence and absence of 100 μM propranolol. Incubations were terminated by vacuum filtration using a Brandel Cell Harvester. Glass-fiber filters (Whatman GF/B) were rinsed three times with assay buffer (5 ml) washes. Samples were dried, and retained radioactivity was measured in a γ-counter. Binding analyses were performed using GraphPAD Software (San Diego, Ca).

Statistical Analysis

All summary values are expressed as the mean ± S.E.M. Comparisons were made using an ANOVA for unpaired data followed by post hoc analysis with Bonferroni's test. A probability level ($p \leq .05$) was considered to be statistically significant. All statistical analyses were done using InStat (GraphPAD Software).

Drugs and Solutions

All drugs were prepared just before use. Carvedilol and bucindolol were synthesized at SmithKline Beecham Pharmaceuticals (King of Prussia, PA). Carvedilol was prepared in a 2% acidified ethanol/8% 2-hydroxypropyl-β-cyclodextrin vehicle. Bucindolol was prepared in sterile water. All other compounds were prepared in a saline vehicle and were obtained from common commercial sources. None of the vehicles used had any significant effects on heart rate.

Results

The basal diastolic blood pressure and heart rate values in the pithed SD rats (n = 68) were 53.4 ± 1.5 mm Hg and 274.4 ± 3.4 beats/min, respectively. In the pithed SHHF rats (n = 14), the basal diastolic blood pressure and heart rate values were 52.1 ± 5.3 mm Hg and 271 ± 16 beats/min, respectively. The basal heart rate and blood pressure did not differ significantly in any of the experimental groups.

Determination of ISA In Vivo. Cumulative dose-response relationships for increases in heart rate were evaluated for bucindolol, xamoterol, bisoprolol, and carvedilol in pithed normotensive SD rats. The administration of bucindolol (1–1000 μg/kg i.v.) and xamoterol (0.3–300 μg/kg i.v.) produced dose-related increases in heart rate (Fig. 1A). Higher doses did not produce further increases in heart rate. The maximum increases in heart rate produced by bucindolol and xamoterol were observed within 3 to 5 min after administration and were prolonged (>20 min). The positive chronotropic potency of bucindolol (ED_{50} = 40 μg/kg i.v.) and xamoterol (ED_{50} = 10 μg/kg i.v.) was approximately 700- and 170-fold less than that of isoproterenolol (ED_{50} = 59 ng/kg i.v.), respectively. The maximal increases in heart rate produced by bucindolol (90 ± 6 beats/min) and xamoterol (84 ± 8 beats/min) were approximately 45% and 42%, respectively, of the isoproterenol maximum (Fig. 1B). In contrast, the cumulative administration of carvedilol and bisoprolol (1–1000 μg/kg i.v.) had no significant effect on resting heart rate in the pithed rat (Fig. 1A).

The mechanism responsible for the positive chronotropic effect of bucindolol (1 mg/kg i.v.) and xamoterol (300 μg/kg i.v.) were explored in animals pretreated with propranolol (1 mg/kg i.v.), carvedilol (1 mg/kg i.v.), betaxolol (0.1 mg/kg i.v.), or ICI 118551 (0.1 mg/kg i.v.). The maximum increase in heart rate elicited by bucindolol (99 ± 9 beats/min) and xamoterol (89 ± 8 beats/min) was similarly attenuated by propranolol (26 ± 2 and 22 ± 4 beats/min, respectively), carvedilol (27 ± 2 and 25 ± 3 beats/min, respectively), and betaxolol (20 ± 12 and 28 ± 8 beats/min, respectively). The selective β_2- adrenoceptor antagonist ICI 118551 had no significant effect on the chronotropic effects of xamoterol and bucindolol.

Cumulative dose-response relationships for increases in heart rate were also evaluated for bucindolol, xamoterol, bisoprolol, and carvedilol in pithed SHHF rats. When compared with SD rats, the SHHF rats had hemodynamic and morphological changes consistent with heart failure (i.e., left ventricular end-diastolic pressure = 22.9 ± 4.4 mm Hg in SHHF versus 6.3 ± 1.5 mm Hg in SD; and heart weight index = 0.55 ± 0.06 in SHHF versus 0.27 ± 0.01 in SD). As in the SD rats, only bucindolol and xamoterol produced a dose-related increase in heart rate (Fig. 3). The potency of bucindolol (ED_{50} = 30 μg/kg i.v.) and xamoterol (ED_{50} = 6 μg/kg i.v.) was similar to that observed in SD rats, however, the efficacy in SHHF rats was significantly reduced (approximately 50%).

cAMP Stimulation in Neonatal Rat Cardiomyocytes. The effects of bucindolol, xamoterol, bisoprolol, and carvedilol on cAMP generation were evaluated in neonatal rat cardiomyocyte cell culture (Fig. 4). Bucindolol and xamoterol, but not bisoprolol or carvedilol, produced a concentration-related increase in cAMP generation in the cardiomyocyte

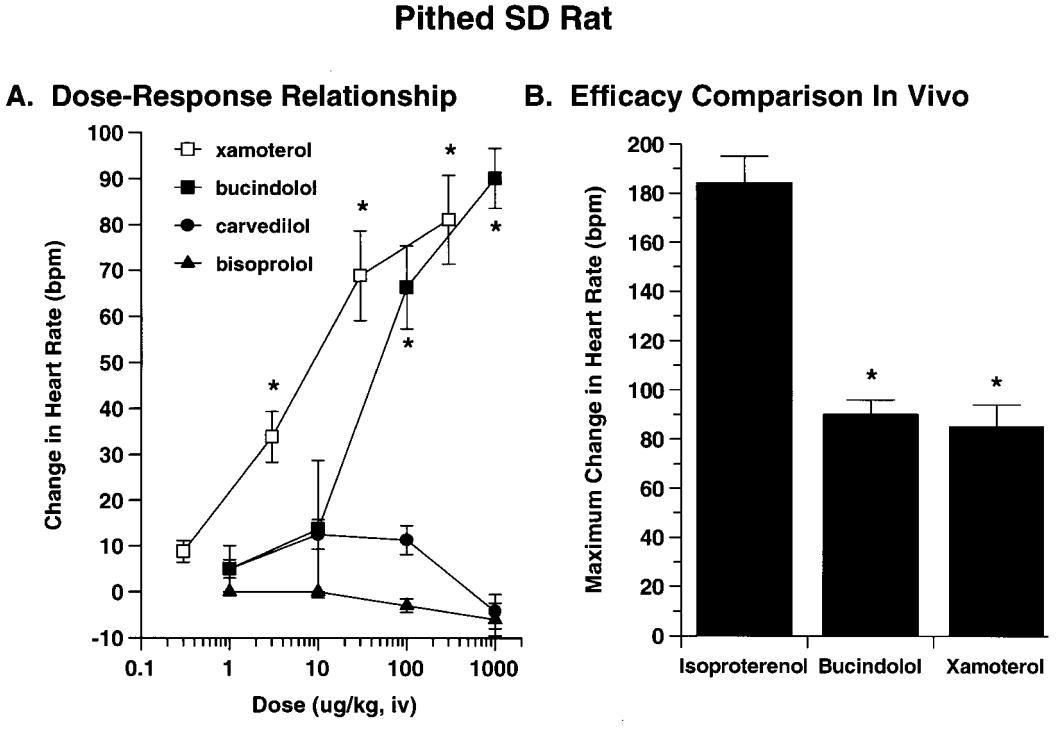

Fig. 1. Cumulative dose-response curves for increases in heart rate induced by xamoterol, bucindolol, bisoprolol, and carvedilol in the pithed SD rat (A). The maximum increase in heart rate elicited by xamoterol and bucindolol was compared with the full β-adrenoceptor agonist isoproterenol (B). *$p <$ .01 indicates a statistically significant difference compared with basal values (A) or isoproterenol (B) (n = 4–6/group).

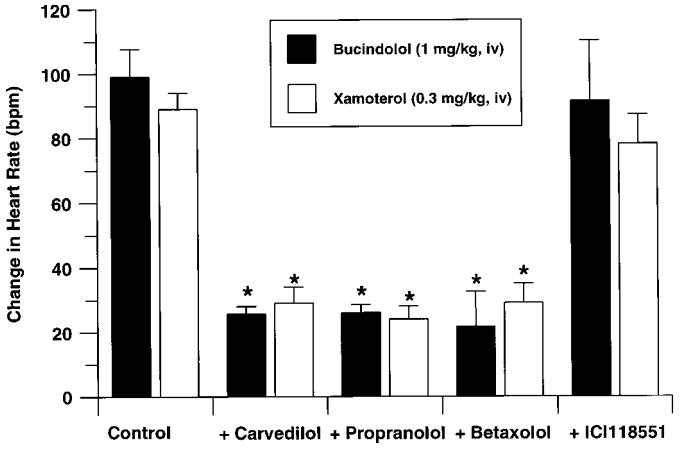

Fig. 2. Treatment with carvedilol (1 mg/kg i.v.), propranolol (1 mg/kg i.v.), or betaxolol (0.1 mg/kg i.v.), the selective β_1-adrenoceptor antagonist, inhibited the positive chronotropic effects of bucindolol (1 mg/kg i.v.) and xamoterol (0.3 mg/kg i.v.). Treatment with the selective β_2-adrenoceptor antagonist ICI 118551 (0.1 mg/kg i.v.) had no significant effect. $^*p < .01$ indicates a statistically significant difference compared with the control group (n = 3–6/group).

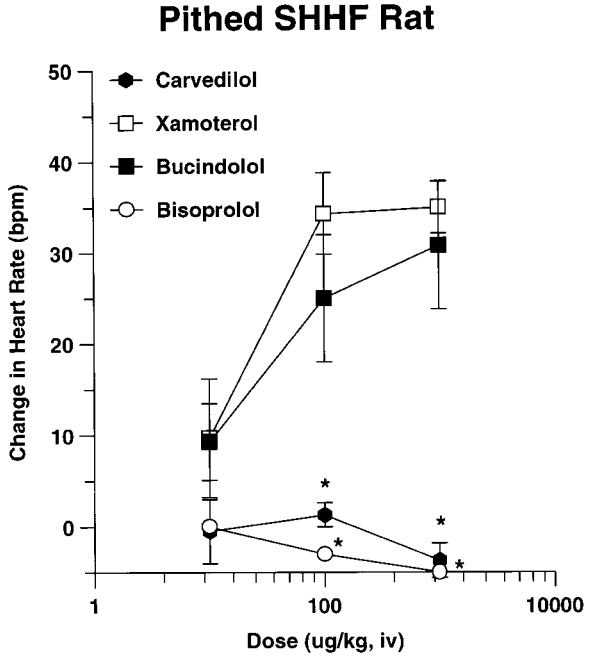

Fig. 3. Cumulative dose-response curves for increases in heart rate induced by xamoterol, bucindolol, bisoprolol, and carvedilol in the pithed SHHF rat. $^*p < .01$ indicates a statistically significant difference compared with basal values (n = 3–4/group).

cell culture. The approximate EC_{50} values of bucindolol and xamoterol were 250 and 80 nM, respectively (Fig. 6A). The maximum cAMP responses obtained with bucindolol and xamoterol were approximately 14% and 26%, respectively, of the maximum isoproterenolol response (Fig. 6B). As illustrated in Fig. 5, the increases in cardiomyocyte cAMP induced by bucindolol and xamoterol were inhibited by preincubation with propranolol (1 μM), carvedilol (1 μM), and betaxolol (0.1 mM) but not by ICI 118551 (0.1 μM).

[^{125}I]Cyanopindolol Radioligand Binding. Radioligand binding competition curves between [^{125}I]cyanopindolol and carvedilol (Fig. 6A), bucindolol (Fig. 6B), and isoproterenol (Fig. 6C) were evaluated in left ventricular myocardial membranes prepared from SHHF rats (Fig. 6) and SD rats (data not shown). The radioligand-binding experiments were performed in the presence and absence of GTPγS to define agonist modulatable binding. The competition curves obtained for carvedilol (Fig. 6A) in the presence and absence of GTPγS were described by a one-site fit with K_i values of 0.39 and 0.40 nM, respectively. Similar results were obtained with bucindolol (Fig. 6B); K_i values are 0.69 nM without GTPγS and 0.64 nM in the presence of GTPγS (Fig. 6B). In contrast, the competition curves obtained with the full β-adrenoceptor agonist isoproterenol were best described with a high- and low-affinity two-site fit; K_i values equal 0.78 and 120 nM, respectively (Fig. 6C). In the presence of GTPγS, isoproterenol competition curves were shifted to the right and best described by a one-site fit with K_i value equal to 280 nM (Fig. 6C). Similar results were obtained when myocardial membranes were prepared from SD rats.

Discussion

This study provides side-by-side comparisons of the ISAs of bucindolol, xamoterol, bisoprolol, and carvedilol in normal and heart failure pithed rats. By examining the heart rate response in these preparations, it was possible to demonstrate that bucindolol and xamoterol stimulate β_1-adrenoceptors in the heart and thereby increase heart rate. Thus, bucindolol, like xamoterol, is a β-adrenoceptor antagonist with ISA or, alternately, a β_1-adrenoceptor partial agonist, similar in profile to xamoterol. Bucindolol and xamoterol also produced ISA in SHHF rats, however, the maximum efficacy for both compounds was 50% of that observed in the normal SD rats. This observation is consistent with the lack of spare β_1-adrenoceptors in the heart and their down-regulation in this heart failure model (Bristow et al., 1982). In contrast, carvedilol and bisoprolol were devoid of ISA in these preparations. Furthermore, carvedilol, as well as propranolol and betaxolol (a selective β_1-adrenoceptor antagonist), blocked the positive chronotropic effects of bucindolol and xamoterol. The β_2-adrenoceptor antagonist ICI 118551 did not alter bucindolol and xamoterol ISAs. Thus, the ISA of bucindolol differentiates this compound from a pure competitive β-adrenoceptor antagonist and places it in a class of partial agonists such xamoterol, pindolol, and celiprolol (Hicks et al., 1987; Louis et al., 1990).

In vitro cAMP studies performed in primary cultures of rat neonatal cardiomyocytes correlated very well with the in vivo results. Both xamoterol and bucindolol acted as partial agonists to increase cAMP via β_1-adrenoceptor activation, and carvedilol and bisoprolol had no effect. In fact, identical agonist and antagonist profiles were observed in vitro in the cardiomyocyte preparation and in vivo in the pithed rat. In contrast, rat atrial membrane adenylate cyclase activity did not correlate with cAMP and in vivo results. In this preparation, the partial agonist activity of xamoterol and bucindolol could not be demonstrated consistently (data not shown). We were also unable to demonstrate GTP-dependent β-adrenoceptor binding with bucindolol and xamoterol in cardiac membranes from normal and heart failure rats. In addition, functional in vitro preparations (i.e., paced and spontaneously beating rat atria) were not sensitive methods for detecting β-adrenoceptor partial agonists (our unpublished observation).

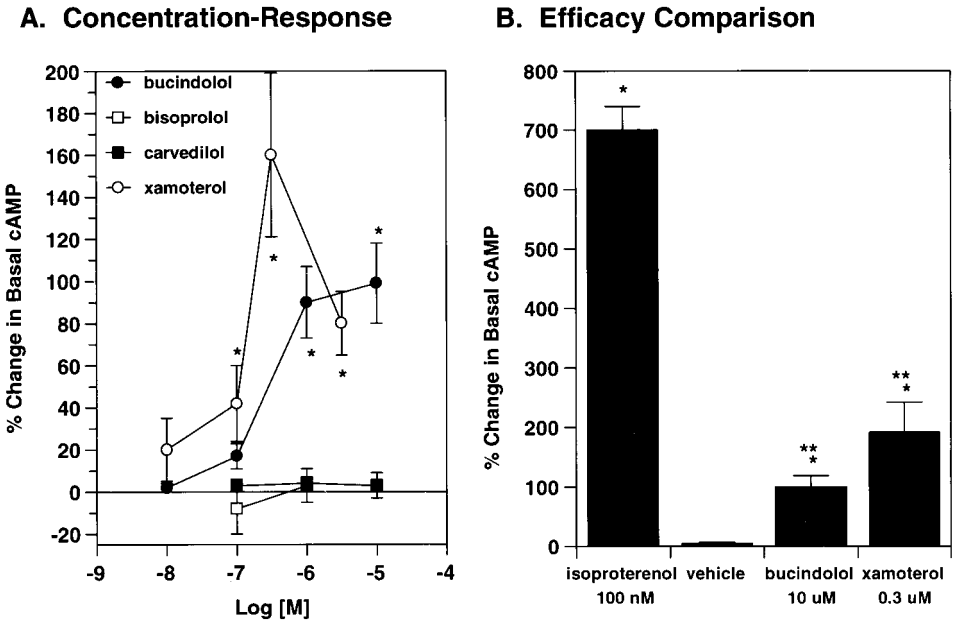

Fig. 4. Concentration-dependent effects of bucindolol, xamoterol, bisoprolol, and carvedilol on cAMP generation were determined in neonatal rat cardiomyocytes (A). The maximum increase in cAMP produced by bucindolol and xamoterol were compared with that observed with isoproterenol (B). *$p < .01$ indicates a statistically significant difference compared with basal (A) and vehicle (B). **$p < .001$ compared with isoproterenol (n = 3–4/group).

The observations discussed above indicate that it is important to conduct side-by-side ISA comparisons for the following reasons. First, the ISAs of various β-adrenoceptor blockers are often defined and compared based on results obtained from disparate experimental conditions with greater or lesser sensitivities for the detection of ISA. In this regard, ISA can be difficult to demonstrate in some in vitro assays (as mentioned above), and results are often equivocal (Hicks et al., 1987). For example, celiprolol and bucindolol have little or no effects in isolated cardiac tissue (adenylate cyclase activity) but consistently produce propranolol-sensitive positive chronotropy in vivo (Deitchman et al., 1980; Hicks et al., 1987; Hershberger et al., 1990). The same can be said for xamoterol, which alone has no effect on contractility in isolated human myocardium in vitro yet is known to possess ISA in vivo (Bohm et al., 1990). Thus, it is difficult to make meaningful comparisons across a variety of experimental conditions.

Second, recent evidence suggests that both carvedilol and bucindolol may possess "agonist-like" characteristics in vitro. For example, carvedilol and bucindolol, but not metoprolol, exhibit guanine nucleotide-modulatable β-adrenoceptor binding in myocardial membranes prepared from human ventricles (Hershberger et al., 1990; Bristow et al., 1992a, b). The reductions in carvedilol and bucindolol binding affinities produced by high concentrations of stable guanine nucleotide analogs are similar to those observed with β-adrenoceptor agonists (Dickinson and Nahorski, 1983). These results are consistent with the ISA of bucindolol, but they are not consistent with repeated demonstrations (as in the present study) that carvedilol is devoid of ISA (Strein et al., 1987; Nichols et al., 1989; Bristow et al., 1992b). Thus, the functional significance of these observations is questionable. In the present study, GTP-sensitive β-adrenoceptor binding was not correlated with ISA and therefore is not a sensitive method for characterizing β-adrenoceptor partial agonists. A similar lack of GTP-modulatable binding has been observed for celiprolol (known to possess ISA) in failing human myocardium (Bohm et al., 1992).

Finally, bucindolol and xamoterol ISAs and the absence of ISA observed with carvedilol and bisoprolol may have clinical relevance. A meta-analysis indicates that β-adrenoceptor blockers with ISA are less likely to reduce mortality rates after myocardial infarction than are β-adrenoceptor blockers lacking ISA (Soriano et al., 1997). In addition, it has been suggested that the ISA of xamoterol contributed significantly to the enhanced mortality observed with the use of this β-adrenoceptor partial agonist in severe heart failure (The Xamoterol Study Group, 1990). Hence, the long-term administration of carvedilol or bisoprolol should not produce adverse effects associated with ISA.

In conclusion, the results of the present in vivo and in vitro studies demonstrate clearly that bucindolol and xam-

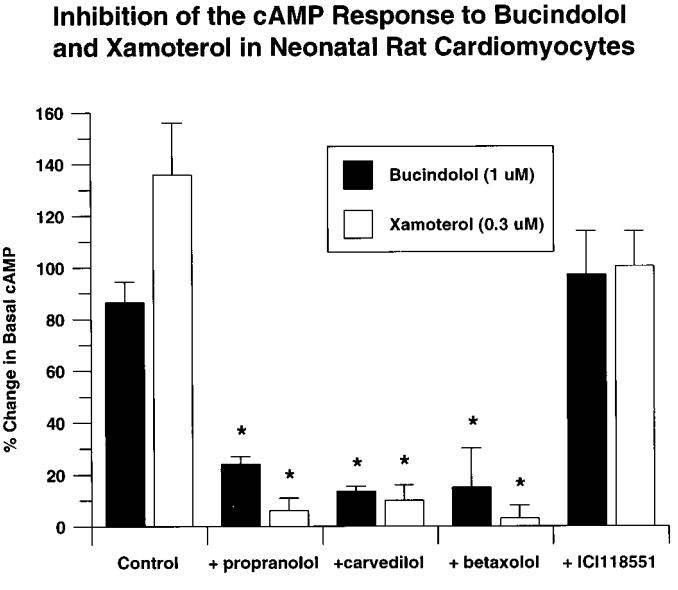

Fig. 5. The accumulation of cAMP in neonatal rat cardiomyocytes induced by bucindolol and xamoterol was evaluated after preincubation with propranolol (1 μM), carvedilol (1 μM), betaxolol (0.1 μM), or ICI 118551 (0.1 μM). *$p < .01$ indicates a statistically significant difference compared with the respective bucindolol or xamoterol control (n = 3–4).

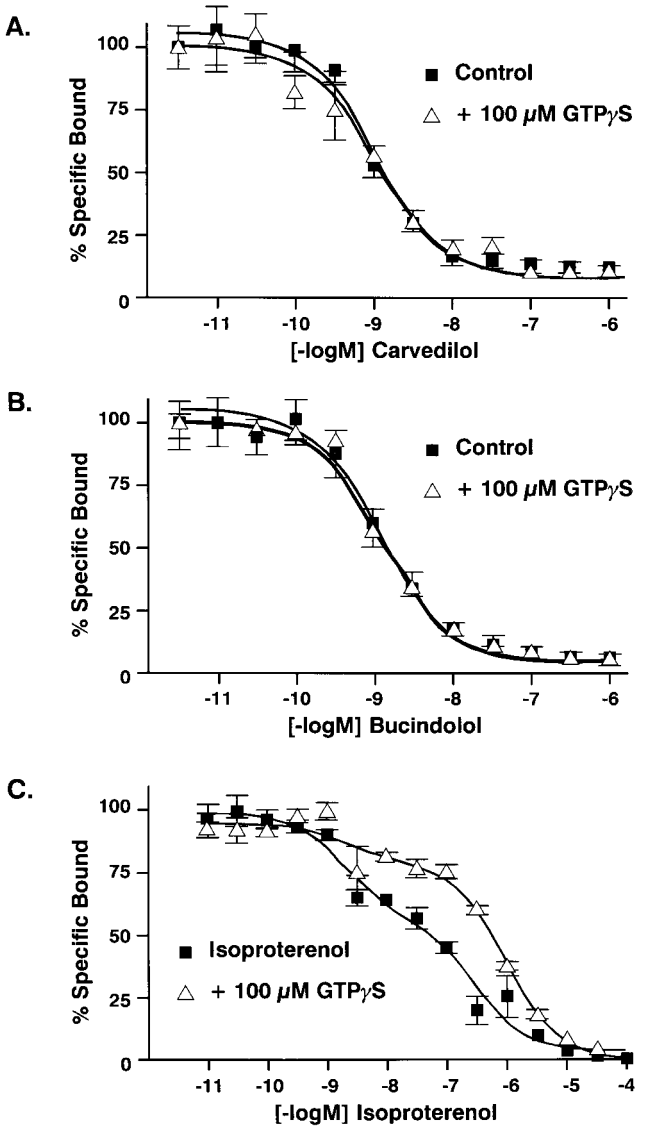

Fig. 6. Radioligand-binding competition curves between [^{125}I]cyanopindolol and carvedilol (A), bucindolol (B), and isoproterenol (C) in left ventricular myocardial membranes from SHHF rats were constructed in the presence and absence of GTPγS (100 μM) (n = 3 separate membrane preparations).

oterol have the capacity to directly activate myocardial β_1-adrenoceptors. This activity was maintained in the heart failure group despite a decrease in β_1-arenoceptor density. Accordingly, bucindolol, like xamoterol, must be classified as either a β-adrenoceptor antagonist with ISA or a β-adrenoceptor partial agonist. In contrast, carvedilol and bisoprolol have no agonist activity at myocardial β_1-adrenoceptors and in fact can inhibit the ISA of bucindolol and xamoterol. The lack of ISA with carvedilol may have important clinical relevance and may be responsible, at least in part, for the large reduction in mortality rates obtained with this drug in congestive heart failure clinical trials.

Acknowledgments

We dedicate this report to the fond memory of our colleague and friend, Dr. Jeffery M. Stadel.

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