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Beta-Blocker Selectivity at Cloned Human Beta$_1$- and Beta$_2$-Adrenergic Receptors

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Summary. **The ratio between the affinities of beta-blockers for the beta$_2$- and beta$_1$-receptors is often used to predict the cardioselectivity and the potential consequences of blocking beta$_2$-receptor–mediated effects of adrenergic receptor blockers. These ratios have been traditionally determined using various in vitro models of beta$_2$ and beta$_1$-receptor antagonist activity, including isolated organ preparations and radioligand binding in tissues from various species. The data from these studies, while useful, are complicated by the use of different preparations, techniques, and nonhuman models. Recombinant cell lines expressing human beta$_2$ and beta$_1$ receptors have been developed, allowing for the direct comparison of the affinities of the beta-blockers for the beta$_2$ and beta$_1$ receptors under identical conditions, and allowing a precise determination of the beta$_1$-receptor selectivity of the beta-blockers. Bisoprolol, atenolol, propranolol, betaxolol, metoprolol, carvedilol, and ICI 118, 551 were compared for their beta-receptor selectivity using membranes prepared from recombinant cells selectively expressing human beta$_2$ and beta$_1$ receptors. Bisoprolol was found to have the highest selectivity for the beta$_1$ receptor, displaying a beta$_2$/beta$_1$ ratio of 19 (a 19-fold higher affinity for the beta$_1$ receptor than for the beta$_2$ receptor). Atenolol, metoprolol, and betaxolol displayed lower, selectivity for the beta$_1$ receptor, whereas propranolol and carvedilol displayed no signiicant beta-adrenergic selectivity. ICI 118,55 was selective for the beta$_2$ receptor. The equilibrium dissociation constants of the beta-blockers for the beta$_1$ and beta$_2$ receptors were generally similar to previously reported values. The affinity ratios were also generally similar to previously reported values.**

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Key Words. beta-blocked, selectivity, cloned beta receptors, cardioselectivity.

Beta-blockers are widely prescribed for the treatment of a wide-rangeing cardiovascular problems [1]. The therapeutic activity of the beta-blackers is attributed to the blockade of beta$_1$-adrenergic receptors predominantly expressed by cardiac tissue [1–7]. The beta$_2$-adrenergic receptor is expressed by the bronchiolor smooth muscle of the lung, among other tissues, and resembles the beta$_1$ receptor in its molecular and pharmacological properties [8–11]. Whereas data have been generated and reported that beta$_1$ and beta$_2$ receptors are distinct from the standpoint of coupling to adenyl cyclase, calcium channels, and G_1 G_I, there is a pharmacological overlap in the beta-blocker interactions with these receptors. The pharmacological overlap of the beta$_2$ receptors presented an important complication in the use of beta$_1$-adrenergic receptor blockers as therapeutic tools, because the beta$_2$-receptor blockade activity generally associated with beta$_1$ blockers may precipitate increases in airway resistance in patients with impaired pulmonary function [1,3,6,12]. Therefore, there has been an ongoing search for a truly selective beta$_1$ blocker devoid of beta$_2$-receptor activity when administered to patients.

Propranolol was the first beta-blocker but is nonselective for the beta$_1$- and beta$_2$-adrenergic receptors [13]. A series of drugs, including bisoprolol and betaxolol [5,14,15], were developed that possessed greater selectivity for the beta$_1$-adrenergic receptor, and these are being used for treating cardiovascular dysfunction. Preclinical determinations of the selectivity of these drugs for beta$_1$- and beta$_2$-adrenergic receptors have been determined in various ways. Classically, isolated cardiac tissue preparations have been used to test for the potencies of the drugs at the beta$_1$-adrenergic receptor activity [4,14–16]. Isolated lung and tracheal preparations have been used to test for the potencies of the drugs at the beta$_2$-adrenergic receptors, because these tissues mainly express and respond to beta$_2$-adrenerg ic receptor stimulation [4,14–16]. The development of radioligand binding assays for the beta$_1$- and beta$_2$-adrenergic receptors allowed for the direct determination of the equilibrium dissociation constants (K_1 values), using various tissue preparations as the source of the receptors [17]. However, these membrane preparations contain mixtures of beta-adrenergic receptors, confounding the interpretation of the K_1 values determined in these experiments.

The cloning of the cDNA and the production of recombinant cells selectively expressing hamster lung beta$_2$-adrenergic receptors was reported [18–20]. These technological advances have allowed the rapid, precise determination of drugs for the human forms of the beta$_1$- and beta$_2$-adrenergic receptors in vitro. To our knowledge, there has been no systematic analysis

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of the selectivity of the commonly prescribed beta-blockers for the human $beta_1$ and $beta_2$-adrenergic receptors, utilizing the recombinant cells expressing the human beta-adrenergic receptors. Therefore, it was decided to determine the human $beta_2/beta_1$-adrenergic receptor selectivity ratio of commonly prescribed beta-blockers, using membranes from recombinant cells selectively expressing human $beta_2$- or $beta_1$-adrenergic receptors.

Methods

Radioligand binding assays were performed as reported previously with minor modifications [21]. Each drug was tested three times at each receptor. Membranes prepared from S49 cells transfected with and expressing the gene coding for human $beta_1$- or $beta_2$-adrenergic receptors were purchased from Receptor Biology, Inc. (Montreal, Canada) or RBI (Natick, MA). These cells express no endogneous beta-adrenergic receptors; thus 100% of the expressed beta-adrenergic receptors result from transfection. The pellet was suspended in buffer, and then centrifuged at 30,000 xg for 30 minutes. The final pellet was resuspended in 50 mM Tris-HCl, 0.5 mM EDTA, 10 mM $MgCl_2$, and 0.1% ascorbate. Radioligand binding assays were performed in triplicate in a volume of 1.0 ml (each tube containing 10 ug of protein). Then 0.5 nM 3_{10}H-CGP 12177 (NEN) was used to label the $beta_1$- and $beta_2$-adrenergic receptors expressed on membranes from cells expressing the human $beta_1$- and $beta_2$-adrenergic receptors (RBI). Propranolol 1 μM was used to determine nonspecific binding, which represented less than 5% of the total binding. Samples were incubated for 30 minutes at 37°C and filtered on a Brandel cell harvester. 5mL of Ecoscint cocktail (National Diagnostics) was added to each sample and radioactivity was determined in a Beckman scintillation counter at 40% efficiency. Data were, analyzed using GraphPad Prism with data reported as ±SEM.

Results

The affinities of seven beta-blockers were determined using radioligand binding assays for the human $beta_1$- and $beta_2$-adrenergic receptors (Table 1). Bisoprolol, metoprolol, betaxolol, and atenolol displayed selectivity for the $beta_1$-adrenergic receptor versus the $beta_2$-adrenergic receptor. The $beta_1$ selectivity was quantified by calculating the ratio of the equilibrium dissociation constant (K_I) for the $beta_2$ receptor by the K_I for the $beta_1$ receptor (Figure 1). Thus the higher the ratio, the more selective the drug is for the $beta_1$ receptor. Bisoprolol appears to be the most $beta_1$ selective, displaying a 19-fold selectivity ratio. Metoprolol, betaxolol, and atenolol also displayed $beta_1$ selectivity, but were less $beta_1$ selective than bisoprolol. Propranolol and carvedilol did not display $beta_1$ selectivity (see Figure 1), and ICI 118,551 displayed pronounced $beta_2$ selectivity.

Table 1. K_I values (nM) of beta-blockers for the radiolabeled human $beta_1$- and $beta_2$-adrenergic receptors expressed in recombinant cells

Beta-blocker	β_1	β_2	Selectivity (β_2/β_1)
Bisoprolol	25 ± 2	480 ± 100	19.6
Betaxolol	32 ± 2	236 ± 56	7.5
Metoprolol	204 ± 24	1227 ± 270	6.0
Atenolol	1520 ± 110	8600 ± 1360	5.7
Carvedilol	0.32 ± .06	0.18 ± .04	.6
Propranolol	3.6 ± 0.3	1.1 ± 0.2	.3
ICI 118,551	148 ±1–9	148 ± 1–2	.01

^{3}H-CGP12177 0.5 nM was used to radiolabel the receptors, and 1 μM propranolol was used to determine nonspecific binding. Results are the means SEM of three independent experiments.

Discussion

The treatment of cardiovascular dysfunction has been revolutionized by the development of beta-blockers [1,2,4,5]. Conditions such as angina pectoris and hypertension are commonly alleviated with drugs that block the $beta_1$-adrenergic receptors expressed in cardiac tissues. However, because of the pharmacological similarity between the $beta_1$- and $beta_2$-adrenergic receptors, a potential side effect of beta-blockers is the precipitation of increased airway resistance [12]. This is caused by the blockade of the sympathetic stimulation of the bronchiolar smooth muscle, which maintains the diameter of the air passages, resulting in the loss of smooth muscle relaxation and narrowing of the air passages. The original beta-blockers were nonselective for the $beta_1$- and $beta_2$-adrenergic receptors, and their therapeutic util-

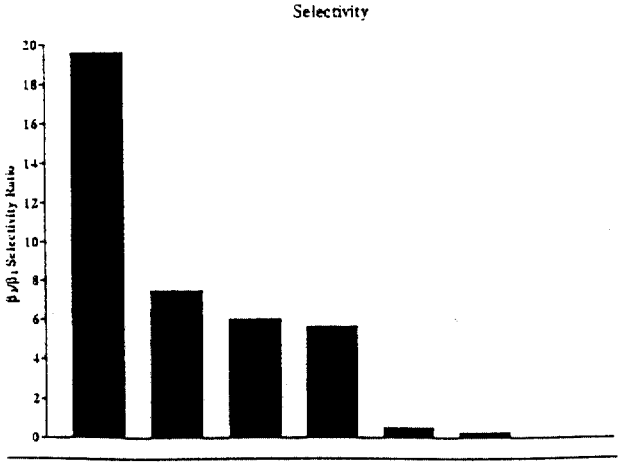

Fig. 1. Selected competition curves of beta-blockers for radiolabeled human recombinant β_1- and β_2- adrenergic receptors. Results are the means ± SEM of three independent experiments performed in triplicate.

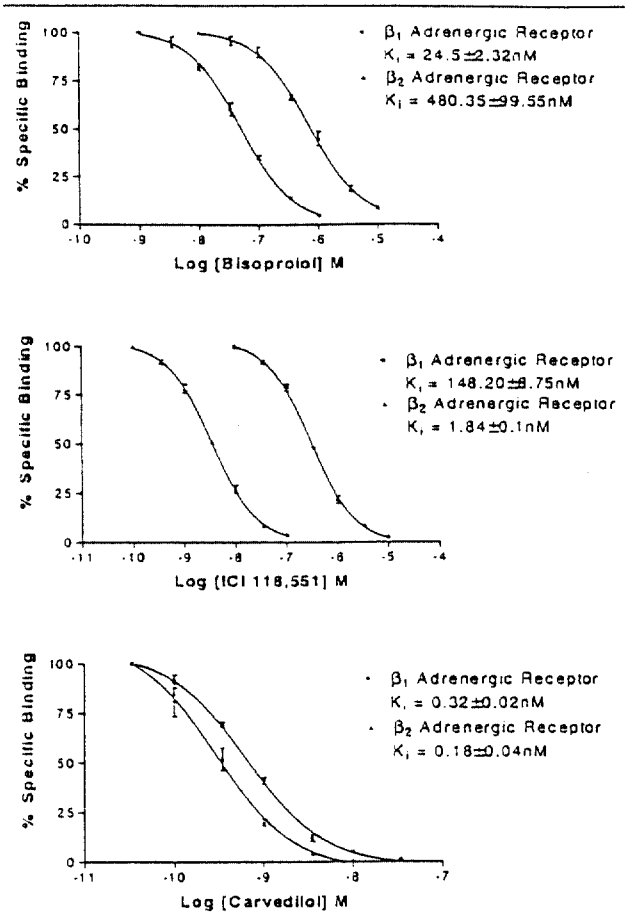

Fig. 2. Beta$_1$ selectivity determined by dividing K_i (beta$_2$) by K_i (beta$_1$). Values >1 indicate beta$_1$ selectivity; bars <1 indicate beta$_2$ selectivity. For the ICI 118,551 selectivity ratio, see Table 1.

ity was limited by the beta$_2$-adrenergic receptor–mediated side effects. The second-generation beta-blockers possess higher affinities for the beta$_1$-adrenergic receptors than for the beta$_2$-adrenergic receptors. The recent development of recombinant cell lines selectively expressing high levels of human beta$_1$- or beta$_2$-adrenergic receptor presented the opportunity to determine the beta$_2$/beta$_1$-receptor affinity ratio for the commonly used beta-blockers under ideal laboratory conditions, using radioligand binding methodology.

The results presented here are generally consistent with data accumulated over the last several years using less direct means to determine the beta$_2$/beta$_1$-receptor ratio. Absolute values for affinities and absolute values for receptor selectivities do vary to some degree from previously reported values; however, there are no qualitative differences in categorizing the drugs as beta$_1$ or beta$_2$ selective. The quantitative differences are undoubtedly caused by the use of other methodologies, such as isolated organ preparations and whole-tissue membrane preparations to determine K_i values.

Propranolol, the original beta-blocker, has no beta$_1$ selectivity. Carvedilol, a newer beta-blocker, also has no beta$_1$ selectivity as determined in this study, consistent with the results of physiological measurements of carvedilol adrenergic receptor blockade activity [13]. Bisoprolol appears to be the most selective beta$_1$ blocker in this group of commonly used beta-blockers, displaying a 19-fold higher affinity for the beta$_1$ receptor than for the beta$_2$ receptor (Figure 2) Metoprolol, betaxolol, and atenolol appear to have a lesser, but substantial, degree of beta$_1$-receptor blockade, consistent with their commonly accepted usage as beta$_1$-receptor blockers. ICI 118,551, a beta$_2$-selective drug, displayed a 82-fold selectivity for beta$_2$ receptors, consistent with its known pharmacological profile [22]. In summary, the use of the recombinant cell lines selectively expressing the human beta$_1$ and beta$_2$ receptor allows precise quantitation of the beta$_2$/beta$_1$-receptor selectivity ratios of potential beta-blockers and indicates that bisoprolol is the most beta$_1$-selective agent tested in this study. The clinical relevance of these data is not known and requires further testing.

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