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Comparison of the β-Adrenoceptor Affinity and Selectivity of Cetamolol, Atenolol, Betaxolol, and ICI-118,551

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Summary: The objective of the present study was to compare the quantitative differences in the β_1- vs. β_2-adrenoceptor affinity and selectivity of cetamolol and its enantiomers to the reference compounds atenolol, betaxolol, and ICI-118,551, using isolated tissues obtained from the dog, guinea pig, and rat. Cetamolol antagonized the β-adrenoceptor-mediated responses induced by isoproterenol, epinephrine, norepinephrine, and salbutamol, in tissues from both the dog and guinea pig, in a concentration-dependent manner. For a given tissue, the β-adrenoceptor antagonist activity of cetamolol (measured as a pA_2 or pK_B value) was independent of the agonist used. In the dog tissues, cetamolol was more potent at inhibiting responses in the coronary artery (β_1-adrenoceptors) than in the saphenous vein (β_2-adrenoceptors). In the guinea pig tissues, the potency of cetamolol was approximately the same in the trachea (mixed β_1- and β_2-adrenoceptors) and atria (predominately β_1-adrenoceptors), but lower in the soleus muscle (β_2-adrenoceptors). Studies with the S-(−) and R-(+) enantiomers of cetamolol demonstrated that the S-(−) enantiomer was approximately 100-fold more potent at β_1-adrenoceptors than the R-(+) enantiomer. In rat brain, cetamolol displaced [^{3}H]-dihydroalprenolol bound to homogenates of cortex (β_1-adrenoceptor binding sites) and cerebellum (β_2-adrenoceptor binding sites). The potency of cetamolol at β_1-adrenoceptors was found to be similar to that of betaxolol but greater than that of atenolol. However, the magnitude of the β_1-adrenoceptor selectivity displayed by atenolol and betaxolol was greater than that displayed by cetamolol. In contrast, ICI-118,551 was found to possess potent and selective affinity for β_2-adrenoceptors. **Key Words:** Cetamolol—Atenolol—Betaxolol—ICI-118,551—β_1-Adrenoceptors—β_2-Adrenoceptors.

Cetamolol (Betacor, Ayerst Laboratories, Princeton, NJ, U.S.A.) is a β-adrenoceptor antagonist currently undergoing clinical trials. Previous preclinical studies have demonstrated that the compound is a potent β-adrenoceptor antagonist possessing a degree of β_1-adrenoceptor selectivity (1–3). In humans, cetamolol has been shown to be a potent, long-acting, and cardioselective β-adrenoceptor antagonist (4–6).

The present study was designed to evaluate and compare potential differences in the β_1- vs. β_2-adrenoceptor antagonist potency and selectivity of cetamolol at β-adrenoceptors in various tissues isolated from different species. The ability of cetamolol to antagonize β-adrenoceptor responses induced by different agonists (e.g., isoproterenol and epinephrine) was also determined to aid further in the verification of the receptor subtype classification of cetamolol's activity (see ref. 7).

MATERIALS AND METHODS

Organ chamber experiments

General methodology. Tissues were suspended in 10 ml organ chambers containing a physiological salt solution (PSS) of the following composition (m*M*): NaCl, 118.4; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; and dextrose, 11.1. The bathing solution was maintained at 37°C and aerated with 95% O_2/5% CO_2. Individual resting tensions were applied to the tissues as detailed below. The tissues were connected via silk sutures to isometric force displacement transducers, and tissue responses were recorded on a polygraph. In all experiments, cocaine (3×10^{-6} *M*), hydrocortisone (1×10^{-5} *M*), and phentolamine (1×10^{-5}

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M) [or rauwolscine (5×10^{-5} *M*) and prazosin (5×10^{-5} *M*) for the dog saphenous vein] were included in the PSS to inhibit cellular uptake$_1$, uptake$_2$, and α-adrenoceptors, respectively. In experiments using guinea pig trachea, indomethacin (2.8×10^{-6} *M*) and calcium disodium EDTA (2.6×10^{-5} *M*) were added to inhibit spontaneous tone and to serve as an antioxidant, respectively.

Mongrel dogs (15–25 kg, Haycock Kennels, Quakertown, PA, U.S.A.) of either sex and male Hartley guinea pigs (250–400 g, Charles River, Wilmington, MA, U.S.A.) were used. The animals had free access to food and water prior to the experiments. Tissues were removed from guinea pigs euthanized by cervical dislocation and from dogs after exsanguination following anesthesia with sodium pentobarbital (30 mg/kg, i.v.).

Dog coronary artery. The method detailing the procedure used to study β_1-adrenoceptors in this tissue has been described elsewhere (8). Briefly, left circumflex and anterior descending coronary arteries were cleaned of adherent connective tissue and cut into rings (5 mm in length) without disturbing the intima (endothelium). A resting tension of 10 g was applied to each tissue and maintained throughout the experiment. After a brief stabilization period, 40 m*M* KCl was added to contract the tissues as a check for tissue viability. Following the washout of KCl and return to baseline, $PGF_{2\alpha}$ ($2–4 \times 10^{-6}$ *M*) was added, and the tissues were allowed to attain a stable contractile response (10 to 20 min). A control concentration–relaxation–response curve to a β-adrenoceptor agonist (e.g., isoproterenol, 1×10^{-9} to 1×10^{-4} *M*) was then obtained. After washout and return to baseline, various concentrations (e.g., 1×10^{-7} to 1×10^{-5} *M*) of a test compound (e.g., cetamolol) were added to the organ baths and allowed a 45–60 min contact time with the tissues. The concentration–relaxation–response curve to the agonist was then repeated in the continued presence of the test compound. One or two tissues in each experiment did not receive a test compound and served as a time/solvent control(s). All responses were calculated as a percentage of the maximum relaxation response to the test agonist. The ability of a test compound to alter baseline tension and $PGF_{2\alpha}$-induced contractions was also noted.

Dog saphenous vein. The method detailing the procedure used to study β_2-adrenoceptors in this tissue has been described elsewhere (9). Briefly, lateral saphenous veins were cleaned of adherent connective tissue and cut into rings (5 mm in length) without disturbing the intima. A resting tension of 2 g was applied to each tissue and maintained throughout the experiment. The experimental protocol and the test compounds used were identical to those described for the dog coronary artery, except that 7×10^{-7} *M* $PGF_{2\alpha}$ was used to contract the tissues.

Guinea pig right atria. The method detailing the procedure used to study β_1-adrenoceptors in this tissue has been described elsewhere (9). Briefly, spontaneously beating right atria were removed and placed under 1 g of basal tension. After a brief stabilization period, the tissues were subjected to a sensitizing concentration of isoproterenol (3×10^{-7} *M*). Following washout and restabilization of the tissues, a control cumulative concentration–response curve to the chronotropic effect of the test agonist (e.g., isoproterenol, 1×10^{-9} to 1×10^{-4} *M*) was established. The atrial beating rate was assessed 3 min after the addition of each successive concentration of agonist. The tissues were then allowed a 60-min period with repeated washouts to restabilize, after which various concentrations (e.g., 1×10^{-7} to 1×10^{-5} *M*) of test compound were introduced to the organ chamber and allowed a 45–60-min contact time with the tissues. The concentration–response curve to the agonist was then repeated in the presence of the test compound. All responses were calculated as a percentage of the maximum chronotropic response to the test agonist. The ability of a test compound to alter basal beating rate was also noted.

Guinea pig trachea. The method detailing the procedure used to study β-adrenoceptors in this tissue has been described elsewhere (10). Briefly, tracheal tissue was removed and dissected free of connective tissue. Each trachea was cut into four to six rings approximately 3 mm (2–3 cartilaginous segments) in width. Two pieces of silk suture were passed through the lumen of each ring and tied around the cartilage adjoining either side of the trachealis muscle. A basal tension of 2 g was applied and maintained throughout the experiment. After an equilibration period, the tissues were exposed to various concentrations (e.g., 1×10^{-9} to 1×10^{-5} *M*) of the test compound. One tissue in each experiment did not receive test compound and served as a control. After a 45 to 60 min equilibration with the test compound, the rings were contracted with $PGF_{2\alpha}$ (1×10^{-6} *M*) and allowed to attain a stable response (10–20 min). A concentration–relaxation–response curve to the test agonist (e.g., salbutamol, 1×10^{-8} to 1×10^{-3} *M*) was then obtained. All responses were calculated as a percentage of the maximum relaxation response to the test agonist. The ability of the test compounds to alter baseline tension and $PGF_{2\alpha}$-induced contractions was also noted.

Guinea pig soleus muscle. The procedure used to study β_2-adrenoceptors in this tissue was similar to that described by Waldeck (11,12). Soleus muscles were rapidly dissected and placed under 2 g of basal tension. Tissues were electrically field-stimulated via platinum electrodes (placed on either side of the tissue) with supramaximal pulses of 0.5 ms duration at ~ 10 Hz every 20 s for 1.5 s.

Following a 30-min stabilization period, a frequency response curve was performed to determine the optimum frequency at which subtetanic twitch contractions with a large degree of fusion were seen (usually between 10 and 15 Hz). The degree of fusion of the twitches and the tension attained for a single contraction were determined by increasing the polygraph paper speed. After an equilibration period, the tissues were washed extensively and tension readjustments were made to ensure 2 g of basal tension. Paired tissues from the same animal were used to study the activity of the test compounds. One tissue was treated with an appropriate concentration (1×10^{-7} to 1×10^{-5} *M*) of the test compound for 45–60 min, while the other acted as a time/solvent control. Following a control contraction–twitch reading, a cumulative concentration–twitch–inhibition–response curve to a test agonist (e.g., isoproterenol) was established on both muscles. The guinea pig soleus muscle responds to β-adrenoceptor agonists by a decrease in the tension of the maximal twitch. Changes in tension (in the presence and absence of test compound) were measured for the lower peaks of the

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final twitch in a contraction. Responses were calculated as a percentage of the maximum depression induced by the test agonist (30–70%). The ability of cetamolol to alter the basal twitch response was also noted.

Calculations and presentation of results. To correct for time-dependent changes in tissue sensitivity to the agonist in experiments where two successive concentration response curves were used, a control preparation was studied in parallel (control curves to the various agonists are slightly shifted to the right with time). The time-related shift was measured by obtaining a "time factor" concentration ratio as follows:

$$\text{Time factor (TF)} = \frac{EC_{50} \text{ for 2nd control concentration–response curve}}{EC_{50} \text{ for 1st control concentration–response curve}}$$

The concentration ratios (CR) (EC_{50} in the presence of antagonist/EC_{50} in the absence of antagonist) obtained for the test compound treated tissue were then adjusted according to the following formula:

$$\text{Adjusted concentration ratio} = CR_{ADJ} = \frac{CR}{TF}$$

The CR_{ADJ} values obtained were used to calculate K_B values (13–15), where

$$K_B = \frac{[\text{Test compound}]}{CR_{ADJ} - 1}$$

The K_B values obtained for cetamolol were compared to values obtained for selected standards to estimate relative potency and β_1- vs. β_2-adrenoceptor selectivity.

When two or more concentrations of a test compound were studied, a pA_2 value was calculated. The adjusted concentration ratios (CR_{ADJ}) obtained from the different concentrations of test compound were utilized in the Schild equation (13–15):

$$\log (CR_{ADJ} - 1) = n \log [B] - \log K_B$$

where [B] is the molar concentration of antagonist and K_B the equilibrium dissociation constant of the antagonist (test compound) for the receptor. A regression performed on the line generated by plotting log ($CR_{ADJ} - 1$) vs. log [B] is a representation of the Schild equation and, if linear with a slope not significantly different from unity (n = 1), the intercept of this line with the abscissa (i.e., when CR = 2) can be considered an estimate of the dissociation constant. The mathematical calculations involved in obtaining a pA_2 value were performed using a computer program based on procedures outlined by Tallarida and Murray (16).

Fractional receptor occupancy was calculated from the equation presented below (17):

$$\text{Fractional receptor occupancy} = \frac{[RA]}{[R_T]} = \frac{[A]}{K_D + [A]}$$

where [RA] is the concentration of receptors occupied by the antagonist (A), $[R_T]$ is the concentration of the total receptor population, K_D is the equilibrium dissociation constant for the antagonist–receptor complex, and [A] is the concentration of antagonist.

Mean values for EC_{50} were calculated as the geometric mean. Results are expressed as the mean ± SEM. In all experiments, obs/N equals the number of observations from N animals.

Receptor binding experiments

General methodology. Male Sprague–Dawley rats (200–250 g, Charles River) were used. The animals had free access to food and water prior to the experiments. Whole brains were removed from rats euthanized by decapitation. The methods detailing the procedures used to study β_1-adrenoceptor binding sites in the cortex and β_2-adrenoceptor binding sites in the cerebellum have been described elsewhere (9). The ability of a test compound to compete with [^{3}H]-dihydroalprenolol (1×10^{-9} *M*) at 30°C was taken as an indication of β-adrenoceptor affinity. Propranolol (1×10^{-6} *M*) was used to define nonspecific binding.

Calculations and presentation of results. The value for nonspecific binding in each assay was subtracted from total binding to give a value for specific binding. All specific binding values obtained in the presence of test compound were calculated as a percentage of that in the absence of test compound. The resultant values were plotted on a logit–log plot of concentration of test compound vs. percentage inhibition of specific binding, and an IC_{50} value (concentration of drug producing a 50% inhibition of specific binding) was determined.

Drugs

Drugs were dissolved in distilled water, dilute HCl (atenolol), or dimethylsulfoxide (prazosin) according to their solubility. When solvents were used, a control was run in parallel to determine the solvent's effect on a given tissue. All drug concentrations are expressed as the final molar concentration in the organ chamber. The following drugs were used: cocaine HCl, $PGF_{2\alpha}$–Tris-HCl, hydrocortisone-21-hemisuccinate (sodium salt), (−)-epinephrine-(+)-bitartrate, (−)-norepinephrine-(+)-bitartrate, (−)-isoproterenol(+)-bitartrate, atenolol, and indomethacin (Sigma Chemical Co.); betaxolol HCl (Synthelabo); prazosin HCl (Pfizer); rauwolscine HCl (Atomergic Chemicals Corp.); ICI-118,551 (ICI Pharmaceuticals Division); salbutamol (Schering); cetamolol HCl, propranolol HCl, the S-(−) enantiomer of cetamolol HCl, and the R-(+) enantiomer of cetamolol HCl (Ayerst); and [^{3}H]-dihydroalprenolol (New England Nuclear).

RESULTS

Cetamolol

Cetamolol (1×10^{-7} to 1×10^{-5} *M*) caused a concentration-dependent antagonism of the β-adrenoceptor-mediated responses induced by isoproterenol, epinephrine, norepinephrine, and salbutamol in tissues from both the dog (Fig. 1) and the guinea pig. To quantify this antagonism, pA_2 or pK_B values were calculated from the rightward shifts in the concentration–response curves to the agonists and are presented in Table 1. For a given tissue, the β-adrenoceptor antagonist activity of cetamolol was independent of the agonist used (i.e., similar pA_2

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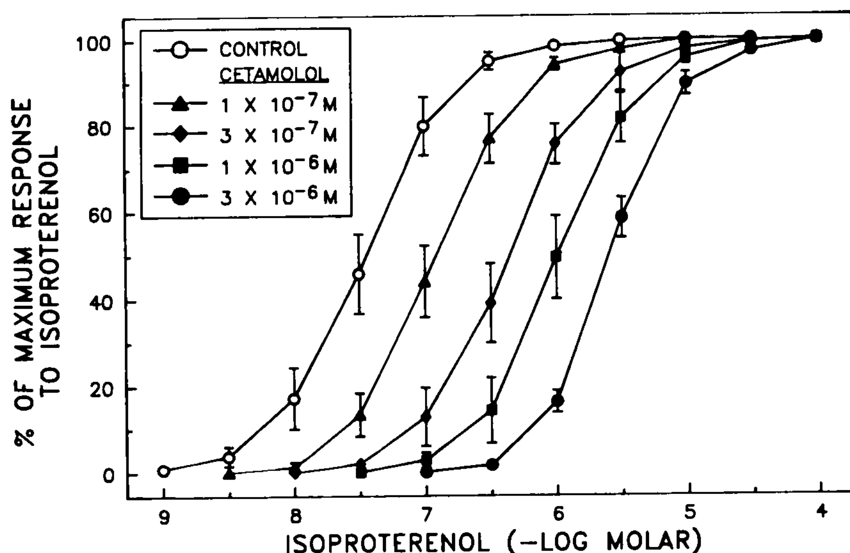

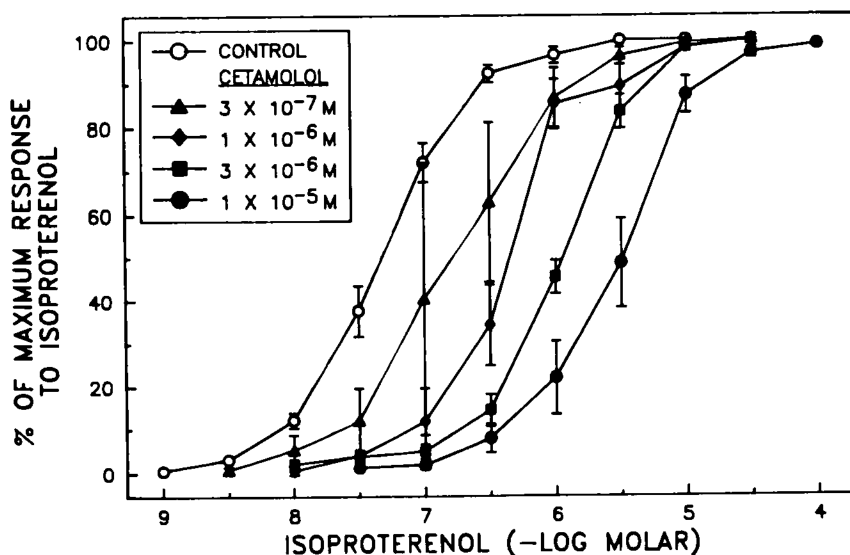

FIG. 1. Cetamolol and β-adrenoceptors in the dog. Representative data for the ability of cetamolol to concentration dependently antagonize the β_1-adrenoceptor (coronary artery) and β_2-adrenoceptor (saphenous vein) effect of isoproterenol. Data are expressed as the mean ± SEM (n = 6–8). See Table 1 for the derived pA_2 values and Schild plot slopes.

and pK_B values). In the dog tissues, cetamolol was more potent at inhibiting β-adrenoceptor-mediated responses in the coronary artery than in the saphenous vein (Table 1). In the guinea pig tissues, the potency of cetamolol was approximately the same in the trachea and atria but lower in the soleus muscle (Table 1). The results of similar studies with the S-(−) and R-(+) enantiomers of cetamolol are presented in Tables 2 and 3, respectively. The S-(−) enantiomer of cetamolol was approximately 100-fold more potent than the R-(+) enantiomer in a given tissue, independent of the agonist used. Cetamolol (1×10^{-7} to 1×10^{-5} *M*) did not significantly alter baseline conditions or $PGF_{2\alpha}$-induced contractions in any of the tissues tested.

Cetamolol also inhibited [^{3}H]-dihydroalprenolol binding in the rat cortex and cerebellum (Table 4). In agreement with the functional studies, the S-(−)

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TABLE 1. *β-Adrenoceptor affinity of cetamolol*

Tissue (receptor subtype)	Agonist											
	Isoproterenol			Epinephrine			Norepinephrine			Salbutamol		
	Schild plot slope	pA_2 or pK_B	Obs./N	Schild plot slope	pA_2 or pK_B	Obs./N	Schild plot slope	pA_2 or pK_B	Obs./N	Schild plot slope	pA_2 or pK_B	Obs./N
Dog coronary artery (β_1)	0.97 ± 0.05	7.6 ± 0.1	17/6	—	7.8 ± 0.0	3/3	—	7.9 ± 0.1	3/3	—	—	—
Dog saphenous vein (β_2)	0.92 ± 0.09	6.9 ± 0.1	17/8	0.96 ± 0.16	6.6 ± 0.1	11/4	—	—	—	—	6.3 ± 0.2	4/4
Guinea pig trachea (β_1 and β_2)	0.85 ± 0.08	8.0 ± 0.2	16/4	0.82 ± 0.08[a]	7.9 ± 0.2	16/4	0.90 ± 0.06	8.0 ± 0.1	16/4	0.88 ± 0.12	8.0 ± 0.2	15/4
Guinea pig atria (β_1)	1.05 ± 0.08	7.8 ± 0.1	15/5	0.88 ± 0.05[a]	8.3 ± 0.1	15/15	0.98 ± 0.05	8.0 ± 0.1	15/15	—	—	—
Guinea pig soleus (β_2)	0.97 ± 0.18	7.4 ± 0.4	12/12	1.00 ± 0.11	7.3 ± 0.2	19/19	—	—	—	—	7.5 ± 0.1	5/5

[a] Slope differs significantly from unity ($p < 0.05$).

enantiomer of cetamolol was about 100-fold more potent than the R-(+) enantiomer (Table 4).

Atenolol

Atenolol (1×10^{-6} to 1×10^{-4} *M*) also caused concentration-dependent β-adrenoceptor antagonism but was significantly less potent in this respect than cetamolol in all cases (Table 5, compare with Table 1). In the dog tissues, atenolol was significantly more potent at inhibiting β-adrenoceptor–mediated responses in the coronary artery than in the saphenous vein. In the guinea pig tissues, atenolol was significantly more potent at inhibiting β-adrenoceptor–mediated responses in the atria than in the soleus muscle, while in the trachea, its expressed potency generally fell between that seen in the atria and soleus muscle. These relative potency differences were independent of the agonist used. However, in a given tissue, the potency of atenolol tended to be greater when norepinephrine was used as the agonist, in comparison to either isoproterenol or epinephrine. Atenolol (1×10^{-6} to 1×10^{-4} *M*) did not significantly alter baseline conditions or $PGF_{2\alpha}$-induced contractions in any of the tissues tested.

In the rat brain, atenolol had a 10-fold greater inhibitory effect on the binding of [^{3}H]-dihydroalprenolol in the cortex when compared with its activity in the cerebellum (Table 4).

Betaxolol and ICI-118,551

The potency of betaxolol was greater in the dog coronary artery and guinea pig atria when compared to the other tissues if isoproterenol was used as the agonist (Table 6). However, in the guinea pig trachea, the potency of betaxolol was significantly greater when norepinephrine was used as the agonist in comparison to isoproterenol, epinephrine, or salbutamol. Betaxolol also tended to be more potent in the dog coronary artery and guinea pig atria

TABLE 2. *β-Adrenoceptor affinity of the S-(−) enantiomer of cetamolol*

Tissue (receptor subtype)	Agonist								
	Isoproterenol			Epinephrine		Norepinephrine		Salbutamol	
	Schild plot slope	pA_2 or pK_B	Obs./N	pK_B	Obs./N	pK_B	Obs./N	pK_B	Obs./N
Dog coronary artery (β_1)	0.90 ± 0.12	8.0 ± 0.2	11/5	7.9 ± 0.1	3/3	—	—	—	—
Dog saphenous vein (β_2)	1.14 ± 0.18	6.8 ± 0.2	10/8	6.7 ± 0.2	4/4	—	—	—	—
Guinea pig trachea (β_1 and β_2)	—	8.0 ± 0.0	4/4	8.2 ± 0.2	4/4	—	—	8.1 ± 0.1	4/4
Guinea pig atria (β_1)	—	7.9 ± 0.1	4/4	7.9 ± 0.2	4/4	8.2 ± 0.1	4/4	—	—
Guinea pig soleus (β_2)	1.16 ± 0.19	7.4 ± 0.3	12/12	—	—	—	—	—	—

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