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The affinity of betaxolol, a β_1-adrenoceptor-selective blocking agent, for β-adrenoceptors in the bovine trachea and heart

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1 The specificity of betaxolol, a β-adrenoceptor antagonist, for β_1- and β_2-adrenoceptors was compared with that of other β-antagonists, atenolol, ICI-118551, butoxamine and (±)-propranolol, in the bovine trachea and heart by competitive interaction with [^{3}H]-CGP12177 as a radioligand.

2 The radioligand K_d values were 0.75 ± 0.12 and 1.60 ± 0.11 nM in the trachea and heart, respectively, and the B_{max} values were 34.00 ± 4.41 and 21.54 ± 2.94 fmol mg^{-1} protein, respectively.

3 Using ICI-118551, we determined the ratio of β_1:β_2-adrenoceptors in the trachea and heart to be approximately 29:71 and 56:44, respectively.

4 In the trachea, a β_2-predominant tissue, betaxolol and atenolol were more selective for β_1-adrenoceptor binding sites than β_2-adrenoceptor binding sites, whereas ICI-118551 and butoxamine were more selective for β_2-adrenoceptor binding sites.

5 The β_1-selectivity of betaxolol was 2.2 and 2.7 fold higher than that of atenolol in the bovine trachea and heart. These findings suggest that betaxolol may be useful in the treatment of hypertension, cardiac arrhythmia and angina pectoris.

Keywords: β-adrenoceptor antagonists; ^{3}H-CGP12177; binding assay; β_1-selectivity

Introduction

β_1- and β_2-adrenoceptors coexist in various tissues; for example, in the heart, where β_1-adrenoceptors predominate (Hedberg *et al.*, 1980; Heitz *et al.*, 1983; Vago *et al.*, 1984; Tsuchihashi *et al.*, 1989a; Bjørnerheim *et al.*, 1989) and in the trachea, where β_2-adrenoceptors predominate (Barnes *et al.*, 1983; Popovich *et al.*, 1984; Davis *et al.*, 1990; Henry *et al.*, 1990). β-Adrenoceptor antagonists are useful drugs for the treatment of hypertension, cardiac arrhythmias and angina pectoris by blocking β_1-adrenoceptors (Prichard *et al.*, 1980), whereas the β_2-blocking action of these drugs aggravates the condition of asthmatic patients (McNeill, 1964). Because of these side effects, β_1-selective adrenoceptor antagonists, such as atenolol, have been developed in clinical therapeutic use. The β_1-selectivity of β-adrenoceptor antagonists has been mainly determined by comparing the pA_2 value against the effects of β-agonists on cardiac muscle with that on tracheal muscle (Boudot *et al.*, 1979; Pringe *et al.*, 1987; Rimele *et al.*, 1988; Bessho *et al.*, 1990). On the other hand, the β_1-selectivity can also be assessed by comparison of p*Ki* values of β-adrenoceptor antagonists for specific binding of radioligands to β_1- and β_2-adrenoceptors, and various β-adrenoceptor antagonists have been compared in detail (Engel *et al.*, 1981; Tsuchihashi *et al.*, 1989a; 1990).

In order to determine accurately the density of β_1- and β_2-adrenoceptor binding sites and the K_d of radioligands for these subtypes in tissues, quantitative analysis of the selectivity of radioligands for subtypes was required (Neve *et al.*, 1986). We also demonstrated that determination of the selectivity of radioligands was useful for assessment of the selectivity of various unlabelled β-antagonists on the β_1-adrenoceptor predominant tissues, rat myocardium (Tsuchihashi *et al.*, 1989a) and cerebral cortex (Tsuchihashi *et al.*, 1990). To determine the β_1- and β_2-selectivity of β-adrenoceptor antagonists in β_2-predominant tissue such as trachea, we have now examined the effects of five β-antagonists on bovine trachea in comparison with their effects on the bovine heart by the binding assay method.

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Methods

Preparation of the membrane-enriched fractions

Membrane-enriched fractions from bovine trachea and heart were prepared by the following method. Bovine trachea and heart were obtained from a local abattoir. In the laboratory, the tracheae were split longitudinally and the trachealis muscle dissected free. The myocardium was dissected from the heart. The trachealis muscle amd myocardium were frozen in liquid nitrogen, and stored at −80°C until use. The trachealis muscle and myocardium (approx. 2 g) were minced with a small pair of scissors in 20 ml of 10 mM Tris-HCl, 250 mM sucrose buffer (pH 7.4) and then homogenized in a Polytron homogenizer, twice for 10 s at setting 8. The homogenate was filtered through 4 layers of gauze. The filtrate was centrifuged at 40,000 *g* for 30 min, and the resultant pellets were rinsed once; then they were homogenized with a Polytron homogenizer, twice for 10 s at setting 8, in 20 ml of 120 mM Tris-HCl, 40 mM $MgCl_2$ buffer (pH 7.4). The membrane-enriched fraction was frozen in liquid nitrogen, stored at −80°C and diluted to appropriate concentrations immediately before use. Protein concentrations were determined by Lowry's methods (Lowry *et al.*, 1951) with bovine serum albumin as the standard.

Binding assays

(a) Saturation binding assays were carried out in duplicate with [^{3}H]-CGP12177 in the presence (non-specific) and absence (total) of 10 μM (−)-propranolol. In brief, 0.25 ml of membrane suspension (0.15–0.2 mg of protein) was incubated for 45 min at 23°C with various concentrations (0.05–10 nM) of [^{3}H]-CGP12177 in a total volume of 0.5 ml containing 60 mM Tris-HCl and 20 mM $MgCl_2$ (pH 7.4).

(b) Displacement experiments were done in the presence of various concentrations of ICI-118551 in duplicate with various concentrations (trachea: 0.06, 0.6 and 12 nM, heart: 0.26, 1.4 and 20 nM) of [^{3}H]-CGP12177. All displacement experiments except those with ICI-118551 were carried out with a single concentration (trachea: 0.6 nM, heart: 1.4 nM) of [^{3}H]-CGP12177. At the end of the incubation period, the

incubation medium was immediately filtered through a GF/C glass fibre filter by the method described by Tsuchihashi *et al.* (1985). The radioactivity was counted with a scintillation counter (Aloka ALC-500). The difference in mean values between the total and non-specific binding was taken as the specific binding.

Drugs

Betaxolol hydrochloride (Mitsubishi Kasei, Japan) was synthesized. Atenolol hydrochloride, ICI-118551 (erythro-DL-1-(7-methylindan-4-yloxyl)-3-(isopropylaminobutan-2-ol) hydrochloride), (±)-propranolol, (−)-propranolol were kind gifts from ICI Pharma (Japan). Butoxamine (Burroughs Wellcome Co., U.S.A.) and (−)-[^{3}H]-CGP12177 ((−)-4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H] benzimidazol-2-one hydrochloride; Amersham, Japan) were purchased from each company. All drugs were dissolved in distilled water.

Kinetic analysis

All kinetic analyses were carried out on an NEC PC-9801 computer system that performs iterative non-linear regression as described previously (Tsuchihashi & Nagatomo, 1987a,b,c; Tsuchihashi *et al.*, 1989b), based on the theory of Munson & Rodbard (1980). Estimates of the dissociation constants (K_d) and maximum binding capacity (B_{max}) of specific [^{3}H]-CGP12177 binding were obtained by Scatchard analysis. In displacement experiments, using various concentrations of the radioligand, parameters describing the competition of ICI-118551 with specific [^{3}H]-CGP12177 binding at two sites (IC_{50} values at β_1- and β_2-adrenoceptors, $\%\beta_1$ and $\%\beta_2$) were estimated by non-linear regression analysis of data which were fitted to a 2-site binding model compared with a 1-site binding model. Then IC_{50} values for various radioligand concentrations (L) were fitted by linear regression using the modified equation of Cheng & Prusoff as follows:

$$IC_{50} = L \cdot K_i/K_d + K_i$$

The K_i values of ICI-118551 for specific [^{3}H]-CGP12177 binding at β_1- and β_2-adrenoceptors were obtained from the intercepts of the line, and K_d values of [^{3}H]-CGP12177 for β_1- and β_2-adrenoceptors were calculated from the slope of the lines. The relative proportion of β_1- and β_2-adrenoceptors within a tissue compartment was derived as $\%\beta_1$- and $\%\beta_2$-adrenoceptors obtained by use of high concentrations ($L/K_d > 10$) of radioligands. An overall estimate of the K_i values of various drugs using displacement analysis was determined by the use of general models with an appropriate concentration of the free radioligand (L) as in the following equation (equation 1):

$$B_1/B_0 = [L \cdot R_1/(L + K_{d_1}(1 + x/K_{i_1})) + L \cdot R_2/(L + K_{d_2}(1 + x/K_{i_2}))]/ (L \cdot R_1/(L + K_{d_1}) + L \cdot R_2/(L + K_{d_2}))$$

where either B_1 or B_0 is the concentration of the radioligand bound with or without the cold ligand, x is the cold ligand concentration; L is the concentration of free radioligand used. R_1 and R_2 are the proportional ratio of receptors 1 and 2 ($R_1 + R_2 = 1$), and K_{d_1} and K_{d_2} are the dissociation constants between a radioligand and receptors 1 and 2. The values of R_1, R_2, K_{d_1} and K_{d_2} were preliminarily determined by the above-mentioned methods and the determined values were substituted in equation 1. By means of these substitutions, the K_i values for two receptor sites can be directly determined by equation 1. In these non-linear or linear regression analyses, the parameter fitting method, termination of iteration, and justification of the models were carried out by previously described methods (Tsuchihashi & Nagatomo, 1987a,c).

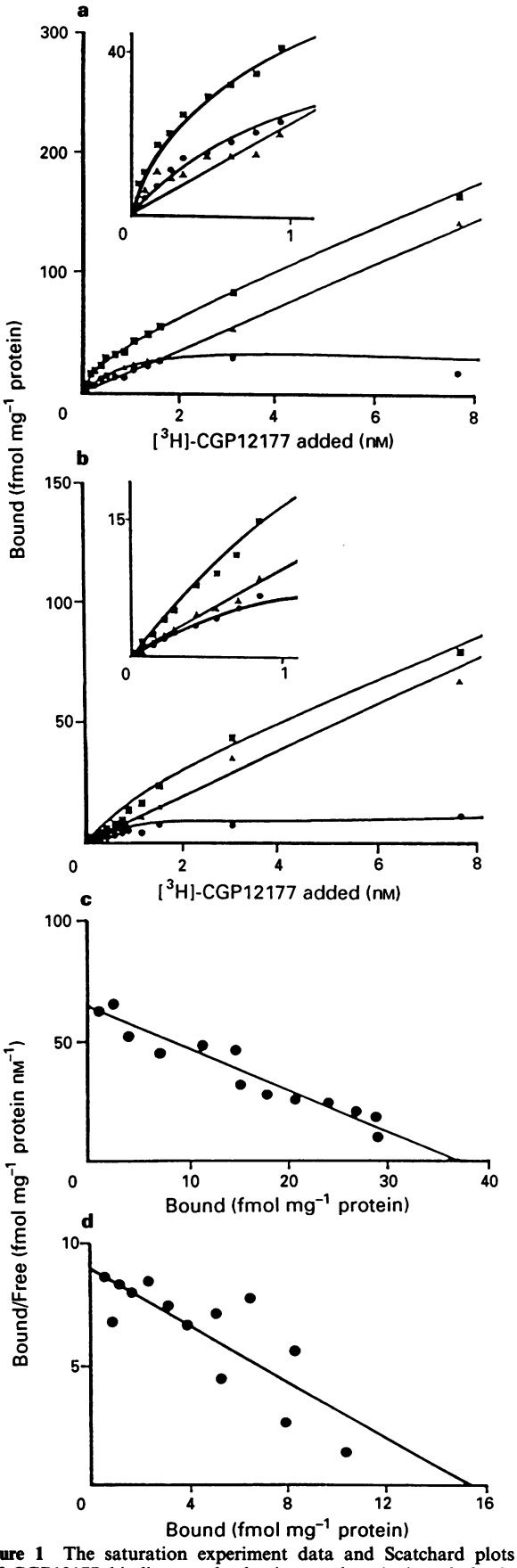

Figure 1 The saturation experiment data and Scatchard plots of [^{3}H]-CGP12177 binding to the bovine trachea (a,c) and the heart (b,d). Specific binding (●) is the difference between the total binding (■) and the binding (▲) in the presence of 10 μM (−)-propranolol (non-specific binding) at [^{3}H]-CGP12177 concentrations between 0.05 and 10 nM. The insets in (a) and (b) show the data points around the K_d values. The data shown are those from a single experiment which is representative of six such experiments in the bovine trachea and the heart, respectively.

Results

Yields of membrane protein per g wet weight of bovine trachea and heart were 6.94 ± 2.45 ($n = 6$) and 7.56 ± 0.58 ($n = 6$) mg protein g^{-1} tissues, respectively. Figures 1a and b show saturation experiments for [^{3}H]-CGP12177 binding to β-adrenoceptors in bovine trachea and heart, respectively. When Scatchard analyses were carried out in the absence (total) and presence (non-specific binding) of 10 μM (−)-propranolol, the curves for specific binding were uniphasic in character (Figure 1c and d). Table 1 summarizes the K_d and B_{max} values in the trachea and heart.

Figure 2 shows the biphasic displacement curves of specific [^{3}H]-CGP12177 binding to the bovine trachea and heart by the β_2-selective antagonist, ICI-118551, using three different concentrations of radioligand. From these results, the values of K_d and B_{max} (%) for [^{3}H]-CGP12177 to the β_1- and β_2-adrenoceptor sites in the trachea and heart were obtained from the modified equation of Cheng & Prusoff (Table 2). [^{3}H]-CGP12177 was 1.5 fold more selective for β_2-adrenoceptors than β_1-adrenoceptors in both tissues, while the proportional percentage of B_{max} of these two binding sites (β_1- and β_2-adrenoceptors) for [^{3}H]-CGP12177 in the trachea and heart were approximately 29:71 and 56:44, respectively.

Displacement curves for five unlabelled ligands, atenolol, betaxolol, butoxamine, (±)-propranolol and ICI-118551 against the radioligand at a single concentration were examined by the fitting method of comparison between a 1-site and a 2-site model in both trachea and heart (Figure 3). The displacement curves for atenolol, betaxolol and ICI-118551 all appeared to fit a 2-site model, while those for butoxamine and (±)-propranolol fitted a 1-site model. The K_i values of these ligands were directly determined by equation 1, and these pK_i values at β_1- and β_2-adrenoceptors against [^{3}H]-CGP12177 binding in the trachea and heart, are summarized in Tables 3 and 4. In both tissues, the β_1-selective antagonists (betaxolol and atenolol) had higher affinity for the β_1- than the β_2-adrenoceptor binding sites, whereas β_2-selective antagonists (ICI-118551 and butoxamine) had higher affinity for the β_2-adrenoceptor binding sites. There was no significant difference between the pK_i values of (±)-propranolol for β_1- and for β_2-binding sites. In the trachea, betaxolol had an approximately 37 fold higher affinity for β_1-adrenoceptors than β_2-adrenoceptors, and it was about 2.2 fold more selective for β_1-adrenoceptors than was atenolol. Similarly in the heart, betaxolol was approximately 2.7 fold more β_1-adrenoceptor-selective than was atenolol.

Table 1 K_d and B_{max} values of [^{3}H]-CGP12177 binding to bovine trachea and heart

	Trachea ($n = 6$)	*Heart* ($n = 6$)
K_d (nM)	0.75 ± 0.12	1.60 ± 0.11
B_{max} (fmol mg^{-1} protein)	34.00 ± 4.41	21.54 ± 2.94

Data are the means ± s.e.

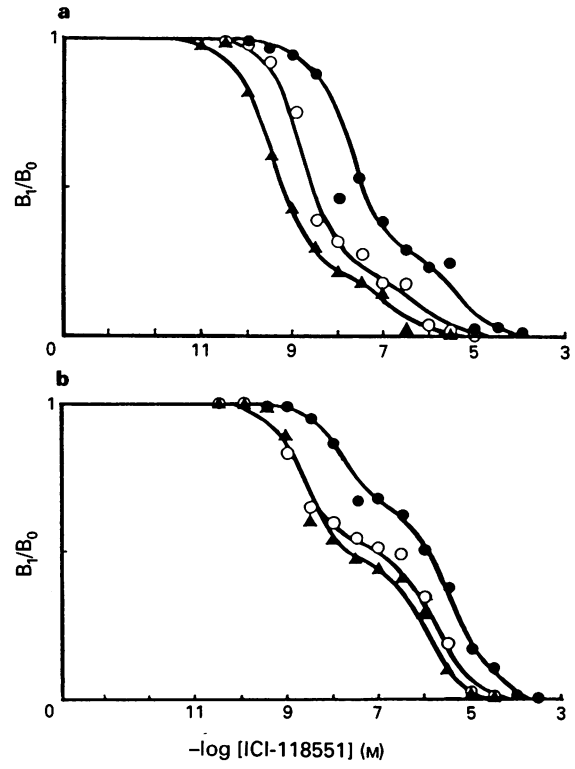

Figure 2 Displacement curves of ICI-118551 for specific [^{3}H]-CGP12177 binding to bovine trachea (a) and heart (b) using three different concentrations of radioligand: 0.06 (▲, slope factor (n_H) = 0.62), 0.6 (○, $n_H = 0.67$) and 12 nM (●, $n_H = 0.66$) in the trachea and 0.26 (▲, $n_H = 0.67$), 1.4 (○, $n_H = 0.65$) and 20 nM (●, $n_H = 0.69$) in the heart. The typical data shown are those from single experiments performed in duplicate and represent the results of three to six such experiments at each radioligand concentration.

Table 2 The K_d and B_{max} of the β_1- and β_2-adrenoceptor binding site of [^{3}H]-CGP12177 in the bovine trachea and heart by displacement experiments using ICI-118551

	Trachea ($n = 3-6$)	*Heart* ($n = 3-6$)
β_1-Adrenoceptor binding sites		
K_d (nM)	1.286	1.866
B_{max} (%)	29.18 ± 3.22	56.20 ± 2.16
β_2-Adrenoceptor binding sites		
K_d (nM)	0.792	1.315
B_{max} (%)	70.82 ± 3.22	43.80 ± 2.16

K_d values were calculated by the modified method of Cheng & Prusoff, $IC_{50} = K_i \times L/K_d \times K_i$, using linear regression analysis in which IC_{50} values and free radioligand (L) at three concentrations were used (trachea; $K_i\beta_1 = 66.67$ nM, $K_i\beta_2 = 0.792$ nM, and heart; $K_i\beta_1 = 681.4$ nM, $K_i\beta_2 = 2.103$ nM). B_{max} values were obtained a high concentration of radioligand (trachea; 12 nM, heart; 20 nM).

Discussion

The coexistence of β_1- and β_2-adrenoceptors in trachea has been reported in the dog (Barnes *et al.*, 1983), pig (Popovich *et al.*, 1984), human (Davis *et al.*, 1990) and mouse (Henry *et al.*, 1990), where the proportions of β_2- relative to β_1-adrenoceptors is between 60 and 90%. In the present study we have also shown that β_1- and β_2-adrenoceptors coexist in the bovine trachea at a β_1- to β_2-adrenoceptor ratio of approximately 30:70. Thus, we found that bovine trachea is also a β_2-adrenoceptor predominant tissue.

In previous studies (Tsuchihashi *et al.*, 1989a; 1990), we reported the pK_i values of several β-antagonists for β_1- and β_2-sites by use of a radioligand binding method. These values correlated closely with the antagonist potencies (pA_2 values) of these β-antagonists against the positive inotropic, chronotropic (β_1) and tracheal relaxant actions (β_2) of isoprenaline (Tsuchihashi *et al.*, 1989a). In the present study, we obtained pK_i values for β-adrenoceptor antagonists at β_1- and β_2-sites by the same method. In the bovine trachea, the order of affinity for β_1-adrenoceptors was (±)-propranolol >> betaxolol, ICI-118551 >> atenolol > butoxamine, whereas that for β_2-receptors was ICI-118551 = (±)-propranolol >> betaxolol > butoxamine >> atenolol. Similar findings were obtained in the bovine heart (present study), rat heart (Tsuchihashi *et al.*, 1989a) and cerebral cortex (Tsuchihashi *et al.*, 1990). However, the relationship between the affinities of [^{3}H]-CGP12177 (K_d value) for β_1-adrenoceptors and for

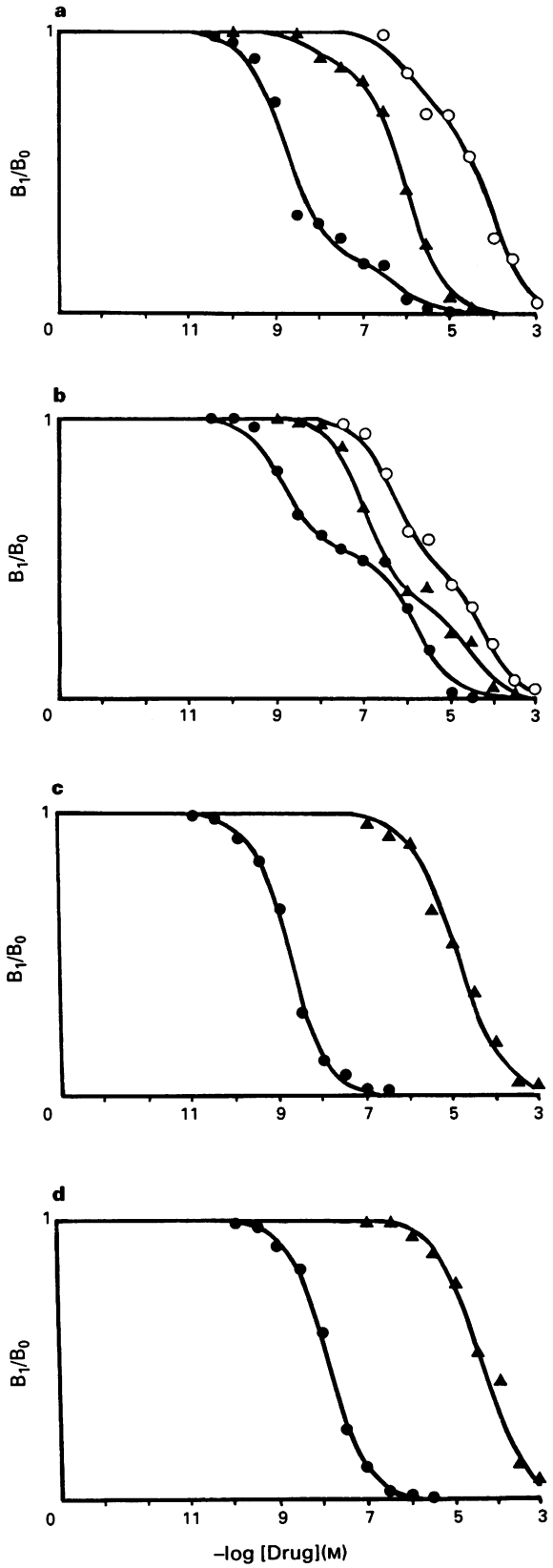

Figure 3 Displacement curves of specific [^{3}H]-CGP12177 (0.6 and 1.4 nM) binding to bovine trachea (a,c) and heart (b,d). (a and b) Betaxolol (▲), atenolol (○) and ICI-118551 (●); (c and d) (±)-propranolol (●) and butoxamine (▲). The slope factors of the plots of β-antagonists were: betaxolol (0.73), atenolol (0.71), ICI-118551 (0.62), (±)-propranolol (1.06) and butoxamine (0.93) in the trachea, and betaxolol (0.69), atenolol (0.75), ICI-118551 (0.71), (±)-propranolol (1.01) and butoxamine (0.92) in the heart. The typical data shown are those from single experiments performed in duplicate and represent the results of six to eight such experiments.

Table 3 p*Ki* values of β-antagonists in the bovine trachea

	pKi values		$K_i\beta_1/K_i\beta_2$
	β_1-sites	*β_2-sites*	*ratio*
Betaxolol ($n = 8$)	7.70 ± 0.21	6.13 ± 0.12	37.2**
Atenolol ($n = 7$)	5.64 ± 0.27	4.41 ± 0.06	17.0**
ICI-118551 ($n = 6$)	7.49 ± 0.07	9.19 ± 0.06	0.02***
Butoxamine ($n = 6$)	4.82 ± 0.18	5.43 ± 0.06	0.25**
(±)-Propranolol ($n = 6$)	9.18 ± 0.06	8.95 ± 0.07	1.70^{NS}

Data are the mean values ± s.e. These data were obtained by the displacement analysis 0.6 nM [^{3}H]-CGP12177 and calculated from equation 1.
Significance of difference between values of p*Ki* for β_1- and β_2-sites was determined by Student's *t* test: $^{**}P<0.01$; $^{***}P<0.001$ and NS: not significant.

Table 4 p*Ki* values of β-antagonists in the bovine heart

	pKi values		$K_i\beta_1/K_i\beta_2$
	β_1-sites	*β_2-sites*	*ratio*
Betaxolol ($n = 6$)	7.56 ± 0.14	5.82 ± 0.21	55.0***
Atenolol ($n = 6$)	5.94 ± 0.14	4.63 ± 0.12	20.4**
ICI-118551 ($n = 5$)	6.46 ± 0.21	9.05 ± 0.25	0.003***
Butoxamine ($n = 6$)	4.29 ± 0.09	4.65 ± 0.13	0.44*
(±)-Propranolol ($n = 7$)	8.32 ± 0.12	8.03 ± 0.18	1.95^{NS}

Data are the mean values ± s.e. These data were obtained by the displacement analysis 1.4 nM [^{3}H]-CGP12177 and calculated from equation 1.
Significance of difference between values of p*Ki* for β_1- and β_2-sites was determined by Student's *t* test: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ and NS: not significant.

β_2-adrenoceptors differed between these studies. Thus the affinity of [^{3}H]-CGP12177 for β_1-sites was higher than that for β_2-sites in rat heart, which was in apparent contrast to that obtained in the bovine trachea and heart, where the affinity was higher at β_2-adrenoceptors. Similarly, the p*Ki* values for (±)-propranolol at β_1- and β_2-adrenoceptors were different. Furthermore, there were inconsistencies in the relative affinities of atenolol and ICI-118551 for β_1-adrenoceptor in various tissues: thus in bovine trachea, ICI-118551 >> atenolol (present study), bovine heart (present study) and rat heart (Tsuchihashi *et al.*, 1989a), ICI-118551 > atenolol; and rat brain, ICI-118551 = atenolol (Tsuchihashi *et al.*, 1990). Furthermore, in a comparison between the *Ki* values for β_1- (rat salivary gland) and β_2-adrenoceptor predominant tissues (rat reticulocyte) (Wellstein *et al.*, 1986) using radioreceptor assay, and between antagonistic potencies for the positive inotropic and chronotropic effects on left and right atria of the guinea-pig, ICI-118551 = atenolol (Tsuchihashi *et al.*, 1989a). These findings could suggest that the β-adrenoceptor conformation and/or the receptor environment differed between species and tissues. We have previously demonstrated that the environment of the receptor site could indeed have a crucial role in ligand-receptor interactions (Tsuchihashi & Nagatomo, 1985a,b,c).

The displacement curves for butoxamine were found to be monophasic when the data were fitted to 1-site and 2-site models in the present study, and the results showed that butoxamine had β_2-adrenoceptor selectivity as assessed by equation 1. We have previously demonstrated that alprenolol has an approximately 7 fold higher affinity for β_2-adrenoceptors than for β_1-adrenoceptors in both rat heart and brain, although it is generally known as a non-selective antagonist, suggesting that the use of equation 1 to directly determine K_i values of ligands is useful for detecting the selectivities of ligands with low affinity differences between β_1- and β_2-adrenoceptors (Tsuchihashi *et al.*, 1989a; 1990).

β_2-Adrenoceptor blockade has been reported to aggravate asthma, whereas β_1-adrenoceptor blockade appears to be less

associated with this side-effect (McDevitt, 1983). In fact, it has been reported that β_1-selective agents are less likely to worsen the condition of asthmatic patients than is propranolol, a non-selective β-antagonist (Johnsson *et al.*, 1975; Singh *et al.*, 1976; Palmiteri & Kaik, 1983). Therefore, β_1-selectivity is believed to be important in a β-adrenoceptor antagonist used clinically for treating cardiovascular disease. Betaxolol, like atenolol, exhibits a high β_1-adrenoceptor selectivity in isolated tissues (Boudot *et al.*, 1979; Pringe *et al.*, 1987; Rimele *et al.*, 1988; Bessho *et al.*, 1990). However, these tissues are thought to contain both β_1- and β_2-adrenoceptors, and therefore these results may not reflect the true β_1-selectivity of the β-antagonists. The net β_1-selectivity of β-antagonists in various tissues has been examined by the receptor binding assay method (Engel *et al.*, 1981; Tsuchihashi *et al.*, 1989a; 1990). We previously reported that betaxolol was a β_1-selective antagonist in rat heart (Tsuchihashi *et al.*, 1989a) and cerebral cortex (Tsuchihashi *et al.*, 1990) and that the affinity for β_1-adrenoceptors was 23 and 170 fold higher than that for β_2-adrenoceptors, respectively. In the guinea-pig lung (Engel *et al.*, 1981), a β_2-predominant tissue, the affinity of β_1-sites was 200 fold higher than that for β_2-sites. In the present study, betaxolol had a 37 fold higher affinity for β_1-adrenoceptors than for β_2-adrenoceptors in the trachea, and 55 fold higher affinity in the heart. Similarly, atenolol was shown to be β_1-selective in both bovine trachea and the heart, but the selectivity was about 2 to 3 fold lower than that of betaxolol. These findings are consistent with our previous data (Tuschihashi *et al.*, 1989a) and those obtained in isolated tissues (Bessho *et al.*, 1990). These findings indicate that betaxolol and atenolol also have a β_1-selective profile in the trachea (β_2-predominance) as well as heart (β_1-predominance).

In conclusion, the results of the present study using a radioligand binding method indicate that β_1- and β_2-adrenoceptors coexist in the bovine trachea and heart, and that not only does betaxolol have a β_1-selective profile in both preparations, but it is more β_1-selective than atenolol. These findings suggest that betaxolol may be a useful drug for the treatment of hypertension, cardiac arrhythmia and angina pectoris.

References

BARNES, P.J., NADEL, J.A., SKOOGH, B. & ROBERTS, J.M. (1983). Characterization of beta adrenoceptor subtypes in canine airway smooth muscle by radioligand binding and physiological responses. *J. Pharmacol. Exp. Ther.*, **225**, 456–461.

BESSHO, H., SUZUKI, J., NARIMATSU, A. & TOBE, A. (1990). Cardioselective β-adrenoceptor blocking action of betaxolol in vitro and in vivo. *Pharmacometrics*, **39**, 521–527.

BJØRNERHEIM, R., GOLF, S. & HANSSON, V. (1989). Apparent lack of β_2 adrenergic receptors in porcine myocardium. *Cardiovasc. Res.*, **23**, 577–583.

BOUDOT, J., CABERO, I., FENARD, S., LEFEVERE-BORG, F., MANOURY, P. & ROACH, A.G. (1979). Preliminary studies on SL 75212, a new potent cardioselective β-adrenoceptor antagonist. *Br. J. Pharmacol.*, **66**, 445P.

DAVIS, P.B., SILSKI, C.L., KERCSMAR, C.M. & INFELD, M. (1990). β-Adrenergic receptors on human tracheal epithelial cells in primary culture. *Am. J. Physiol.*, **258**, C71–C76.

ENGEL, G., HOYER, D., BERTHOLD, R. & WAGNER, H. (1981). (±)[^{125}Iodo]cyanopindolol, a new ligand for β-adrenoceptors: identification and quantitation of subclasses of β-adrenoceptors in guinea-pig. *Naunyn-Schimiedebergs Arch Pharmacol.*, **317**, 277–285.

HEDBERG, A., MINNEMAN, K.P. & MOLINOFF, P.B. (1980). Differential distribution of beta-1 and beta-2 adrenergic receptors in cat and guinea-pig heart. *J. Pharmacol. Exp. Ther.*, **212**, 503–508.

HEITZ, A., SCHWARZ, J. & VELLY, J. (1983). β-Adrenoceptors of human myocardium: determination of β_1 and β_2 subtypes by radioligand binding. *Br. J. Pharmacol.*, **80**, 711–717.

HENRY, P.J., RIGBY, P.J. & GOLDIE, R.G. (1990). Distribution of β_1- and β_2-adrenoceptors in mouse trachea and lung: a quantitative autoradiographic study. *Br. J. Pharmacol.*, **99**, 136–144.

JOHNSSON, G., SVEDMYR, N. & THIRINGER, G. (1975). Effects of intravenous propranolol and metoprolol and their interactions with isoprenalin on pulmonary function, heart rate and blood pressure in asthmatics. *Eur. J. Clin. Pharmacol.*, **8**, 175–180.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–272.

MCDEVITT, D.G. (1983). Clinical significance of cardioselectivity. *Drugs*, **25** (Suppl 2), 219–226.

MCNEIL, R.S. (1964). Effect of a beta-adrenergic-blocking agent, propranolol, on asthmatics. *Lancet*, **ii**, 1101–1102.

MUNSON, P.J. & RODBARD, D. (1980). LIGAND: a versatile computerized approach for the characterization of ligand binding systems. *Anal. Biochem.*, **107**, 220–239.

NEVE, K.A., MCGONIGLE, P. & MOLINOFF, P.B. (1986). Quantitative analysis of the selectivity of radioligands for subtypes of beta adrenergic receptors. *J. Pharmacol. Exp. Ther.*, **238**, 46–53.

PALMITERI, R. & KAIK, G. (1983). Time course of the bronchial response to salbutamol after placebo, betaxolol and propranolol. *Eur. J. Clin. Pharmacol.*, **24**, 741–745.

POPOVICH, K.J., HILLER, A., HOUDH, A., NORRIS, J.S. & CORNETT, L.E. (1984). Characterization of β-adrenergic receptor in porcine trachealis muscle. *Am. J. Physiol.*, **247**, C342–C349.

PRICHARD, B.N.C., OWENS, C.W.I. & TUCKMAN, J. (1980). Clinical features of adrenergic agonist and antagonists. In *Handbook of Experimental Pharmacology: Adrenergic Activators and Inhibitors.* ed. Szekeres, L. Vol 54, pp. 559–697. Berlin: Springer-Verlag.

PRINGE, T.H., RIDDLE, J.G. & SHANKS, R.G. (1987). A comparison of the cardioselectivity of five β-adrenoceptor blocking drugs. *J. Cardiovasc. Pharmacol.*, **10**, 228–237.

RIMELE, T.J., HENRY, D.E., GIESA, F.R., BUCKLEY, S.K., GEIGER, G., HEASLIP, R.J., LEE, D.K.H. & GRIMES, D. (1988). Comparison of the β-adrenoceptor affinity of cetamolol, atenolol, betaxolol and ICI-118,551. *J. Cardiovasc. Pharmacol.*, **12**, 208–217.

SINGH, B.N., WHITLOCK, R.M.L., COMBER, R.H., WILLIAMS, F.H. & HARRIS, E.A. (1976). Effects of cardioselective β-adrenoceptor blockade on specific airway resistance in normal subjects and in patients with bronchial asthma. *Clin. Pharmacol. Ther.*, **19**, 493–501.

TSUCHIHASHI, H. & NAGATOMO, T. (1985a). Influence of polymeric effectors on binding of ^{3}H-dihydroalprenolol to β-adrenergic receptor of rat brain. *Jpn. J. Pharmacol.*, **38**, 17–23.

TSUCHIHASHI, H. & NAGATOMO, T. (1985b). Binding characteristics of ^{3}H-dihydroalprenolol to β-adrenergic receptors of rat brain: influence of lectins. *Jpn. J. Pharmacol.*, **38**, 121–125.

TSUCHIHASHI, H. & NAGATOMO, T. (1985c). Binding characteristics of ^{3}H-dihydroalprenolol to β-adrenergic receptors of rat brain: influence of exo- and endo-glycosidases and glycopeptidase. *Jpn. J. Pharmacol.*, **38**, 403–409.

TSUCHIHASHI, H. & NAGATOMO, T. (1987a). Biphasic binding of ^{125}I-iodocyanopindolol to β-adrenergic receptors in rat cerebral cortical membranes. I. Assessment by the use of agonists. *Chem. Pharmacol. Bull.* (Tokyo), **35**, 2966–2972.

TSUCHIHASHI, H. & NAGATOMO, T. (1987b). Characterization of ^{3}H-dihydroalprenolol binding to β-adrenergic receptors of rat brain: two binding sites of racemic propranolol in displacement experiments. *Chem. Pharmacol. Bull.* (Tokyo), **35**, 2979–2984.

TSUCHIHASHI, H. & NAGATOMO, T. (1987c). Binding characteristics of ^{125}I-iodocyanopindolol to β-adrenergic receptors: biphasic Scatchard plots. II. Effects of selective antagonists. *Chem. Pharmacol. Bull.* (Tokyo), **35**, 3424–3432.

TSUCHIHASHI, H., SASAKI, M. & NAGATOMO, T. (1985). Binding characteristics of [^{3}H]dihydroalprenolol to β-adrenergic receptors of rat brain: comparison with those of rat heart treated with neuraminase. *Chem. Pharmacol. Bull.* (Tokyo), **33**, 3972–3976.

TSUCHIHASHI, H., YOKOYAMA, H. & NAGATOMO, T. (1989a). Binding characteristics of ^{3}H-CGP12177 to β-adrenoceptors in rat myocardial membranes. *Jpn. J. Pharmacol.*, **49**, 11–19.

TSUCHIHASHI, H., NAGATOMO, T. & IMAI, S. (1989b). Three binding sites of ^{125}I-iodocyanopindolol to β_1, β_2-adrenergic and $5HT_{1B}$-receptors in rat brain determined by the displacement and Scatchard analysis. *J. Pharmacodyn.*, **12**, 509–516.

TSUCHIHASHI, H., NAKASHIMA, Y., KINAMI, J. & NAGATOMO, T. (1990). Characteristics of ^{125}I-iodocyanopindolol binding to β-adrenergic and serotonin-1B receptors of rat brain: selectivity of β-adrenergic agents. *Jpn. J. Pharmacol.*, **52**, 195–200.

VAGO, T., BEVILAVILACQUA, M., DAGNI, R., MERONI, R., FRIGENI, G., SANTOLI, C. & NORBIATO, G. (1984). Comparison of rat and human left ventricle beta-adrenergic receptors: subtype heterogeneity delineated by direct radioligand binding. *Biochem. Biophys. Res. Commun.*, **121**, 346–354.

WELLSTEIN, A., PALM, D. & BELZ, G.G. (1986). Affinity and selectivity of β-adrenoceptor antagonists in vitro. *J. Cardiovasc. Pharmacol.*, **8**, S36–S40.

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