Characteristics of ^{125}I -Iodocyanopindolol Binding to β -Adrenergic and Serotonin-1B Receptors of Rat Brain: Selectivity of β -Adrenergic Agents

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Abstract—The present study was designed to examine the specificity of β -adrenergic antagonists for β_1 -, β_2 -adrenergic and $5\text{HT}_{1\text{B}}$ -serotonergic receptors by the competitive interaction with ^{125}I -iodocyanopindolol (^{125}I -ICYP) as a radioligand. The β_1 -adrenoceptors were preferred by acebutolol, atenolol, betaxolol, practolol, and l-, dl- and d-metoprolol, while butoxamine and ICI-118,551 preferred β_2 -adrenoceptors. The selectivities of these β_1 - and β_2 -antagonists are well-known, but alprenolol which is known as a non-selective antagonist was 7.2-fold more selective for the β_2 -adrenoceptors in the present study. All β -antagonists used were more selective towards β -adrenoceptors as compared with $5\text{HT}_{1\text{B}}$ -receptors. Good correlations were observed between the potencies of β -adrenoceptor antagonists for inhibition of ^{125}I -ICYP binding to β_1 - and β_2 -adrenoceptor sites and their potencies for inhibiting the binding of the same radioligand to $5\text{HT}_{1\text{B}}$ -serotonergic receptor sites. These results suggest that β -adrenoceptor antagonists can bind to β -adrenoceptors and $5\text{HT}_{1\text{B}}$ -receptors.

In our previous reports (1–3), the Scatchard plots of ^{125}I -iodocyanopindolol (^{125}I -ICYP) binding to β -adrenoceptors in the rat cerebral cortex were shown to be biphasic and suggested that the high- and low-affinity sites in the biphasic Scatchard plots apparently correspond to β - and $5\text{HT}_{1\text{B}}$ -receptors, respectively. Furthermore, we found that the selectivities of ^{125}I -ICYP were in the order of $\beta_2 > \beta_1 > 5\text{HT}_{1\text{B}}$ -receptors when displacement experiments were done with the competitor l-metoprolol (3). In addition, we found that some β -adrenoceptor antagonists displaced ^{125}I -ICYP bound to $5\text{HT}_{1\text{B}}$ -serotonergic receptors (3). Thus, the present study was designed to examine the preference of β -adrenoceptor antagonists for β_1 -, β_2 -adrenoceptors and $5\text{HT}_{1\text{B}}$ -serotonergic receptors by radioligand binding assay using displacement experiments.

Materials and Methods

Materials: (–)- ^{125}I -ICYP (2200 Ci/mmol) was purchased from New England Nuclear

Co., Ltd.

Preparation of the membrane-enriched fractions: The membrane-enriched fraction from the rat cerebral cortex was prepared by using the previously described method (1). 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 the methods of Lowry et al. (4) using bovine serum albumin as the standard.

Binding assay: (a) Direct K_i determinations of the β -antagonists for the three binding sites (β_1 -, β_2 - and $5\text{HT}_{1\text{B}}$ -receptors) were carried out by duplicate displacement experiments with 20 pM of ^{125}I -ICYP in the presence of various concentrations of β -antagonists such as l-metoprolol. The non-specific binding was determined in the presence of 10 μM l-propranolol. The apparent percentage of β_1 -, β_2 - and $5\text{HT}_{1\text{B}}$ -receptors observed in this experiment were 32, 19 and 50% of the binding sites, respectively.

(b) K_i determinations for the β -antagonists of 5HT_{1B}-receptors by displacement experiments was also carried out in duplicate with 20 pM of [¹²⁵I]-ICYP in the presence of various concentrations of β -antagonists such as l-metoprolol. These experiments were carried out in the presence of 300 μ M l-metoprolol because of the complete inhibition of [¹²⁵I]-ICYP binding to β -adrenoceptors as described previously (3).

Incubation was started by the addition of 0.25 ml of membrane suspension (0.1 mg of protein) from rat cerebral cortex for 60 min in a total volume of 0.5 ml containing 60 mM Tris-HCl and 20 mM MgCl₂ (pH 7.2). At the end of the incubation period, the sample was immediately filtered through a GF/C glass

fiber filter using our improved method (5). The radioactivity was counted with an auto-well gamma counter (Aloka ARC-500). The difference in mean values between the total and non-specific binding was taken as the specific binding.

Kinetic analyses: All kinetic analyses were carried out on an NEC PC-9801 computer with iterative non-linear regression (1-3, 6). The previous results (3) showed that [¹²⁵I]-ICYP bound to β_1 -, β_2 - and 5HT_{1B}-receptors with preferences in the order of $\beta_2 > \beta_1 > 5HT_{1B}$ -receptors. In the present study, the displacement curves were used to analyze the three binding site model, using the following equation:

$$\frac{B_1}{B_0} = \frac{\frac{L \cdot LT_1}{L + K_{d1}(1+x/K_{i1})}}{\frac{L \cdot LT_1}{L + K_{d1}}} + \frac{\frac{L \cdot LT_2}{L + K_{d2}(1+x/K_{i2})}}{\frac{L \cdot LT_2}{L + K_{d2}}} + \frac{\frac{L \cdot LT_3}{L + K_{d3}(1+x/K_{i3})}}{\frac{L \cdot LT_3}{L + K_{d3}}}$$

where either B_1 and B_0 are the concentrations of the radioligand bound with or without the cold ligand, respectively; x and L are the concentration of cold ligand and radioligand, respectively; and K_{i1} , K_{i2} and K_{i3} are the inhibition constants of a cold ligand for receptors 1, 2 and 3, respectively. LT_1 , LT_2 and LT_3 are the proportion of receptors 1, 2 and 3 ($LT_1 + LT_2 + LT_3 = 1$), respectively; and K_{d1} , K_{d2} and K_{d3} are the dissociation constants between a radioligand and receptors 1, 2 and 3, respectively. When receptors 1, 2 and 3 respectively corresponded to β_1 -, β_2 - and 5HT_{1B}-receptors, the values of LT_1 (0.05), LT_2 (0.09), LT_3 (0.86), K_{d1} (30.9 pM), K_{d2} (44.4 pM), K_{d3} (369 pM) were determined as described previously (3), and the determined values were substituted into this equation. By means of these substitutions, the K_i values for the three receptor sites: K_{i1} for β_1 -adrenoceptors, K_{i2} for β_2 -adrenoceptors, K_{i3} for 5HT_{1B}-receptors were directly determined by this equation.

In addition, the determinations of K_i values of various drugs for 5HT_{1B}-receptors were also carried out using the one receptor-one ligand model as follows:

$$\frac{B_1}{B_2} = \frac{1}{1 + \frac{x}{IC50}}$$

where IC50 is an apparent inhibition constant (concentration of the cold ligand which inhibits the radioligand binding by 50%). K_i was determined by the equation of Cheng and Prusoff as follows:

$$K_i = IC50 / (1 + L/K_d)$$

The values of K_d (369 pM) were determined as described previously (3) and used in this study.

In these non-linear or linear regression analyses, the parameter fitting method, termination of iteration, and justification of the models were carried out by the methods described previously (1, 6). The values were sometimes expressed as pK_i ($-\log K_i$) values in this report.

Results

The preference of β -antagonists for β_1 - and β_2 -adrenergic receptors were initially examined by displacement analysis, and the values of pK_i of β -antagonists were directly determined numerically by the model of three binding sites. Table 1 summarizes the results of β -adrenergic antagonists for β_1 - and β_2 -adrenoceptors in [¹²⁵I]-ICYP binding to rat cerebral cortical membranes. Acebutolol, atenolol, betaxolol, practolol, and l-, dl- and d-metoprolol bound β_1 -adrenoceptors selectively, whereas butoxamine and ICI-118,551

Table 1. Selectivity of β -adrenergic agents for β -adrenergic receptors

	pK _i values		Signifi- cance	Selec- tivity	β_1 -K _i / β_2 -K _i ratio
	β_1 -sites	β_2 -sites			
1. Arotinolol (3)	9.74±0.40	9.26±0.07	N	$\beta_1 = \beta_2$	3.0
2. Pindolol (3)	9.18±0.36	9.50±0.50	N	$\beta_1 = \beta_2$	0.48
3. l-Propranolol (3)	9.16±0.21	9.34±0.04	N	$\beta_1 = \beta_2$	0.66
4. dl-Propranolol (3)	8.55±0.02	8.61±0.05	N	$\beta_1 = \beta_2$	0.87
5. Alprenolol (3)	8.38±0.07	9.24±0.13	C	$\beta_1 < \beta_2$	0.14
6. YM-09538 (3)	8.36±0.17	7.88±0.20	N	$\beta_1 = \beta_2$	3.0
7. Oxprenolol (3)	8.11±0.17	8.01±0.26	N	$\beta_1 = \beta_2$	1.3
8. Labetalol (3)	7.92±0.23	7.17±0.19	N	$\beta_1 = \beta_2$	5.6
9. Betaxolol (3)	7.91±0.13	5.69±0.25	C	$\beta_1 > \beta_2$	170
10. l-Metoprolol (3)	7.86±0.32	5.61±0.12	C	$\beta_1 > \beta_2$	180
11. dl-Metoprolol (3)	7.20±0.18	5.77±0.25	C	$\beta_1 > \beta_2$	27
12. d-Propranolol (3)	7.09±0.30	7.36±0.30	N	$\beta_1 = \beta_2$	0.54
13. Atenolol (3)	7.01±0.36	5.55±0.08	B	$\beta_1 > \beta_2$	29
14. Acebutolol (3)	6.90±0.07	5.15±0.02	D	$\beta_1 > \beta_2$	56
15. ICI-118,551 (3)	6.71±0.07	8.75±0.10	D	$\beta_1 < \beta_2$	0.0091
16. Practolol (3)	6.61±0.13	4.71±0.15	D	$\beta_1 > \beta_2$	79
17. Sotalol (3)	5.65±0.36	6.47±0.31	N	$\beta_1 = \beta_2$	0.15
18. Butoxamine (3)	5.43±0.04	6.33±0.14	C	$\beta_1 < \beta_2$	0.13
19. d-Metoprolol (3)	4.89±0.20	4.06±0.19	A	$\beta_1 > \beta_2$	6.8

Each value in parenthesis shows the number of experiments. Data are the mean values±S.E. Significance of difference between values of pK_i to β_1 - and β_2 -sites was determined by Student's *t*-test. A: P<0.05, B: P<0.02, C: P<0.01, D: P<0.001, and N: not significant.

were selective towards β_2 -adrenoceptors. In addition, alprenolol which is well-known as a non-selective antagonist preferred β_2 -adrenoceptors. Arotinolol, pindolol, YM-09538, oxprenolol, labetalol, sotalol, and d-, dl- and l-propranolol were not selective towards β_1 - and β_2 -adrenoceptors. The most β_2 -selective antagonist was dl-metoprolol, and the most β_1 -selective antagonist was ICI-118,551.

Table 2 summarizes pK_i values of these β -adrenergic antagonists used for 5HT_{1B}-serotonergic receptors, determined by the three binding site model and the one receptor-one ligand model carried out in the presence of the β -adrenoceptor competitor 30 μ M l-metoprolol. The values obtained from these two models were identical. All these β -adrenergic antagonists displaced ¹²⁵I-ICYP binding to 5HT_{1B}-receptors; arotinolol was the most potent of the drugs, and d-metoprolol was the least potent.

Figure 1 depicts the relationship between the K_i values of these drugs for β_1 - and β_2 -adrenoceptors determined by the binding assays in the current study and the K_i values of these drugs for β_1 - and β_2 -adrenoceptors

in the rat myocardium determined by the binding assay using ³H-CGP12177 as a radioligand, which were described previously (7). A good correlation (pK_i values for β_1 - (r=0.97) and β_2 -adrenoceptors (r=0.96)) was observed between the pK_i values in this study and those obtained in the previous study.

The relationship between the pK_i values of these β -adrenergic antagonists for β_1 - and β_2 -adrenoceptors determined in this study and their pK_i values (A values in Table 2) for 5HT_{1B}-serotonergic receptors determined in the present study is shown in Fig. 2. A good correlation (β_1 vs. 5HT_{1B}; 0.87 and β_2 vs. 5HT_{1B}; 0.88) was also observed between the pK_i values for β_1 - and β_2 -adrenoceptors and those for 5HT_{1B}-receptors.

Discussion

The selectivities of ¹²⁵I-ICYP binding to β_1 -, β_2 -adrenoceptors and 5HT_{1B}-serotonergic receptors were in the order of $\beta_2 > \beta_1 > 5HT_{1B}$ -receptors as described previously (3). The preferences of β -antagonists for β_1 -, β_2 - and 5HT_{1B}-receptors were assessed by the radioligand binding assay using ¹²⁵I-ICYP as

Table 2. pK_i values of β -antagonists for 5HT_{1B} receptors

	pK_i values		β_1 K_i / 5HT _{1B} K_i ratio	β_2 K_i / 5HT _{1B} K_i ratio
	A	B		
Arotinolol	7.97±0.08 (3)	8.16±0.24 (3)	59	19
dl-Propranolol	7.25±0.21 (3)	7.01±0.05 (3)	20	23
Pindolol	7.23±0.12 (3)	7.19±0.08 (3)	89	180
Alprenolol	7.15±0.08 (3)	7.04±0.03 (3)	17	120
l-Propranolol	7.11±0.05 (3)	7.19±0.03 (3)*	110	170
Oxprenolol	6.76±0.05 (3)	5.93±0.02 (3)	22	18
YM-09538	5.89±0.08 (3)	5.60±0.02 (3)	300	98
Labetalol	5.70±0.07 (3)	5.41±0.06 (3)	170	30
ICI118,551	5.41±0.07 (3)	4.92±0.03 (3)	20	2200
Acebutolol	5.38±0.02 (3)	5.00±0.02 (3)	33	0.59
d-Propranolol	5.15±0.04 (3)	4.89±0.02 (3)	87	160
Betaxolol	4.18±0.09 (3)	3.56±0.10 (3)	5400	32
dl-Metoprolol	4.06±0.02 (3)	3.75±0.06 (3)	1400	51
l-Metoprolol	3.94±0.11 (3)	3.55±0.04 (3)	8300	47
Practolol	3.43±0.07 (3)	3.39±0.04 (3)*	1500	19
Atenolol	3.43±0.12 (3)	3.35±0.08 (3)*	3800	130
Sotalol	3.23±0.11 (3)	3.06±0.08 (3)	260	1700
Butoxamine	3.11±0.03 (3)	3.59±0.17 (3)*	210	1700
d-Metoprolol	2.57±0.08 (3)	2.18±0.11 (3)	210	31

The pK_i values of A and B are the results obtained by the displacement experiments using the one site model in the presence of 30 μ M l-metoprolol and the three site model, respectively. Each value in parenthesis shows the number of experiments. Data are the mean values±S.E. The pK_i values of β -antagonists to β_1 - and β_2 -adrenergic receptors were those in Table 1, and these values were compared with the values (A) for 5HT_{1B}-receptors. The values indicated by * were obtained from the report published by Tsuchihashi et al. (3).

a radioligand. When the preferences of these antagonists for β_1 - and β_2 -adrenoceptors were compared, acebutolol, atenolol, betaxolol, practolol, and l-, dl- and d-metoprolol preferred β_1 -adrenoceptors; alprenolol, butoxamine and ICI-118,551 preferred β_2 -adrenoceptors; and arotinolol, pindolol, YM-09538, oxprenolol, labetalol, sotalol, and d-, dl- and l-propranolol were non-selective towards β_1 - and β_2 -adrenoceptors. Alprenolol is well-known as a non-selective antagonist, but the present study showed that it has 7.2-fold higher affinity for β_2 -adrenoceptors (Table 1), suggesting that the methods in this study are useful for determining the preferences of β -antagonists with low affinity differences between β_1 - and β_2 -adrenoceptors.

All β -antagonists displaced the binding of [¹²⁵I]-ICYP from the 5HT_{1B}-receptors. The non-selective antagonists for β -adrenoceptors had 18- to 300-fold preferences towards β -adrenoceptors as compared to their selec-

tivity towards 5HT_{1B}-receptors, whereas the selective antagonists showed 33–8300-fold higher selectivity towards β_1 - and β_2 -adrenoceptors than 5HT_{1B}-receptors. These results suggested that all β -adrenoceptor antagonists tested in this study displaced [¹²⁵I]-ICYP bound to 5HT_{1B}-serotonergic receptors.

Furthermore, it is possible that these receptors may exist in the brain, heart, lung and spleen (2, 3). In the present study, a good correlation was observed between the pK_i values of β -antagonists for β -adrenoceptors and those of 5HT_{1B}-receptors, suggesting that β -adrenoceptors antagonists may regulate the function of 5HT_{1B}-receptors. Although the existence of an interaction between the function and structure of β -adrenoceptors and those of 5HT_{1B}-receptors is yet to be proven, it would be of interest to examine the function and distribution of 5HT_{1B}-receptors in the body.

A good correlation was also observed be-

Fig. 1. Relationship between the potencies (pK_i) of the chemicals for inhibition of ¹²⁵I-ICYP binding to β_1 - (A) and β_2 -adrenoceptors (B) obtained by the present study in the rat cerebral cortex and those obtained by the binding of ³H-CGP12177 in the rat myocardium, which were reported previously (7). Numbers refer to individual drugs in Table 1. (A) $y=0.91x+0.90$, $r=0.97$ ($P<0.001$). (B) $y=0.96x+0.39$, $r=0.96$ ($P<0.001$).

tween the pK_i values of β -antagonists for β_1 - and β_2 -adrenoceptors obtained in the present study and those obtained by the binding of ³H-CGP12177 in the rat myocardium. In our previous study (5), we found that there was a difference in the IC₅₀ values between rat heart and brain; especially, the affinity of alprenolol for β -adrenoceptors in the brain was much lower than those in the heart. However, the present results did not demonstrate such differences in affinity of alprenolol in the rat myocardium and cerebral cortex, indicating that binding methods may be useful

Fig. 2. Relationship between the potencies (pK_i) of the chemicals for inhibition of ¹²⁵I-ICYP binding to β_1 - (A) and β_2 -adrenoceptors (B) and those to 5HT_{1B}-receptors (A values in the Table 2). Numbers refer to individual drugs in Table 1. (A) $y=0.67x+4.02$, $r=0.87$ ($P<0.001$). (B) $y=0.90x+2.37$, $r=0.88$ ($P<0.001$).

for the assessing the relative potencies of the chemicals as β -adrenoceptor antagonists.

The radioligand ¹²⁵I-ICYP was used as a model ligand in competitive binding experiments. The present results quantitated the affinity of β -adrenoceptor antagonists for β_1 -, β_2 - and 5HT_{1B}-serotonergic receptors and found that the β -adrenoceptors may be related to 5HT_{1B}-receptors.

References

- 1 Tsuchihashi, H. and Nagatomo, T.: Biphasic binding of ¹²⁵I-iodocyanopindolol to β -adren-
ergic receptors in rat cerebral cortical mem-
branes. I. Assessment by the use of agonists.
Chem. Pharm. Bull. (Tokyo) **35**, 2966-2972
(1987)

- 2 Tsuchihashi, H. and Nagatomo, T.: Binding characteristics of ^{125}I -iodocyanopindolol to β -adrenergic receptors: Biphasic Scatchard plots. II. Effects of selective antagonists. *Chem. Pharm. Bull. (Tokyo)* **35**, 3424–3432 (1987)
- 3 Tsuchihashi, H., Nagatomo, T. and Imai, S.: Three binding sites of ^{125}I -iodocyanopindolol to β_1 , β_2 -adrenergic and $5\text{HT}_{1\text{B}}$ -receptors in rat brain determined by the displacement and Scatchard analysis. *J. Pharmacobiodyn.* **12**, 509–516 (1989)
- 4 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275 (1951)
- 5 Tsuchihashi, H., Sasaki, M. and Nagatomo, T.: Binding characteristics of ^3H -dihydroalprenolol to β -adrenergic receptors of rat brain: Comparison with those of rat heart treated with neuraminidase. *Chem. Pharm. Bull. (Tokyo)* **33**, 3972–3976 (1985)
- 6 Tsuchihashi, H. and Nagatomo, T.: Characterization of ^3H -dihydroalprenolol binding to β -adrenergic receptors of rat brain: Two binding sites of racemic propranolol in displacement experiments. *Chem. Pharm. Bull. (Tokyo)* **35**, 2979–2984 (1987)
- 7 Tsuchihashi, H., Yokoyama, H. and Nagatomo, T.: Binding characteristics of ^3H -CGP12177 to β -adrenoceptors in rat myocardial membranes. *Japan. J. Pharmacol.* **49**, 11–19 (1989)