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Antagonist binding at 5-HT_{2A} and 5-HT_{2C} receptors in the rabbit: High correlation with the profile for the human receptors

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Abstract

This study examined the binding of serotonin receptor antagonists at the 5-HT_{2A} and 5-HT_{2C} receptors of the rabbit's cerebral cortex. The 5-HT_{2A} receptor was characterized by the binding of [^{3}H]MDL 100,907 (*R*(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol) to cortical membranes and the 5-HT_{2C} receptor by the binding of [^{3}H]mesulergine in the presence of the selective 5-HT_{2A} receptor ligand spiperone. Both [^{3}H]MDL 100,907 and [^{3}H]mesulergine demonstrated high affinity binding to single sites in rabbit membranes. Based on Scatchard plots of [^{3}H]MDL 100,907 binding, the mean B_{max} was 8.5 ± 0.7 fmol/mg tissue and the mean K_d was 33.1 ± 3.5 pM. For [^{3}H]mesulergine binding the mean B_{max} was 3.70 ± 0.58 fmol/mg tissue and the mean K_d was 0.35 ± 0.05 nM. Binding of [^{3}H]MDL 100,907 to the 5-HT_{2A} receptor and of [^{3}H]mesulergine to the 5-HT_{2C} receptor was confirmed by displacement studies with highly selective 5-HT_{2A} and 5-HT_{2C} receptor ligands. The pharmacological profile of these ligands in rabbits correlated highly with published values for 5-HT_{2A} ($r = 0.91$, $P < 0.001$) and 5-HT_{2C} ($r = 0.94$, $P < 0.001$) receptors in humans. There was also a high correlation between the profiles for human and rat 5-HT_{2C} receptor ($r = 0.92$, $P < 0.001$), but not for 5-HT_{2A} receptors ($r = 0.53$, $P > 0.10$). It was concluded that the rabbit provides an appropriate animal model for studies attempting to predict the pharmacology of human 5-HT_{2A} and 5-HT_{2C} receptors.

Keywords: 5-HT_{2A} receptor; 5-HT_{2C} receptor; Frontal cortex; (Rabbit); (Human)

1. Introduction

Both the rat and the rabbit are currently being employed in a variety of experiments aimed at clarifying the role of 5-HT receptors in learning and memory (Harvey, 1996; Meneses, 1998). Currently, this laboratory is investigating the role of 5-$HT_{2A/2C}$ receptor agonists, antagonists and inverse agonists in a rabbit model of associative learning (Welsh et al., 1998a,b; Harvey et al., 1999; Romano et al., 2000). Extrapolation of results employing the rabbit to the human would be facilitated if the rabbit 5-HT_{2A} and 5-HT_{2C} receptors had a similar pharmacological profile to that of the corresponding human receptors. Binding affinities of various antagonists at the rat and human 5-HT_{2A} or 5-HT_{2C} receptor have been published (see Table 1), however, there are no data available for the rabbit. Since it is well established that the pharmacological properties of some serotonin receptors diverge widely across animal species (Nelson et al., 1993; Wainscott et al., 1996; Pauwels, 1997), it is not clear whether the rabbit model is appropriate for understanding the possible role of 5-$HT_{2A/2C}$ receptors in human associative processes. Therefore, we examined the binding affinities of a number of 5-HT_{2A} and 5-HT_{2C} receptor ligands in the rabbit.

The 5-HT_{2A} receptor was defined by the binding of [^{3}H]MDL 100,907 (*R*(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol), since it has been demonstrated to be a highly selective 5-HT_{2A} receptor ligand in both the rat (Johnson et al., 1996; López-Giménez et al., 1997a) and human (López-Giménez et al., 1997b). Based on previous studies in the rat (Pranzatelli et al., 1992), the 5-HT_{2C} receptor was defined by the binding of [^{3}H]mesulergine in the presence of spiperone, which is highly selective for the 5-HT_{2A} receptor as compared with the 5-HT_{2C} receptor. Additionally, we conducted a literature search for the binding affinities of a number of 5-HT_2 receptor ligands for the rat and human 5-HT_{2A} and 5-HT_{2C} receptors (see Table 1). These values were used to deter-

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Table 1
Binding affinities at the rabbit, human and rat 5-HT_{2A} and 5-HT_{2C} receptors

Drug	Rabbit 5-HT_{2A}^{a}	Human 5-$HT_{2A}^{b,c}$	Rat 5-$HT_{2A}^{b,c}$	Rabbit 5-HT_{2C}^{d}	Human 5-$HT_{2C}^{c,e}$	Rat 5-$HT_{2C}^{c,e}$
MDL 100,907	0.03 ± 0.004	0.14	0.40	ND	ND	ND
BOL	0.13 ± 0.02	ND	0.48[f]	4.40 ± 0.53	7.14[f]	7.9
Spiperone	0.19 ± 0.02	2.00	1.27	134 ± 33	650	1257
Ergonovine	0.32 ± 0.02	1.38	16.7	7.91 ± 0.71	19	ND
Ketanserin	0.54 ± 0.10	2.50	1.49	15.8 ± 0.67	102	59
MDL 11,939	0.54 ± 0.06	6.06	ND	81.6 ± 6.7	1021	ND
Ritanserin	0.71	0.79	8.07	ND	0.35	1.99
Mianserin	1.46 ± 0.37	8.9	6.50	0.70 ± 0.10	4.85	3.72
Methysergide	1.80 ± 0.05	18.1	2.60	0.26 ± 0.03	1.33	1.26
LY 53,857	3.91 ± 0.63	ND	16.7	5.83 ± 1.41	ND	ND
Mesulergine	6.19 ± 0.52	64.2	6.00	0.35 ± 0.05	1.75	1.97
RS 102221	445 ± 116	876	141	1.07 ± 0.42	2.9	3.55

ND, determinations were not carried out or were not available in the literature.

[a]Values are the K_i (nM) for displacement of [^{3}H]MDL 100,907, except that the value for MDL 100,907 is reported as the K_d. Each value is the mean ± S.E.M. of three to eight determinations, except for ritanserin, which is the mean of duplicate determinations.

[b]Values are the K_i (nM) for displacement of [^{3}H]ketanserin as obtained from the literature, except that the value for MDL 100,907 in the human is reported as the K_d.

[c]K_i values for human and rat cortical tissue were obtained from the following references (Almaula et al., 1996; Bonhaus et al., 1997; Choudhary et al., 1995; Hagen et al., 1994; Hoyer, 1988; Hoyer et al., 1985, 1986; Johnson et al., 1994, 1996; Kao et al., 1992; Labrecque et al., 1995; Leonhardt et al., 1992; Leysen et al., 1982; López-Giménez et al., 1997b; McKenna and Peroutka, 1989; Newton and Elliott, 1997; Newton et al., 1996; Pazos et al., 1984; Pranzatelli et al., 1992; Schotte et al., 1983; Schreiber et al., 1995; Siegel et al., 1996; Sleight et al., 1996; Teitler et al., 1990; Wainscott et al., 1996; Weinhardt et al., 1996; Westphal and Sanders-Bush, 1994).

[d]Values are the K_i (nM) for displacement of [^{3}H]mesulergine, except for the value for mesulergine, which is reported as the K_d. Each value is the mean ± S.E.M. of three to eight determinations.

[e]Values are the K_i (nM) for displacement of [^{3}H]mesulergine as obtained from the literature.

[f]Values are the K_i (nM) for displacement of [^{125}I]DOI (Wainscott et al., 1996).

mine the degree of similarity between the pharmacology of the human 5-HT_{2A} and 5-HT_{2C} receptors with that of the rabbit and rat.

2. Materials and methods

2.1. Animals

Adult, New Zealand white rabbits of both sexes were obtained from Covance (Denver, PA). Rabbits were housed individually with free access to rabbit chow and water under a 12:12-h light/dark cycle in a colony maintained at 22°C. Samples of rabbit frontal cerebral cortex were dissected, frozen on dry ice within 10 min of sacrifice, and stored at −70°C. These studies were carried out in accordance with the National Institute of Health Guide "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and were approved by our Institutional Animal Care and Use Committee.

2.2. Chemicals

[^{3}H]MDL 100,907 (specific activity 83 Ci/mmol) and [^{3}H]mesulergine (specific activity 74 Ci/mmol) were obtained from Amersham Life Sciences (Arlington Heights, IL). Ketanserin tartrate, LY 53857 maleate (6-methyl-1-(1-methylethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate), mesulergine hydrochloride, methysergide maleate, mianserin hydrochloride, ritanserin, and spiperone hydrochloride were obtained from Research Biochemicals International (Natick, MA). MDL 11,939 (α-phenyl-10(2-phenylethyl)-4-piperidinemethanol) and RS 102221 (8-[5-(2,4-dimethoxy-5-(4-trifluoromethyphenylsulphonamido) were purchased from Tocris Cookson (Ballwin, MO). Ergonovine was obtained from Sigma (St. Louis, MO). BOL (*d*-2-bromolysergic acid diethylamide hydrogen tartrate) was supplied by the National Institute on Drug Abuse (Rockville, MD).

2.3. Tissue preparation

On the day of the binding assay, the frozen samples of cerebral cortex were placed in 10 volumes (by weight) of ice-cold buffer (50 mM Tris–HCl, pH 7.4 at 0°C) and immediately homogenized using a Brinkman Polytron (10 s at half power). All subsequent steps were performed at 0–4°C. Tissue homogenate was centrifuged at 40,000 × *g* for 20 min. The resulting pellet was resuspended in 50 volumes of the 50 mM Tris buffer using a Brinkman Polytron (10 s at half power) followed by centrifugation as described above. The washed membrane fraction was dispersed in a room temperature assay buffer (20 mM Tris–HCl, pH 7.4 at 20°C) using a Polytron.

2.4. 5-HT_{2A} receptor analysis

All binding assays were performed at 25°C in the 20 mM Tris–HCl, pH 7.4 buffer. 5-HT_{2A} receptors were analyzed using the selective antagonist, [^{3}H]MDL 100,907. Saturation analyses were performed using eight concentrations of [^{3}H]MDL 100,907 ranging from 7 to 1200 pM. The assay was initiated by the addition of washed membranes (1 mg) in a total volume of 1 ml. For competition studies, each tube contained 30–60 pM [^{3}H]MDL 100,907, eight concentrations of unlabelled drug, and washed membranes derived from 1 mg of tissue in a total volume of 1 ml. Nonspecific binding was defined by the addition of 100 nM spiperone. The mixture was incubated for 120 min at 25°C before being terminated by rapid filtration through Whatman GF/B filters (presoaked in 0.5% polyethylenimine) followed by three washes, each consisting of 5 ml of wash buffer (20 mM Tris–HCl, pH 7.4 at 4°C). The amount of radioactivity retained on the filter was determined by liquid scintillation counting.

2.5. 5-HT_{2C} receptor analysis

All binding assays were performed at 25°C in the 20 mM Tris–HCl, pH 7.4 buffer. 5-HT_{2C} receptors were analyzed using [^{3}H]mesulergine in the presence of spiperone (30 nM, final concentration) to prevent mesulergine binding to 5-HT_{2A} and dopamine D_2 receptors. Saturation analyses were performed using eight concentrations of [^{3}H]mesulergine (0.03–4.2 nM) in a 1-ml final assay volume. The binding was initiated by the addition of washed membranes (4–6 mg). For competition studies, each tube contained 0.2–0.4 nM [^{3}H]mesulergine, eight concentrations of unlabelled drug, and washed membranes derived from 4 mg of tissue in a total volume of 1 ml. Nonspecific binding was defined by 1 μM methysergide. The assay was performed in glass tubes and all pipettes were siliconized to prevent loss of the [^{3}H]mesulergine. The mixture was incubated for 120 min at 25°C before being terminated by rapid filtration followed by three washes as described in Section 2.4. The amount of radioactivity retained on the filter was determined by liquid scintillation counting.

2.6. Literature survey

The K_i values for the inhibition of ketanserin binding to rat and human 5-HT_{2A} receptors or the inhibition of mesulergine binding to rat and human 5-HT_{2C} receptors were determined from literature reports where the species used was clearly identified. Manuscripts reporting inhibition of binding to 5-HT_{2A} or 5-HT_{2C} receptors in native tissues or receptors expressed in various cell lines were utilized. If more than one value for a given ligand was reported, the mean value was used for the comparative analysis.

2.7. Data analysis

The binding data were analyzed using the nonlinear, curve fitting program LIGAND (Munson and Rodbard, 1980) as modified for Macintosh computers, which allows calculation of the K_d, B_{max} and K_i. For the calculation of K_d and B_{max}, nonspecific binding was a computer-fitted parameter. The Pearson product moment coefficient of correlation was employed to test the degree of association between variables (Hays, 1981).

3. Results

3.1. Characterization of rabbit cortical 5-HT_{2A} receptors

Scatchard analysis revealed that the selective 5-HT_{2A} receptor antagonist [^{3}H]MDL 100,907 bound to a single class of binding sites in rabbit cerebral cortex (Fig. 1, upper panel). Based on five separate determinations, the mean value for B_{max} was 8.5 ± 0.7 fmol/mg tissue and the mean K_d was 33.1 ± 3.5 pM. Eleven 5-$HT_{2A/2C}$ receptor antagonists were found to dose-dependently inhibit [^{3}H]MDL 100,907 binding to rabbit cortical 5-HT_{2A} receptors. Nonlinear curve fitting analyses demonstrated that all of the drugs tested inhibited [^{3}H]MDL 100,907 binding from a single class of sites. Based on the K_i values, there was a large range of estimated affinities ranging from 0.13 nM for BOL to 445 nM for RS 102221 (Table 1). Of relevance to this study is the finding that the 5-HT_{2A} receptor selective ligands, spiperone and MDL 11,939 (Leysen et al., 1982) demonstrated high affinities (K_i values of 0.19 and 0.54 nM, respectively) whereas the 5-HT_{2C} receptor selective ligand, RS 102221 (Bonhaus et al., 1997) displayed a low affinity ($K_i = 445$ nM) for the 5-HT_{2A} receptor.

3.2. Characterization of rabbit cortical 5-HT_{2C} receptors

Scatchard analysis revealed that [^{3}H]mesulergine (in the presence of 30 nM spiperone) bound to a single class of binding sites in rabbit cortex (Fig. 1, bottom panel). The results of eight separate experiments gave a mean K_d of 0.35 ± 0.05 nM and a B_{max} of 3.70 ± 0.58 fmol/mg tissue. Ten 5-$HT_{2A/2C}$ receptor antagonists examined dose-dependently inhibited [^{3}H]mesulergine binding from a single class of sites. The K_i values of these drugs for displacement of [^{3}H]mesulergine are presented in Table 1. Importantly, the 5-HT_{2C} receptor selective ligand RS 102221 displayed a high affinity ($K_i = 1.07$ nM) whereas the 5-HT_{2A} receptor selective ligands, spiperone and MDL 11,939 displayed low affinities, the K_i values being 134 and 81.6 nM, respectively.

3.3. Comparison of rabbit affinities with those of rat and human obtained from the literature

The K_i values (or in a few cases K_d values) determined from published values for human and rat 5-HT_{2A}

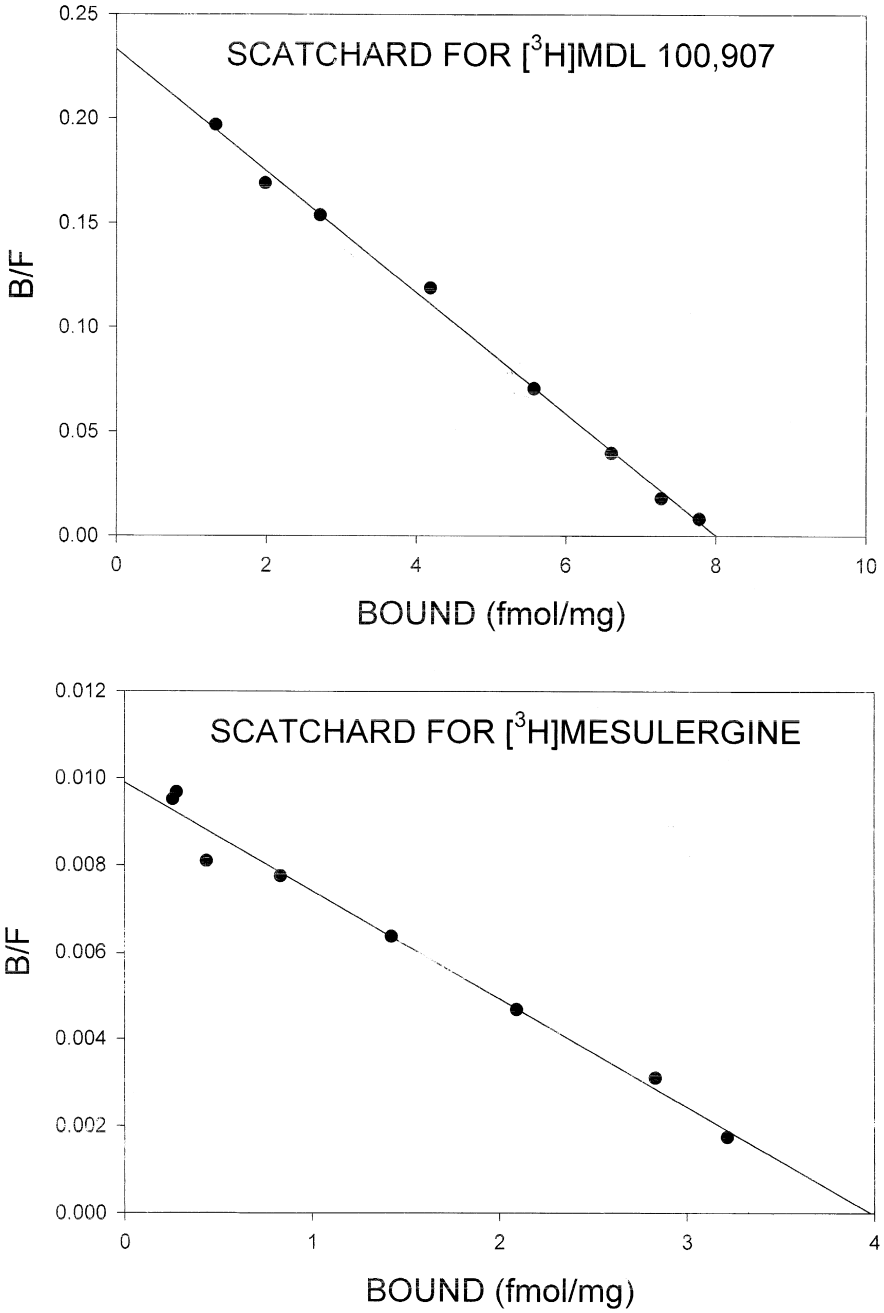

Fig. 1. Upper panel, Scatchard curve for [^{3}H] MDL 100,907 binding to rabbit cortical membranes. Membranes were incubated with eight concentrations of [^{3}H]MDL 100,907 (range 7–1200 pM). A representative Scatchard plot of the specific binding is shown. These data are from one experiment, which was repeated five times. Bottom panel, Scatchard analysis of [^{3}H]mesulergine binding to rabbit cortical membranes. Rabbit cortical membranes were incubated with eight concentrations of [^{3}H]mesulergine (range 0.03–4.2 nM) in the presence of 30 nM spiperone. These data are from one experiment, which was repeated eight times.

and 5-HT$_{2C}$ receptors are listed in Table 1. Except as noted, the affinities for human and rat tissue reported in Table 1 were based on the displacement of [^{3}H]ketanserin for the 5-HT$_{2A}$ receptors and on the displacement of [^{3}H]mesulergine for the 5-HT$_{2C}$ receptors. We then calculated Pearson product moment coefficients of correlation between the affinities of the various 5-HT$_2$ receptor antagonists for the human receptors and their affinities for the rat and rabbit receptors. There was a high degree of similarity between the affinity of these drugs for the human 5-HT$_{2C}$ receptor and their affinity for the rabbit ($r = 0.94$, $P < 0.001$) and rat ($r = 0.92$, $P < 0.001$) recep-

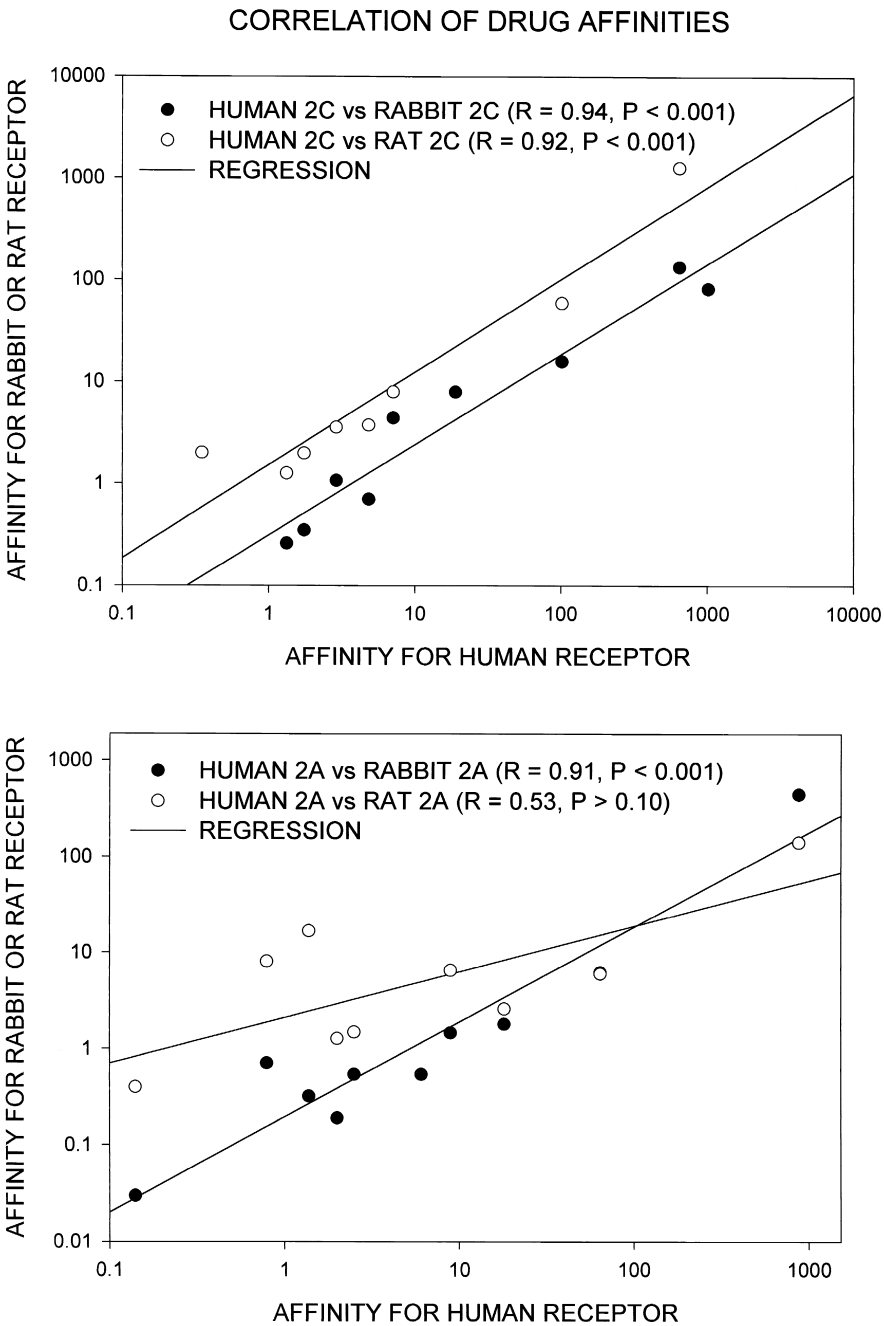

Fig. 2. The degree of association between the affinities of drugs for the human receptor and their affinities for the rabbit and rat receptors. Top panel: the affinities of drugs for the human 5-HT_{2C} receptor are plotted versus their affinities for rabbit and rat 5-HT_{2C} receptors. Affinities are expressed as the K_i or K_d for displacing specifically bound [^{3}H] mesulergine from the human, rabbit and rat 5-HT_{2C} receptors as presented in Table 1. Bottom panel: the affinities of drugs for the human 5-HT_{2A} receptor are plotted versus their affinities for the rabbit and rat 5-HT_{2A} receptors. Affinities for the rabbit are expressed as the K_i or K_d for displacing specifically bound [^{3}H]MDL 100,907 and affinities for the human and rat as the K_i or K_d for displacing specifically bound [^{3}H]ketanserin as presented in Table 1.

tors (Fig. 2, upper panel). There was also a highly significant correlation ($r = 0.91$, $P < 0.001$) between the affinity of drugs for the human and rabbit 5-HT_{2A} receptors (Fig. 2, lower panel). The correlation between the human and rat 5-HT_{2A} receptors, however, was not significant ($r = 0.53$, $P > 0.10$).

4. Discussion

4.1. Characterization of the rabbit's 5-HT_{2A} and 5-HT_{2C} receptors

The present study has provided the first data on the pharmacology of rabbit 5-HT_{2A} and 5-HT_{2C} receptors. In

agreement with previous studies in the rat (Johnson et al., 1996; López-Giménez et al., 1997a) and human (López-Giménez et al., 1997b), the highly selective 5-HT_{2A} receptor ligand [^{3}H]MDL 100,907 bound with very high affinity ($K_d = 0.03$ nM) to a single class of binding sites in cortical membranes from rabbits. Previous studies have demonstrated that [^{3}H]mesulergine (in the presence of a selective 5-HT_{2A} receptor ligand) labels the 5-HT_{2C} receptor in the rat (Pranzatelli et al., 1992). Also, we found that [^{3}H]mesulergine (in the presence of the selective 5-HT_{2A} receptor ligand spiperone) bound with high affinity ($K_d = 0.35$ nM) to a single class of binding sites in rabbit cortex. Displacement studies with various 5-$HT_{2A/2C}$ receptor ligands confirmed that [^{3}H]MDL 100,907 was labeling the 5-HT_{2A} receptor, and that [^{3}H]mesulergine was labeling the 5-HT_{2C} receptor. For example, as shown in Table 1, the selective 5-HT_{2A} receptor antagonists, spiperone and MDL 11,939 were potent inhibitors of [^{3}H]MDL 100,907 binding in rabbit cortex ($K_d = 0.19$ and 0.54 nM, respectively) but poor inhibitors of [^{3}H]mesulergine binding ($K_d = 134$ and 82 nM, respectively). In contrast, RS 102221, the highly selective 5-HT_{2C} receptor ligand, was a potent inhibitor of [^{3}H]mesulergine binding in rabbit cortex ($K_d = 1.07$ nM), but a weak inhibitor of [^{3}H]MDL 100,907 binding ($K_d = 445$ nM). In addition, a comparison of the ratio of the relative density of cortical 5-HT_{2A} and 5-HT_{2C} receptors in rabbits and humans reveals similarities. The results of our study demonstrate that the density of 5-HT_{2A} receptors in rabbit cortex ($B_{max} = 8.5 \pm 0.7$ fmol/mg of tissue) is twice that of 5-HT_{2C} receptors ($B_{max} = 3.70 \pm 0.58$ fmol/mg of tissue), which is similar to the ratio of the density of these two receptors in human cortex (Pranzatelli and Balletti, 1992). In contrast, in rat cortex, the density of 5-HT_{2C} receptors was only 20% of the density of 5-HT_{2A} receptors (Pranzatelli and Balletti, 1992).

4.2. Pharmacology of the 5-HT_{2A} and 5-HT_{2C} receptors in the rabbit is identical with the human

The high degree of correlation obtained between the binding affinities of various 5-HT_2 receptor ligands for the rabbit and human 5-HT_{2A} and 5-HT_{2C} receptors ($r = 0.91$ and 0.94, respectively) indicates that the rabbit provides an excellent animal model for predicting drug effects in the human. There have been no previous comparisons between the pharmacology of the rat and human 5-HT_{2C} receptor. However, the data we obtained from the literature indicated that the antagonist affinities at the rat and human 5-HT_{2C} receptor were highly correlated ($r = 0.92$). In contrast, there was no significant correlation ($r = 0.53$) between the binding affinities of these ligands for the rat and human 5-HT_{2A} receptor. This non-significant correlation is in agreement with previously reported differences between the pharmacology of the rat and human 5-HT_{2A} receptor (Kao et al., 1992; Nelson et al., 1993; Hagen et al., 1994; Johnson et al., 1994).

In summary, our data indicate that the pharmacological profiles of the rabbit 5-HT_{2A} and 5-HT_{2C} receptors are similar to those of the human receptors. This suggests that the rabbit provides an appropriate model for examining the pharmacology of drugs acting at these receptors.

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