SELECTIVE BINDING OF YM-09151-2, A NEW POTENT NEUROLEPTIC, TO D2-DOPAMINERGIC RECEPTORS

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Abstract—Effects of YM-09151-2 and five other neuroleptics (haloperidol, spiperone, chlorpromazine, sulpiride and clozapine) on the binding of [3H]-ligands to nine different receptors (α1-adrenergic, α2-adrenergic, β-adrenergic, muscarinic, D2-dopaminergic, H1-histaminergic, 5HT1-serotonergic, 5HT2-serotonergic and opiate receptors) and on dopamine-sensitive adenylate cyclase were determined using brain membranes in the rat, guinea-pig and dog. The affinity of YM-09151-2 for D2-receptors with a Kd value of 0.1 nM was more than 1000-times higher than that for the other receptors and dopamine-sensitive adenylate cyclase, and it was the greatest among the neuroleptics tested.

A close relationship between antidopaminergic and antischizophrenic effects of neuroleptics has been demonstrated by pharmacological, electrophysiological, histochemical and biochemical studies (1). Recent studies have also shown multiplicity of the dopaminergic receptors, referred to as D1 (adenylate cyclase-linked), D2 (non-adenylate cyclase-linked), D3 (non-adenylate cyclase-linked) and D4 (inversely adenylyl cyclase-linked) according to the terminology proposed by Seeman (1, 2), in the brain; and the antischizophrenic effect seems to be at least partly attributable to a blockade of a particular type (D2) of the dopaminergic receptors. However, usefulness of the drugs in the treatment of schizophrenic patients is often limited, partly due to insufficient specificity in the affinity for the D2-dopaminergic receptors, which occasionally results in undesirable side effects such as orthostatic hypotension and thirst. Lack of specificity of neuroleptics for the D2-receptor also makes it difficult to attribute their dopaminergic functions to the D2-receptor. Therefore, a highly D2-selective dopamine blocker is desirable for clinical use as well as for neurochemical studies. Sulpiride, a benzamide derivative seems to be a relatively specific blocker of the D2-receptor, but shown to hardly cross the blood brain barrier; whereas YM-09151-2, N-[(2RS, 3RS)-1-benzyl-2-methyl-3-pyrrolidinyl]-5-chloro-2-methoxy-4-methylaminobenzamide, which is also a benzamide substitute, can readily pass through the barrier and exhibits potent neuroleptic effects in animals (3, 4). The present paper describes the extremely high specificity of YM-09151-2 for the D2-receptor.

Materials and Methods

Materials: Dopamine hydrochloride, (−)-alprenolol bitartarate and (+)-propranolol hydrochloride were obtained from Sigma, St Louis, MO, U.S.A. [3H]Mepyramine (24.1 Ci/m mole) and [3H]prazosin (20.2 Ci/m mole) were from Amersham, England. [3H]Clonidine (23.8 Ci/m mole). [3H] (−)-dihydroalprenolol (31.5 Ci/m mole). [3H]-
naloxone (40.0 Ci/mmole), [3H]quinuclidinyl benzilate (33.1 Ci/mmole), [3H]serotonin (26.4 Ci/mmole) and [3H]spiperone (27.6 Ci/mmole) were purchased from New England Nuclear, Boston, MA, U.S.A. The following materials were also commercially obtained: atropine (Nakarai, Kyoto, Japan), chlorpromazine (Rhône-Poulenc, Paris, France) diphenhydramine (Kongo Chem., Toyama, Japan), haloperidol (Dainippon, Osaka, Japan), morphine (Sankyo, Tokyo, Japan), phentolamine (Takeda, Osaka, Japan), serotonin (Tokyo Kasei, Tokyo, Japan), spiperone (Eisai, Tokyo, Japan), sulpiride (Fujisawa, Osaka, Japan) and Whatman GF/B filters (Whatman, Kent, U.K.). We acknowledge generous gifts of the following compounds: cis-flupentixol (H. Lundbeck & Co., Copenhagen, Denmark), methysergid (Sandoz, Basel, Switzerland), mianserin (Organon, Oss, Netherlands) and prazosin (Pfizer, New York, NY, U.S.A.). YM-09151-2, clonidine and clozapine were prepared by Dr. S. Iwanami in our laboratories. YM-09151-2 was dissolved in 99.5% ethanol at the concentration of 2 mM and then diluted with 10% ethanol; the final ethanol concentration in the reaction mixture was kept below 0.5%. Other drugs hardly soluble in water were also dissolved similarly.

Membrane preparation: Male Wistar rats (7 to 9 weeks old) or Hartley guinea-pigs (300 to 400 g) were killed by decapitation. Brain without cerebellum, cerebral cortex, frontal cortex, striatum and cerebellum were quickly removed and chilled in ice-cold 0.85% NaCl. The tissues were homogenized in a motor-driven Teflon homogenizer in 9 volumes of 0.32 M sucrose. The homogenates were centrifuged at 900×g for 10 min and the precipitates were washed once. The combined supernatant fluids were further centrifuged at 12,000×g for 20 min. The pellets were washed once with 0.32 M sucrose and then twice with 5 mM Tris-HCl, pH 7.5. The washed pellets were suspended in the binding reaction buffer and frozen at −80°C. A hypotonic lysate (M1) of the crude mitochondrial fraction of canine caudate nucleus was prepared as described before (5).

Receptor binding assays: All of the binding assays described here were conducted according to known methods: [3H]Prazosin (0.4 nM) binding to α1-adrenergic receptors in the rat brain was determined by the method of Greengrass and Bremner (6); 1.0 nM [3H]clonidine binding to α2-adrenergic receptors in the rat cerebral cortex by the method of U'Prichard et al. (7); 0.06 nM [3H]dihydroalprenolol binding to β-adrenergic receptors in the rat cerebral cortex by the method of Bylund and Snyder (8); 0.06 nM [3H]quinuclidinyl benzilate binding to muscarinic cholinergic receptors in the rat brain by the method of Yamamura and Snyder (9); 0.4 nM [3H]spiperone binding to D2-dopaminergic receptors in the rat striatum in the presence of 0.3 μM mianserin, a 5-HT2 blocker (10), by the method of Leysen et al. (11); 0.5 nM [3H]-mepyramine binding to H1-histaminergic receptors in the guinea-pig cerebellum by the method of Tran et al. (12); 0.6 nM [3H]naloxone binding to opiate receptors in the rat frontal cortex by the method of Chang and Cuatrecases (13). Both 1.5 nM [3H]-serotonin binding to 5HT1-serotonergic receptors in the rat cerebral cortex and 0.4 nM [3H]spiperone binding to 5HT2-serotonergic receptors in the rat frontal cortex were determined by the method of Peroutka and Snyder (14). Specific binding was defined as the excess over blank in the presence of 6 μM prazosin for α1-receptors, 3 μM clonidine for α2-receptors, 10 μM (−)-alprenolol for β-receptors, 1 μM atropine for muscarinic receptors, 10 μM cis-flupentixol for D2-receptors, 10 μM diphenhydramine for H1-receptors, 10 μM levallorphan for opiate.
receptors, 10 μM serotonin for 5HT₁-receptors and 10 μM methysergid for 5HT₂-receptors. The IC₅₀ values, the concentrations required to inhibit specific binding by 50%, were computed by the logit-log analysis (15); and the inhibition constant (Kᵢ) was obtained according to the following equation:

\[
Kᵢ = \frac{IC₅₀}{1 + \frac{[3H]-ligand concentration}{Kᵦ of [3H]-ligand}}
\]

in which Kᵦ is the dissociation constant.

**Dopamine-sensitive adenylate cyclase:**
Dopamine-sensitive adenylate cyclase of the membranes from canine caudate nucleus was determined as described previously (5). The standard reaction mixture contained (in a final volume of 0.5 ml) 80 mM Tris-maleate buffer, pH 7.4; 8 mM MgSO₄; 2 mM ATP; 0.02% ascorbic acid; 0.6 mM EGTA; 0.5 mM 3-isobutyl-1-methylxanthine; 50 μM GTP and an appropriate amount of membranes with and without 20 nM dopamine. The reaction was carried out at 30°C for 6 min, and the amount of cyclic AMP was determined either by a radioimmunoassay kit (Yamasa, Choshi, Japan) or by the protein binding assay of Brown et al. (16) using Millipore filters (17).

**Protein amount:** Protein was determined by the method of Lowry et al. (18), using bovine serum albumin as a standard.

**Results**
When plotted in a logit-log scale as a function of drug concentration (−log concentration), the inhibitions of [³H]spiperone binding to rat striatal membranes (D₂-dopaminergic receptor) by YM-09151-2 and five other neuroleptics were linearly increased within the range of concentrations tested, as shown in Fig. 1. Of the neuroleptics tested, YM-09151-2 was the most potent in the inhibition of [³H]spiperone binding with an IC₅₀ of 0.28 nM, followed in order of potency by spiperone, haloperidol, chlorpromazine, sulpiride and clozapine. Similar inhibitions by the neuroleptics of [³H]spiperone binding to the canine caudate nucleus membranes were also obtained (Table 1). For an easy comparison of the neuroleptics in their affinity

![Fig. 1. Logit-log inhibition plot (Hill plot) of [³H]spiperone binding to the D₂-receptors in rat striatum membranes in the presence of 0.3 μM mianserin. B₀=total binding, Bᵢ=total binding in the presence of the drugs, Bᵢₐ=nonspecific binding in the presence of 10 μM cis-flupentixol. The abscissa indicates -log concentration of the drugs. Inhibition of the binding by YM-09151-2 (●—●), spiperone (○—○), haloperidol (▲—▲), chlorpromazine (△—△), sulpiride (■—■) and clozapine (□—□) were determined under the standard conditions. Slopes of the line give the Hill coefficient. Each point is the mean±standard error of six experiments.](image-url)
for the D<sub>2</sub>-receptors in different animal species, the K<sub>i</sub> values are presented in Table 1.

YM-09151–2 was studied for its inhibitory effects on dopamine-sensitive adenylate cyclase (D<sub>1</sub>-dopaminergic receptors) in the membranes from the rat striatum and canine caudate nucleus. As shown in Table 1, the K<sub>i</sub> value for the rat membrane was computed to be 0.7 μM, in agreement with the result of Jenner and Marsden (19). For the five different membrane preparations from mongrel dogs, the K<sub>i</sub> value of YM-09151–2 varied from 0.07 μM to 0.33 μM with a mean value of 0.2 μM. Thus, there was no substantial species difference in the inhibition of the cyclase by YM-09151–2 between the dog and the rat. Under the same experimental conditions, sulpiride was ineffective on the dopamine-stimulated activity of adenylate cyclase at 10<sup>-5</sup> M. YM-09151–2 and spiperone were equipotent, but 10-times less active than haloperidol in the inhibition of dopamine-sensitive adenylate cyclase in canine caudate nucleus. These results indicated that YM-09151–2 in contrast to other neuroleptics acted rather selectively on the D<sub>2</sub> type of the two dopaminergic receptors.

The inhibition of binding of other [3H]-ligands to their specific receptors was also studied by the same kinetic analysis as that for D<sub>2</sub>-receptor binding. The pK<sub>i</sub> values (−log K<sub>i</sub>) and the Hill coefficients (the slope of line in the logit-log plot) were calculated by a linear regression of the data and presented in Table 2. The receptors tested were α<sub>1</sub>-adrenergic, α<sub>2</sub>-adrenergic, β-adrenergic, muscarinic-cholinergic, H<sub>1</sub>-histaminergic, 5HT<sub>1</sub>-serotonergic, 5HT<sub>2</sub>-serotonergic and opiate receptors. Chlorpromazine, clozapine, spiperone and haloperidol were potent inhibitors of α<sub>1</sub>-adrenergic receptor binding with the K<sub>i</sub> values of 10 nM or below, whereas the affinity of YM-09151–2 for the α<sub>1</sub>-receptor (K<sub>i</sub>=0.2 μM) was only one-twentieth that of haloperidol. The affinity of YM-09151–2 and haloperidol for the 5HT<sub>2</sub>-receptor (K<sub>i</sub>=0.1 μM for both) was much smaller than that of chlorpromazine (K<sub>i</sub>=7.6 nM) and spiperone (K<sub>i</sub>=1.3 nM). Chlorpromazine and clozapine exhibited a high affinity with the K<sub>i</sub> values of lower than 40 nM for muscarinic and H<sub>1</sub>-receptors, whereas micromolar concentrations of YM-09151–2, haloperidol and spiperone were required for 50% inhibition in both receptor binding assays. Affinities of the neuroleptics with an exception of clozapine for α<sub>1</sub>-, β-, 5HT<sub>1</sub>- and opiate receptors were relatively low or negligibly small at such concentrations.

Table 1. Inhibition by neuroleptics of [3H]spiperone binding to and dopamine-sensitive adenylate cyclase in the membranes from canine caudate nucleus and rat striatum

|                | Canine caudate nucleus | Rat striatum | Canine caudate nucleus | Rat striatum |
|----------------|------------------------|--------------|------------------------|--------------|
| YM-09151–2    | 0.00024                | 0.00010      | 0.21                   | ND<sup>∗</sup> |
| Haloperidol   | 0.0020                 | 0.0021       | 0.021                  | ND<sup>∗</sup> |
| Chlorpromazine| 0.017                  | 0.010        | 0.065<sup>**</sup>     | ND<sup>∗</sup> |
| Sulpiride     | 0.22                   | 0.068        | No effect at 10<sup>-5</sup> M | No effect at 10<sup>-5</sup> M |
| Clozapine     | 0.42                   | 0.45         | 0.43<sup>**</sup>      | ND<sup>∗</sup> |
| Spiperone     | 0.00054                | 0.00014      | 0.17                   | ND<sup>∗</sup> |

<sup>∗</sup>: Not determined.  **: Data from Sano et al. (5). The value indicates K<sub>i</sub> in a micromolar concentration. K<sub>i</sub> values were obtained from IC<sub>50</sub> as described in the text using at least four different concentrations of the drugs.
as to block D2-receptors. Clozapine blocked $a_1$, $a_2$, muscarinic-cholinergic, $H_1$-histaminergic and 5HT2-serotonergic more potently than D2-dopaminergic receptors.

**Discussion**

The present experiments demonstrate that YM-09151–2 has a highly selective affinity for D2-dopaminergic receptors with a $K_i$ of 0.1 nM, which is rather similar to that of spiperone. The affinity of YM-09151–2 for other receptors was by at least three orders of magnitude smaller than that for the D2-receptor. This selective binding was consistent with the pharmacological data such as antagonism against phenylephrine-induced hypertension, histamine-induced contraction of the ileum, acetylcholine-induced contraction of the ileum or serotonin-induced head twitch (manuscript in preparation). Sulpiride which is a benzamide derivative like YM-09151–2 also exhibits higher affinity for the D2-receptor, with a $K_i$ of 68 nM, than for any other receptors tested. However, it must be mentioned that the affinities of YM-09151–2 and sulpiride for D3-receptors were 0.98 $\mu$M and 1.2 $\mu$M, respectively, in our previous report (3) (in reference 3, the term "D2" was used instead of D3). Thus, the selectivity of YM-09151–2 between the D2- and D3-receptors is far greater than that of sulpiride. The other

Table 2. Comparison in the ability of YM-09151–2 and other neuroleptics to bind to nine different types of receptors

| $[^{3}H]$-ligand       | YM-09151–2 | Haloperidol | Chlorpromazine | Sulpiride | Clozapine | Spiperone |
|------------------------|------------|-------------|----------------|-----------|-----------|-----------|
| $[^{3}H]$ Spiperone ($D_2$) | 10.0±0.03  | 8.68±0.08   | 7.99±0.07      | 7.17±0.09 | 6.35±0.07 | 9.85±0.06 |
|                        | (0.90)     | (0.83)      | (1.16)         | (0.82)    | (1.01)    | (0.76)    |
| $[^{3}H]$ Prazosin ($a_1$) | 6.61±0.05  | 7.85±0.05   | 9.36±0.05      | No effect* | 8.40±0.06 | 8.21±0.05 |
|                        | (1.10)     | (1.02)      | (0.81)         |           | (0.87)    | (0.88)    |
| $[^{3}H]$ Clonidine ($a_2$) | 5.45±0.10  | <5          | 5.82±0.03      | No effect* | 6.56±0.03 | <5        |
|                        | (1.53)     |             | (0.88)         |           | (0.84)    |           |
| $[^{3}H]$ DHA ($\beta$) | 5.67±0.05  | <5          | No effect*     | No effect*|           |           |
|                        | (0.82)     |             |                |           |           |           |
| $[^{3}H]$ QNB (muscarinic) | 5.90±0.04  | 6.09±0.04   | 7.26±0.09      | No effect*| 8.04±0.08 | 5.80±0.09 |
|                        | (0.99)     | (1.05)      | (1.32)         |           | (1.10)    | (1.29)    |
| $[^{3}H]$ Mepyramine ($H_1$) | 5.67±0.06  | 6.56±0.02   | 8.75±0.05      | No effect*| 8.76±0.10 | 5.93±0.09 |
|                        | (1.06)     | (1.08)      | (1.25)         |           | (1.52)    | (1.09)    |
| $[^{3}H]$ Naloxone (opioida) | 8.03±0.09  | 6.27±0.07   | 5.25±0.04      | No effect*| No effect*| 6.90±0.07 |
|                        | (0.90)     | (0.95)      | (1.49)         |           |           | (0.85)    |
| $[^{3}H]$ Serotonin (5HT_1) | 5.91±0.12  | <5          | <5             | No effect*| 5.75±0.08 | <5        |
|                        | (0.42)     |             |                |           | (0.80)    |           |
| $[^{3}H]$ Spiperone (5HT_2) | 6.99±0.05  | 7.02±0.07   | 8.12±0.05      | No effect*| 7.58±0.06 | 8.89±0.08 |
|                        | (0.91)     | (0.76)      | (0.98)         |           | (1.09)    | (1.52)    |

*: Less than 15% inhibition at $10^{-5}$ M. DHA: Dihydroalprenolol. QNB: Quinuclidinyl benzilate. The values represent -log $K_i$ and its confidence limits at $P=0.05$. Figures in parenthesis show the Hill coefficient. At least four different concentrations of the drugs were used to determine the inhibition of $[^{3}H]$-ligand binding. Under the standard binding conditions, -log $K_i$ values of representative positive control drugs for their specific receptors were: 8.23±0.04 for phenolamine in $[^{3}H]$prazosin binding, 8.12±0.06 for phenolamine in $[^{3}H]$clonidine binding, 8.82±0.07 for (±)propranolol in $[^{3}H]$-diydroalprenolol binding, 9.34±0.06 for atropine in $[^{3}H]$quinuclidinyl benzilate binding, 8.04±0.07 for diphenhydramine in $[^{3}H]$mepyramine binding, 9.03±0.12 for morphine in $[^{3}H]$naloxone binding, 6.92±0.08 for methysergid in $[^{3}H]$serotonin binding and 8.02±0.05 for mianserin in $[^{3}H]$spiperone binding (5HT2).
neuroleptics are not so selective in the binding to D1-, D2-, and D3-receptors as YM-09151-2 and also considerably block a1-adrenergic, muscarinic, H1-histaminergic or 5HT2-serotonergic receptors at such concentrations as to block the D2-receptor. In general, YM-09151-2 resembles spiperone in the receptor binding, except that the former has much less affinity for the a1-adrenergic receptor. YM-09151-2 also resembles sulpiride, except for the selectivity between D2- and D3-receptors. Thus, it is of much interest to know what differences are obtained in the clinical efficacy between YM-09151-2 and the other neuroleptics. Since the antischizophrenic effect of neuroleptics is generally correlated to a blockade of D2-dopaminergic receptors, a potent D2-selective blocker, YM-09151-2, may be clinically effective with less side effects resulting from the blockade of other receptors and may be useful for a better understanding of the mechanism for dopamine actions.

There is a discrepancy between the present and previous data (3) with respect to the Kd values of YM-09151-2 for dopamine-sensitive adenylate cyclase in the canine caudate nucleus membranes. The affinity of the cyclase for YM-09151-2 in the present experiment is evidently lower than the previous value. There are some possible reasons for this difference. The cyclic AMP assay method using the crude binding protein and charcoal may be partly responsible in the particular YM-09151-2 inhibition experiment, as generally discussed by Oka and Kaneko (20). By this method, the reproducible inhibition by YM-09151-2 has been found to be occasionally difficult. Because of this difficulty, we now use the membrane filtration technique for separation of bound from free cyclic AMP instead of charcoal, as demonstrated in the present experiment. Other responsible factors may be ages of the mongrel dogs and the season in which the animal was sacrificed.

In summary, YM-09151-2 is the most selective and potent blocker of the D2-dopaminergic receptor.

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