Comparison of Effects of Tiapride and Sulpiride on D-1, D-2, D-3 and D-4 Subtypes of Dopamine Receptors in Rat Striatal and Bovine Caudate Nucleus Membranes

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Abstract—To determine the affinity of tiapride to D-1, D-2, D-3 and D-4 subtypes of dopamine (DA) receptors, inhibitory effects of tiapride on [3H]-cis-flupenthixol, [3H]spiperone and [3H]N-propylapomorphine binding were examined in the rat striatum and bovine caudate nucleus membranes and compared to those of sulpiride and haloperidol. The IC50 values of tiapride, sulpiride and haloperidol were estimated as follows: 1440, 132 and 0.295 µM for D-1; 45.8, 8.8 and 0.004 µM for D-2; greater than 100, greater than 100 and 0.64 µM for D-3; 11.7, 2.88 and 0.0044 µM for D-4, respectively. It is suggested that the affinity of tiapride is high to D-2 and D-4, but is not high to D-1 and D-3. The affinity pattern of tiapride to each DA receptor subtype is similar to but lower than those of sulpiride and haloperidol. In the D-2 receptor assay, the IC50 values of tiapride and sulpiride were 1/22.7 and 1/19.1 of those in the presence of 100 mM NaCl, respectively, suggesting that benzamide drug binds to the D-2 subtype with higher affinity in the presence of Na+ than in the control.

Tiapride (N-[2-(diethylamino)ethyl]-5-(methylsulfonyl)-o-anisamide) has been demonstrated as a dopamine (DA) receptor antagonist which selectively suppresses dyskinesia (1). DA receptors are classified as D-1, D-2, D-3 and D-4 subtypes (2). D-1 and D-2 subtypes cause a stimulation and inhibition in adenylate cyclase activity, respectively (3, 4). D-3 receptors seem to be presynaptically located to regulate DA release (2). In addition, it is also suggested that D-4 subtypes may be an interconvertible state of D-2 subtypes (5), although the details of the functional significances cannot be clarified.

To identify the affinity of tiapride to D-1, D-2, D-3 and D-4 subtypes as well as those of sulpiride and haloperidol, the inhibitory effects of each drug on [3H]cis-flupenthixol (FPT), [3H]spiperone and [3H]N-propylapomorphine (NPA) were examined in the membranes of rat striatum and bovine caudate nucleus.

Male adult rats of the Wistar strain were used for D-1 and D-2 receptor binding assay. Since specific binding of [3H]NPA to rat striatal membranes was too low in both D-3 and D-4 receptor assays of preliminary experiments, bovine brain obtained from a local slaughterhouse was used for D-3 and D-4 receptor assay. The striatum from rats or caudate nucleus from bovine brain were rapidly dissected and homogenized in 50 vol. of ice-cold 50 mM Tris-HCl buffer (pH 7.7) using a Polytron® (setting at No. 6 for 30 sec) and then centrifuged at 50,000 × g for 15 min, followed twice by resuspension in fresh buffer and centrifugation.

D-1 DA receptor assay was carried out by
the modified method of Murrin (6). The pellets from rat striatum were resuspended in assay buffer (50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂ and 1 mM MgCl₂, pH 7.4). Aliquots (0.8 ml) of the suspension (0.13–0.2 mg/ml), 0.1 ml of drug or buffer and 0.1 ml of [³H] FPT (final concentration, 1.0 nM) were incubated at 37 °C for 5 min. Whatman GF/C filters were soaked in the assay buffer containing Tween 80 (polyoxyethylene sorbitan monooleate) at room temperature for at least 15 min prior to use. The incubation was terminated by rapid vacuum filtration over treated GF/C filters followed by four 5 ml rinses with ice-cold assay buffer. The radioactivity on the filters was assayed by liquid scintillation counting. Blank values were generated by inclusion of 20 μM (+)-butaclamol. Specific binding was 60.0% of the total binding.

D-2 DA receptor assay was carried out by the modified method of Usdin et al. (7). The rat striatal membrane pellets described above were resuspended in 50 mM Tris-HCl buffer with 0.1% ascorbic acid (pH 7.1). The membrane suspension (0.3–0.4 mg/tube) was incubated with 0.4–0.5 nM [³H] spiperone, 0.1 μM ketanserin and test drug at 37 °C for 15 min. Membranes were recovered by rapid filtration (Whatman GF-B filters) under vacuum with three 5 ml rinses of ice-cold buffer. Specific binding was measured as the excess over blanks taken in the presence of 1 μM (+)-butaclamol. Specific binding was estimated as 69.0% of the total binding.

D-3 and D-4 DA receptor assays were performed by the modified method of Seeman (2). Membrane pellets from bovine caudate nucleus were resuspended in 15 mM Tris-HCl buffer (pH 7.4) containing 5 mM EDTA-Na₂, 0.02% ascorbic acid and 12.5 nM nialamide. In the D-3 receptor assay, membrane suspension (0.3–0.4 mg protein/tube) containing 0.1 μM spiperone was incubated with 0.29 nM [³H] NPA and each test drug at 22 °C for 30 min. To assay D-4 receptors, membrane suspension (0.3–0.4 mg protein/tube) containing 25 nM DA was incubated with 0.29 nM [³H] NPA and each test drug at 22 °C for 30 min. Specific bindings of D-3 and D-4 receptor assays were defined as the binding displaceable by 25 nM DA and by 0.1 μM spiperone, respectively. The specific bindings of D-3 and D-4 were estimated as 57.0 and 71.0%, respectively.

Protein content was determined by the method of Lowry et al. (8). Statistical significances were analyzed by Student's t-test.

[³H] cis-Flupenthixol (10.4 Ci/mmol, New England Nuclear), [³H] spiperone (16.0 Ci/mmol, Amersham) and [³H] N-propylapomorphine (53.1 Ci/mmol, New England Nuclear) were used. Tiapride hydrochloride, sulpiride and haloperidol were purchased from Fujisawa Pharmaceutical Co., Ltd.; ketanserin from Kyowa Hakko Kogyo Co., Ltd.; spiperone from Eisai Pharmaceutical Co., Ltd.; (+)-butaclamol hydrochloride from Ayerst Res. Lab. (Canada); dopamine hydrochloride from Nakarai Chemicals Co., Ltd. Sulpiride was dissolved in 0.1 N HCl and diluted with buffer. Haloperidol and ketanserin were dissolved in ethylalcohol, followed by dilution with buffer. Ethylalcohol did not affect the binding. Tiapride and dopamine were dissolved in buffer. Preliminary experiments showed that neither 0.1% (D-2 assay) nor 0.02% (D-3 and D-4 assay) ascorbic acid significantly affected the binding.

Effects of tiapride, sulpiride, haloperidol and DA on D-1 sites were investigated by examining effects of each drug on [³H] FPT binding to rat striatal membranes. From concentration-inhibition curves, the IC₅₀ values of tiapride, sulpiride, haloperidol and DA were 1440±370, 132±44, 0.295±0.052 and 2.53±0.37 μM, respectively (Table 1). To avoid [³H] spiperone binding to S-2 serotonin binding sites, [³H] spiperone binding to D-2 sites in rat striatal membranes was carried out in the presence of 0.1 μM ketanserin. As shown in Table 1, the IC₅₀ of tiapride, sulpiride, haloperidol and DA were estimated as 45.8±4.3, 8.8±1.24, 0.004±0.0006 and 1.41±0.32 μM, respectively. In the presence of 100 mM NaCl, inhibitory effects of tiapride and sulpiride were enhanced, and the IC₅₀ values of these drugs become 1/22.7 and 1/19.1, respectively. Previously reported results (9) showed that the addition of 0.1 μM unlabeled spiperone into the binding medium was enough to avoid [³H] NPA binding to
D-4 binding sites. Inhibitory effects of test drugs on [3H]NPA binding were examined in the presence of 0.1 μM spiperone, and the IC50 values are shown in Table 1. In comparison to the potent inhibitory action of DA (IC50=0.002±0.0009 μM) and to the relatively potent action of haloperidol (IC50=0.640±0.050 μM), neither tiapride nor sulpiride caused 50% inhibition at 100 μM. Inhibitory effects of test drugs on [3H]NPA binding to D-4 sites of bovine caudate nucleus membranes were determined in the presence of 25 nM unlabeled DA to exclude [3H]NPA binding to D-3 sites as described in the previous report (9). The IC50 values of tiapride, sulpiride, haloperidol and DA were estimated as 11.7±4.1 μM, 2.88±1.10 μM, 0.0044±0.0014 μM and 0.798±0.272 μM, respectively (Table 1).

Table 1. The IC50 values of tiapride, sulpiride, haloperidol and dopamine on D-1, D-2, D-3 and D-4 sites

| Drug       | D-1       | D-2       | D-3       | D-4       |
|------------|-----------|-----------|-----------|-----------|
| Tiapride   | 1,440±370 | 45.8±4.3  | >100.0    | 11.7±4.1  |
| Sulpiride  | 132±44    | 8.80±1.24 | >100.0    | 2.88±1.10 |
| Haloperidol| 0.295±0.052| 0.004±0.0006| 0.640±0.050| 0.0044±0.0014|
| Dopamine   | 2.53±0.37 | 1.41±0.32 | 0.002±0.0009| 0.798±0.272|

The IC50 values were estimated by concentration-inhibition curves of tiapride, sulpiride, haloperidol and dopamine in D-1, D-2, D-3 and D-4 binding experiments. Each value shows the mean±S.E. of at least three independent experiments.

A recent paper reported that D-1 sites are subdivided into two types of sites: one type is D-1s (low affinity sites for agonist) and the other is D-1a (high affinity sites for agonist) (10). D-1s and D-1a may correspond to D-1 and D-3 sites, respectively, and D-1a may convert into D-1s by GTP. At the moment, however, it is difficult to identify both sites (11). Regarding the relationship between D-2 and D-4 sites classified by Seeman (2), it is consistent that D-2 and D-4 sites are the same sites but different affinity sites: D-2s and D-2a for agonists probably correspond to D-2 and D-4 sites, respectively. (12, 13).

Although there are several problems in the classification of DA receptor subtypes, effects of test drugs on D-1, D-2, D-3 and D-4 sites were examined by the modified method of Seeman (2). Based on the very close relationship between inhibitory effects of several neuroleptics on [3H]FPT binding and on adenylate cyclase (14, 15), [3H]FPT binding seems to bind to D-1 sites. Although FPT binds to D-2 DA sites with higher affinity than to D-1 sites (6, 16), the maximum number of D-1 binding sites is much higher (over 2,000 fmol/mg protein) than that of D-2 sites (334 fmol/mg protein) in the rat striatum (2). In fact, several authors have suggested that 80% of [3H]FPT binding seems to be D-1 sites (10, 15, 17). Furthermore, the present assay was carried out by the methods reducing [3H]FPT binding to D-2 sites suggested by Murrin as follows: 1) filtration was done using GF/C instead of GF/B, 2) filters were pretreated with Tween 80. Therefore, almost [3H]FPT binding in the present experiments could be to D-1 sites. D-2 sites were assayed using [3H]spiperone binding in the presence of 0.1 μM unlabeled ketanserin to avoid S-2 serotonin binding sites. To separate [3H]NPA binding to D-3 and D-4 sites, the addition of 0.1 μM spiperone for D-3 sites and 25 nM DA were adopted (9). The results clearly showed that DA binds to D-3 with high affinity and D-4 with low affinity (Table 1), suggesting that each subtype could be reasonably assayed by the present binding methods.

The results show that tiapride binds to D-2 and D-4 subtypes with high affinity and D-1 and D-3 subtypes with low affinity. Selectivity of tiapride regarding each subtype
seems to be similar to sulpiride and haloperidol. The affinity of tiapride to each subtype is markedly low, and so is that of sulpiride, compared to that of haloperidol. The addition of 100 mM NaCl resulted in an approximately 20-fold decrease in the IC50 values of tiapride and sulpiride. Increasing effects of Na+ on the affinity of D-2 sites have been observed to be great in sulpiride (18-20), but slight in spiperone (7). It is of interest to clarify how Na+ enhances the affinity of benzamide to D-2 sites. Tiapride inhibited [3H]FPT binding at high concentration (IC50=1.44 mM), suggesting substantially no inhibitory effects of tiapride on D-1 sites. D-3 sites cannot be inhibited by tiapride either. Thus, neither stimulatory effects of DA on adenylate cyclase nor effects on autoreceptors could be inhibited by tiapride. On the other hand, tiapride showed high affinity to D-2 and D-4 sites as well as sulpiride. Since D-4 sites may be agonist high affinity states (5), tiapride has relatively high affinity to D-4 sites.

Although behavioral studies show that tiapride has more inhibitory effects on apomorphine-induced stereotypy than sulpiride (21), the present results show lower affinity of tiapride to D-2 as well as other sites than sulpiride. The mechanism underlying pharmacologically different effects of tiapride and sulpiride remains to be clarified.

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