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Pharmacological profile of neuroleptics at human monoamine transporters

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Abstract

Using radioligand binding techniques, we determined the equilibrium dissociation constants (K_D) for 37 neuroleptics and one metabolite of a neuroleptic (haloperidol metabolite) for the human serotonin, norepinephrine, and dopamine transporters with [^{3}H]imipramine, [^{3}H]nisoxetine, and [^{3}H]WIN35428, respectively. Among neuroleptics, the four most potent compounds at the human serotonin transporter were triflupromazine, fluperlapine, chlorpromazine, and ziprasidone (K_D 24–39 nM); and at the norepinephrine transporter, chlorpromazine, zotepine, chlorprothixene, and promazine (K_D 19–25 nM). At the human dopamine transporter, only pimozide ($K_D = 69 \pm 3$) ziprasidone ($K_D = 76 \pm 5$) had notable potency. These data may be useful in predicting therapeutic and adverse effects, including drug interactions of neuroleptics.

Keywords: 5-HT (5-hydroxy-hyptamine, serotonin), human; Norepinephrine transporter, human; Dopamine transporter, human; Neuroleptic; Radioligand binding assay

1. Introduction

Many different types of neurotransmitter receptors at synapses in brain and elsewhere in the body are targets for blockade by neuroleptics. Some of these receptor blocking effects may relate to the therapeutic and adverse effects of these antipsychotic drugs. Less well known is that some neuroleptics have effects on another target in some synapses, namely, re-uptake or transport sites for serotonin, norepinephrine, and dopamine (Haggendal and Hamberger, 1967; Richelson and Pfenning, 1984). Re-uptake is a process that prevents overstimulation of synaptic receptors, and occurs through the action of unique transporter proteins that have been molecularly cloned from several species, including human (Pacholczyk et al., 1991; Ramamoorthy et al., 1993; Pristupa et al., 1994; Eshleman et al., 1995). Although the exact mechanism of action of neuroleptics remains uncertain, neuroleptics may also exert some of their therapeutic and adverse effects in the body by blocking human transporters for serotonin, norepinephrine, or dopamine.

We (Richelson and Pfenning, 1984; Bolden-Watson and Richelson, 1993) and many others have obtained data for the inhibitory potency of antidepressants at blocking re-uptake into rat brain synaptosomal preparations. However, with numerous examples in the literature of species differences for binding of compounds to molecularly cloned proteins, including the norepinephrine (Tatsumi et al., 1997) and serotonin (Barker et al., 1994; Barker and Blakely, 1996) transporters, we were interested in determining the binding potencies of neuroleptics and related compounds for the human serotonin, norepinephrine, and dopamine transporters. Here we report the K_D values for a large series of neuroleptics for binding to these transporters. We recently published a similar study for antidepressants (Tatsumi et al., 1997).

2. Materials and methods

2.1. Materials

The following drugs were generously provided by the manufacturers or other sources as indicated: bromperidol, carpipramine, clocapramine, moperone and perazine from Yoshitomi (Osaka, Japan); chlorpromazine, prochlorpera-

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zine, trifluoperazine from Smith Kline and French Laboratories (Philadelphia, PA); chlorprothixene from Hoffman-LaRoche (Nutley, NJ); *cis*-thiothixene from Pfizer (New York, NY); fluperlapine from Sandoz (Basel, Switzerland); fluphenazine, triflupromazine from E.R. Squibb and Sons, (Princeton, NJ); haloperidol, haloperidol metabolite, risperidone from Research Diagnostics (Flanders, NJ); loxapine from Lederle Laboratories (New York, NY); melperone from National Institute of Mental Health (Rockville, MD); mesoridazine from Boehringer Ingelheim (Ridgefield, CT); molindone from Dupont Pharmaceuticals (Wilmington, DE); olanzapine from Eli Lilly (Indianapolis, IN); promazine from Wyeth Laboratories (Philadelphia, PA); quetiapine from Zeneca Pharmaceuticals (Wilmington, DE); sertindole from Abbott Laboratories (North Chicago, IL); sultopride from Mitsui (Tokyo, Japan); tiospirone from Dr. H.Y. Meltzer (Psychiatric Hospital at Vanderbilt, Nashville, TN); timiperone from Daiichi (Tokyo, Japan); ziprasidone HCl from Pfizer Central Research (Groton, CT); zotepine from Fujisawa (Osaka, Japan). All other biochemicals were purchased from either Sigma (St. Louis, MO) or Research Biochemicals International (Natick, MA). [^{3}H]imipramine (imipramine hydrochloride, [benzene ring-^{3}H, specific activity 46.5 Ci/mmol) and [^{3}H]WIN35428 (WIN35428, [*N*-methyl-^{3}H], specific activity 83.5 Ci/mmol were from Dupont New England Nuclear (Boston, MA); [^{3}H]nisoxetine (nisoxetine HCl, [*N*-methyl-^{3}H], specific activity 85.0 Ci/mmol) from Amersham (Arlington Hts., IL). The human serotonin transporter cDNA and the cell line of the human norepinephrine transporter were provided to us by co-author Randy D. Blakely, PhD. The human dopamine transporter cDNA was provided to us by Zdenek B. Pristupa, PhD and H.B. Niznik, PhD (University of Toronto, Toronto, Canada).

2.2. Methods

2.2.1. Expression of the human transporters

For this study, we directionally ligated the human serotonin transporter cDNA into the expression vector pRc/CMV and transfected it into HEK293 (human embryonic kidney) cells by the calcium phosphate method (Chen and Okayama, 1987). For the expression of the human dopamine transporter cDNA, we directionally ligated the human dopamine transporter cDNA into the expression vector pcDNA3 and transfected it into HEK293 cells, also by the calcium phosphate method. All cell lines used were stably transfected.

2.2.2. Cell culture

Our cell lines were grown, passaged, and harvested in 150-mm petri dishes with 17.5 ml of Dulbecco's Modified Eagle's Medium (Mediatech, Herndon, VA) containing 0.1 mM Non-Essential Amino Acid Solution For MEM (Mediatech), 5% (v/v) Fetal CloneBovine serum product (Hyclone Laboratories, Logan, UT), and 1 U/μl Penicillin and Streptomycin Solution (Mediatech). They were incubated in 10% CO_2, 90% air at 37°C and 100% humidity. The selecting antibiotic geneticin sulfate (250 μg/ml) was used continuously for cell culture of cells expressing the human norepinephrine transporter.

2.2.3. Membranal preparations

For the preparation of the homogenates, medium was removed by aspiration. The cells were washed with 4 ml modified Puck's D1 solution (solution 1) (Pfenning and Richelson, 1990) and the cells were then incubated for 5 min at 37°C in 10 ml solution 1 and 100 mM ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetra-acetic acid (EGTA). Afterwards, cells were removed from the surface by scraping with a rubber spatula, placed in a centrifuge tube, and collected by centrifugation at $110 \times g$ for 5 min at 4°C. The supernatants were decanted. The pellets were resuspended in each binding assay buffer by use of a Polytron (Brinkmann Instruments, Westbury, NY) for 10 s at setting 6. The mixture was then centrifuged at $35\,600 \times g$ for 10 min at 4°C. The pellets were suspended in the same volume of the respective buffer and the centrifugation was repeated. The supernatants were decanted and the final pellets were suspended in the respective buffer and stored at −80°C until assayed. The final protein concentration was determined by Lowry assay (Lowry et al., 1951), using bovine serum albumin as a standard.

2.2.4. Radioligand binding assays

2.2.4.1. Experimental design. All radioligand binding assays were run at 11 different concentrations of competitor (drug) in duplicate. Each binding assay included a curve for competition with the radioligand by its non-radioactive form, also at 11 different concentrations in duplicate. Thus, each assay generated a value for the equilibrium dissociation constant (K_D) of the radioligand, the means of which is presented. For all drugs K_D values (which are theoretically equivalent to K_i values) were averaged from at least three independent experiments.

2.2.4.2. [^{3}H]imipramine binding to human serotonin transporter. Radioligand binding assays were performed by a modification of the method of O'Riordan et al. (1990). Binding buffer contained 50 mM Tris, 120 mM NaCl, and 5 mM KCl (pH 7.4). Compounds to be tested were dissolved in 5 mM HCl (Bylund and Yamamura, 1990) and run in duplicate over at least 11 different concentrations against 1 nM [^{3}H]imipramine with 15 μg/tube membranal protein for 30 min at 22°C. Non-specific binding was determined in the presence of 1 μM imipramine. With the use of a 48-well Brandel cell harvester (Gaithersburg, MD), we terminated the assay by rapid filtration through a GF/B filter strip that had been pre-soaked with 0.2% polyethylenimine. The filter strips were rinsed five times

with ice-cold 0.9% NaCl. Finally, each filter was placed in a scintillation vial containing 6.5 ml of Redi-Safe (Beckman Instruments, Fullerton, CA) and counted in a Beckman liquid scintillation counter (LS 5000TD).

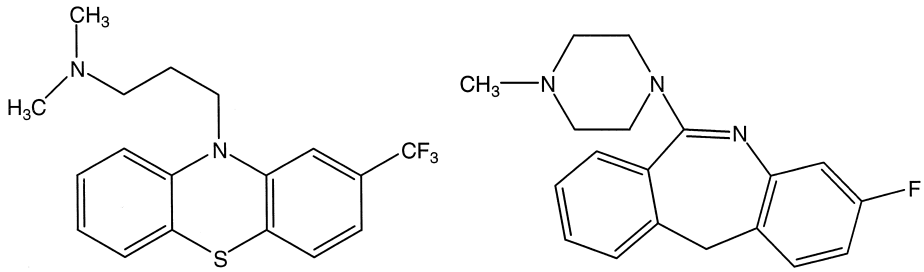
2.2.4.3. [^{3}H]nisoxetine binding to human norepinephrine transporter. Radioligand binding assays were performed by a modification of the method of Jayanthi et al. (1993). Binding buffer contained 50 mM Tris, 300 mM NaCl, and 5 mM KCl (pH 7.4). [^{3}H]nisoxetine at 0.5 nM was incubated with competing drugs and 25 μg/tube membranal protein for 60 min at 22°C. Non-specific binding was determined in the presence of 1 μM nisoxetine. The remainder of the assay was exactly as described in Section 2.2.4.1.

2.2.4.4. [^{3}H]WIN35428 binding to human dopamine transporter. Radioligand binding assays were performed by a modification of the method of Pristupa et al. (1994). This modification included the use of a binding buffer containing 50 mM Tris and 120 mM NaCl (pH 7.4) (Madras et al., 1989). [^{3}H]WIN35428 at 1 nM was incubated with

Table 1
Neuroleptics: equilibrium dissociation constants (K_D) for the human serotonin, norepinephrine, and dopamine transporters

Compound	Geometric mean of $K_D \pm$ S.E.M. (nM)		
	Serotonin transporter	Norepinephrine transporter	Dopamine transporter
Bromperidol	2200 ± 100	8600 ± 400	1660 ± 60
Carpipramine	1370 ± 30	5100 ± 200	1300 ± 100
Chlorpromazine	30 ± 2	**19 ± 2**	1750 ± 40
Chlorprothixene	110 ± 10	21 ± 1	1690 ± 50
cis-Thiothixene	3190 ± 40	30 000 ± 2000	3600 ± 100
Clocapramine	560 ± 10	3200 ± 200	370 ± 30
Clozapine	1330 ± 50	2700 ± 300	> 10 000
Fluperlapine	30 ± 4	128 ± 3	> 100 000
Fluphenazine	400 ± 30	4600 ± 100	1690 ± 20
Haloperidol	3000 ± 100	6500 ± 300	2400 ± 70
Haloperidol meta2	6300 ± 300	> 10 000	> 10 000
Levomepromazine	2800 ± 100	> 10 000	4100 ± 200
Loxapine	2400 ± 100	380 ± 30	9000 ± 200
Melperone	> 10 000	> 10 000	> 10 000
Mesoridazine	> 100 000	> 10 000	> 10 000
Molindone	> 100 000	> 100 000	> 100 000
Moperone	2680 ± 80	7690 ± 60	> 10 000
Olanzapine	1310 ± 40	4500 ± 100	> 10 000
Oxypertine	> 10 000	4000 ± 200	630 ± 320
Perazine	3200 ± 100	1540 ± 80	5800 ± 100
Perphenazine	610 ± 30	740 ± 20	1290 ± 30
Pimozide	74 ± 8	370 ± 10	**69 ± 3**
Prochlorperazine	940 ± 30	510 ± 10	590 ± 20
Promazine	190 ± 20	25 ± 2	8400 ± 100
Quetiapine	> 10 000	> 10 000	> 10 000
Risperidone	> 10 000	> 10 000	> 10 000
Sertindole	1200 ± 200	640 ± 40	200 ± 20
Spiperone	7500 ± 300	> 10 000	4200 ± 300
Sulpiride	> 100 000	> 100 000	> 10 000
Sultopride	> 100 000	> 100 000	> 10 000
Thioridazine	650 ± 30	1620 ± 70	1130 ± 30
Tiapride	> 100 000	> 100 000	> 10 000
Timiperone	> 100 000	> 10 000	5400 ± 300
Tiospirone	2200 ± 200	1900 ± 300	460 ± 20
Trifluoperazine	810 ± 10	3000 ± 100	570 ± 50
Triflupromazine	**24 ± 1**	110 ± 4	1830 ± 40
Ziprasidone	39 ± 3	59 ± 7	76 ± 5
Zotepine	45 ± 3	20 ± 3	2350 ± 80
Some Reference compounds[a]			
Paroxetine	0.13 ± 0.01	–	–
Desipramine	–	0.83 ± 0.05	–
Mazindol	–	–	8.1 ± 0.4
Cocaine	–	–	220 ± 9

The most potent neuroleptic in each category is in bold.
[a] Data from Tatsumi et al. (1997).



Trifluprazine Fluperlapine

Fig. 1. Structures of the two most potent neuroleptics at the human serotonin transporter.

competing drugs and 30 μg/tube membranal protein for 120 min at 4°C. Non-specific binding was determined in the presence of 10 μM WIN35428. The remainder of the assay was exactly as described in Section 2.2.4.1.

2.2.5. Analysis of data

We analyzed the data by using the LIGAND program (Munson and Rodbard, 1980) to provide the K_D values. The program has been modified by us to calculate the Hill coefficients (n_H). Data are presented as geometric mean ± S.E.M. (Fleming et al., 1972; De Lean et al., 1982) of at least three independent experiments. One- and two-component models were compared using the root mean square error of each fit and the *F*-test.

3. Results

The data are summarized in Table 1. The K_D values for neuroleptics and related compounds in this study were derived from competition experiments with varying concentrations of the compounds and fixed concentrations of the respective radioligands. Hill coefficients (n_H) for all of the compounds at each binding site were close to unity (data not shown), suggesting that the binding of the drugs in the radioligand binding assays obeyed the law of mass action.

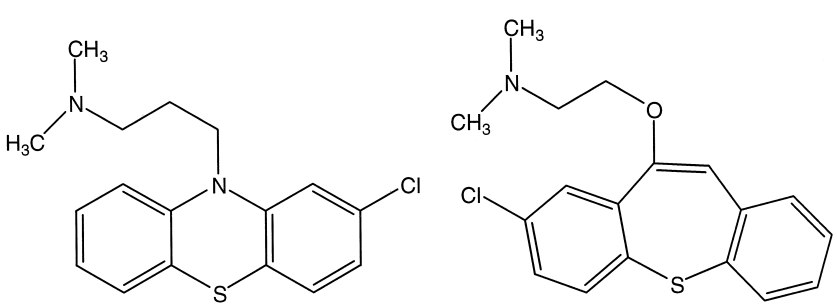
For imipramine, the K_D and n_H were 1.40 ± 0.03 nM and 0.980 ± 0.004 ($n = 51$, i.e., 51 independent determinations of these numbers), respectively; for nisoxetine, 1.85 ± 0.03 nM and 0.950 ± 0.003 ($n = 43$), respectively; and for WIN35428, 24.0 ± 0.4 nM and 0.930 ± 0.003 ($n = 42$), respectively. A one-component binding model was statistically preferred over a two-component model for each radioligand at its respective monoamine transporter. Most neuroleptics we tested were not very potent for the human monoamine transporters. The structures of the two most potent neuroleptics for the respective human monoamine transporter are shown in Figs. 1–3.

3.1. Neuroleptics and the human serotonin transporter (Table 1)

At the human serotonin transporter, the several compounds—trifluprazine, fluperlapine, chlorpromazine, ziprasidone, and zotepine—were relatively potent with K_D values in the range of 20 to 40 nM.

3.2. Neuroleptics and the human norepinephrine transporter (Table 1)

At the human norepinephrine transporter, four compounds—chlorpromazine, zotepine, chlorprothixene, and promazine—were relatively potent and had about the same affinity for the human norepinephrine transporter with K_D values in the range of 20 to 25 nM.



Chlorpromazine Zotepine

Fig. 2. Structures of the two most potent neuroleptics at the human norepinephrine transporter.

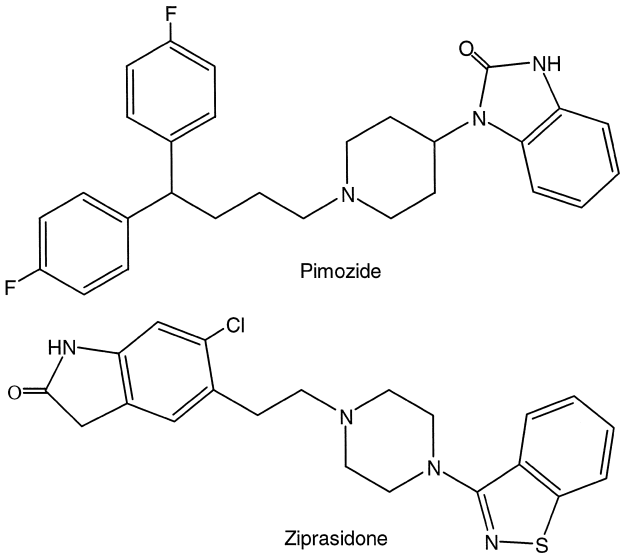


Fig. 3. Structures of the two most potent neuroleptics at the human dopamine transporter.

3.3. Neuroleptics at the human dopamine transporter (Table 1)

Pimozide and ziprasidone were clearly the two most potent compounds among neuroleptics that we tested at the human dopamine transporter with K_D values of 69 ± 3 and 76 ± 5 nM, respectively. Most neuroleptics were very weak at this transporter. The benzamides sulpiride, sultopride, and tiapride, which are selective D_2 receptor antagonists, had no detectable competition at this or the other human monoamine transporters.

4. Discussion

This study reports the data for the binding potencies of 37 neuroleptics and one metabolite of a neuroleptic (haloperidol meta2) at the human serotonin, norepinephrine, and dopamine transporters. As we have discussed previously (Tatsumi et al., 1997), our K_D value for imipramine ($K_D = 1.40$ nM) was similar to that of a high-affinity binding site ($K_D = 1.2$ nM) reported by Brust et al. (1995) and to that ($K_D = 3.2$ nM) reported by Barker et al. (1994). Also, the K_D for nisoxetine at the human norepinephrine transporter was close to that at the rat norepinephrine transporter (Tejani-Butt, 1992; Gehlert et al., 1995). However, our K_D value for WIN35428 was higher by about 6, 2, and two-fold compared to the values reported by Pristupa et al. (1994), Eshleman et al. (1995), and Reith et al. (1996), respectively. Differences in assay conditions (i.e., temperature of incubation and composition of the buffer) could account for these observed differences.

In addition, some published studies on the binding of [^{3}H]WIN35428 to the molecularly cloned, human, dopamine transporter show the presence of two binding sites for this compound (Pristupa et al., 1994; Eshleman et al., 1995; Reith et al., 1996), depending upon the cell type used for the expression of this protein and whether intact cells or membranal preparations were used in the assay. However, under the conditions of our assay, we could identify only one binding site of high affinity for [^{3}H]WIN35428.

The data show that some neuroleptics were relatively potent at binding to the three human monoamine transporters (Table 1). However, none was as potent or more potent than some antidepressants (Table 1; Tatsumi et al., 1997), including some tricyclic antidepressants, which have structural analogy to phenothiazine neuroleptics. Thus, it was not unexpected that the phenothiazine chlorpromazine, and its close structural analogs chlorprothixene and triflupromazine were relatively potent at both the human serotonin and norepinephrine transporters. This property of chlorpromazine at the norepinephrine transporter has been known for many years (Haggendal and Hamberger, 1967). In addition, although binding to the transporter strongly suggests that these compounds will inhibit the transport of substrate by these transporters, no direct evidence of these effects was presented here. Also, to know whether these compounds will affect these transporters in vivo, concentrations at the site of action (the synapse) would need to be known. In the absence of such data, one can extrapolate from the sparse published data on the therapeutic plasma concentrations of neuroleptics (Van Putten et al., 1991). Based on this information for chlorpromazine (therapeutic plasma concentration of 80–300 nM), one could expect that this neuroleptic would bind to the serotonin and norepinephrine transporters in vivo. From plasma concentrations of pimozide after a single oral dose of 2 mg (Sallee et al., 1987), one could also expect this drug to have effects in vivo at the dopamine transporter.

Interestingly, at the human dopamine transporter, the two most potent drugs, pimozide and ziprasidone, were each about three-fold more potent than cocaine at this transporter. These two compounds grossly have some structural similarity (Fig. 3). Pimozide was also relatively potent at the human serotonin transporter. However, the one compound that was near the top of the list for potency at each transporter was ziprasidone, which is considered an atypical neuroleptic. Other atypical neuroleptics such as clozapine, olanzapine and risperidone were weak at these transporters. Pimozide appears to be effective for Tourette's syndrome (Sallee et al., 1997) and for schizophrenic patients with negative signs and symptoms (Kolivakis et al., 1974; Feinberg et al., 1988). Ziprasidone also appears to improve negative signs and symptoms of schizophrenia, as well as both positive ones.

As has been suggested (Kapur and Remington, 1996), increasing dopamine function in the striatum may alleviate neuroleptic-induced extrapyramidal symptoms, while increasing dopamine function in the prefrontal cortex may ameliorate negative signs and symptoms of schizophrenia.

Blocking dopamine transporters and blocking 5-HT_{2A} receptors in these regions may be ways of increasing this dopamine function. This latter effect of receptor blockade has been postulated as conferring atypical properties to some neuroleptics (Meltzer et al., 1989).

Antidepressants, most of which block monoamine transport to varying degrees (Tatsumi et al., 1997), are useful for schizophrenic patients with post-psychotic depression (Siris et al., 1994) or with negative signs and symptoms (Siris et al., 1991). Our data suggest that some neuroleptics may have antidepressant effects and would be of use in schizophrenic patients who are also depressed or have negative signs and symptoms. Indeed, an early placebo-controlled study with chlorpromazine, the most potent neuroleptic at the human norepinephrine transporter (Table 1) showed that this phenothiazine was comparable to imipramine, and much superior to placebo in agitated, non-psychotic, depressed patients (Fink et al., 1965). These results were replicated in several subsequent studies. (Klein and Davis, 1969) However, like antidepressants, neuroleptics that are relatively potent at blocking monoamine transporter can potentially, as a result, cause certain adverse effects and drug interactions (Richelson, 1994). The clinician needs to be aware of these possible adverse pharmacodynamic effects.

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