ELSEVIER

European Journal of Pharmacology 340 (1997) 249–258

ejp

Pharmacological profile of antidepressants and related compounds at human monoamine transporters

Masahiko Tatsumi [a,b], Karen Groshan [a], Randy D. Blakely [c], Elliott Richelson [a,*]

[a] *Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224, USA*
[b] *Department of Psychiatry, Showa University School of Medicine, Tokyo, Japan*
[c] *Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA*

Received 17 July 1997; revised 23 September 1997; accepted 3 October 1997

Abstract

Using radioligand binding assays, we determined the equilibrium dissociation constants (K_D's) for 37 antidepressants, three of their metabolites (desmethylcitalopram, desmethylsertraline, and norfluoxetine), some mood stabilizers, and assorted other compounds (some antiepileptics, Ca^{2+} channel antagonists, benzodiazepines, psychostimulants, antihistamines, and monoamines) for the human serotonin, norepinephrine, and dopamine transporters. Among the compounds that we tested, mazindol was the most potent at the human norepinephrine and dopamine transporters with K_D's of 0.45 ± 0.03 nM and 8.1 ± 0.4 nM, respectively. Sertraline ($K_D = 25 \pm 2$ nM) and nomifensine (56 ± 3 nM) were the two most potent antidepressants at the human dopamine transporter. We showed significant correlations for antidepressant affinities at binding to serotonin ($R = 0.93$), norepinephrine ($R = 0.97$), and dopamine ($R = 0.87$) transporters in comparison to their respective values for inhibiting uptake of monoamines into rat brain synaptosomes. These data are useful in predicting some possible adverse effects and drug–drug interactions of antidepressants and related compounds. © 1997 Elsevier Science B.V.

Keywords: 5-HT (5-hydroxytryptamine, serotonin) transporter, human; Norepinephrine transporter, human; Dopamine transporter, human; Antidepressant; Radioligand binding assay

1. Introduction

After the early work of Kuhn (1958), researchers discovered that most antidepressants were inhibitors of transporters for serotonin and norepinephrine in the presynaptic nerve ending (Glowinski and Axelrod, 1964; Ross and Renyi, 1969). Although blockade by antidepressants of biogenic amine uptake into nerve endings is one of the cornerstones of the biogenic amine hypothesis of affective illness (Maas, 1975), the exact mechanism of action of antidepressants in alleviating depression remains uncertain. Nonetheless, this property of antidepressants clearly relates to some of their adverse effects and some of their drug–drug interactions (Richelson, 1994).

Re-uptake of serotonin, norepinephrine, and dopamine into nerve endings is a process that prevents overstimulation of receptors in the synapse. This process of re-uptake occurs through the action of unique 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).

We (Richelson and Pfenning, 1984; Bolden-Watson and Richelson, 1993) and many others (e.g., see Koe, 1976; Shank et al., 1987) 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 serotonin transporter (Barker et al., 1994; Barker and Blakely, 1996), we were interested to determine the binding potencies of antidepressants and related compounds for the human serotonin, norepinephrine, and dopamine transporters and to compare these data with our previously obtained data using rat brain synaptosomal preparations. The results, which include some interesting differences from our previous data (Richelson and Pfenning, 1984; Bolden-Watson and Richelson, 1993), are presented here.

* Corresponding author. Tel.: +1-904-9532439; fax: +1-904-9532482; e-mail: richel@mayo.edu.

2. Materials and methods

2.1. Materials

The following drugs were generously provided by the manufactures: alprazolam from Upjohn Co. (Kalamazoo, MI); butriptyline from Ayerst Laboratories (New York, NY); carbamazepine, oxaprotiline from CIBA Pharmaceuticals (Summit, NJ); citalopram, desmethylcitalopram from Lundbeck and Co. (Kobenhaun, Denmark); desmethylsertraline maleate, doxepin HCl, and sertraline HCl from Pfizer Central Research (Groton, CT); dothiepin from Marion Laboratories (Kansas City, MO); etoperidone HCl from Angelini Pharmaceuticals (Riveredge, NJ); femoxetine HCl from Novo Nordisk (Måløv, Denmark); fluoxetine HCl, norfluoxetine maleate, olanzapine and tomoxetine HCl from Eli Lilly (Indianapolis, IN); fluvoxamine from Duphar (Amsterdam); iprindole from Wyeth Laboratories (Philadelphia, PA); lofepramine HCl from Kabi Pharmacia (Helsingborg); mirtazapine, (+)-mirtazapine, and (−)-mirtazapine from Organon (West Orange, NJ); nefazodone HCl from Bristol–Myers Squibb (Wallingford, CT); paroxetine HCl from Smith Kline Beecham Pharmaceuticals (Surrey); venlafaxine HCl from Wyeth–Ayerst (Princeton, NY); viloxazine from Stuart Pharmaceuticals (Wilmington, DE); and zimelidine from Astra Lakemedel AB (Södertälje, Sweden). 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 83.5Ci/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, Ph.D. The human dopamine transporter cDNA was provided to us by Zdenek B. Pristupa, Ph.D. and H.B. Niznik, Ph.D. (University of Toronto, Toronto).

2.2. Methods

2.2.1. Expression of the human transporters

In 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 Ca^{2+} phosphate method (Chen and Okayama, 1987). For the expression of the human dopamine transporter, we directionally ligated the human dopamine transporter cDNA into the expression vector pcDNA3 and transfected it into HEK293 cells, also by the Ca^{2+} 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 nonessential 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 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′*-tetraacetic 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 the respective binding assay buffer by use of a Polytron (Brinkmann Instruments, Westbury, NY) for 10 s at setting 6. The mixture was then centrifuged at $35600 \times g$ 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. [^{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. Nonspecific binding was determined in the presence of 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 presoaked 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).

2.2.4.2. [^{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. Nonspecific binding was determined in the presence of 1 μM nisoxetine. The remainder of the assay was exactly as described above Section 2.2.4.1.

2.2.4.3. [^{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 (pH7.4) (Madras et al., 1989). [^{3}H]WIN35428 at 1 nM was incubated with competing drugs and 30 μg/tube membranal protein for 120 min at 4°C. Nonspecific binding was determined in the presence of 10 μM WIN35428. The remainder of the assay was exactly as described above 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 values for the equilibrium dissociation constants (K_D's). The program has been modified by us to calculate the Hill coefficients (n_H). Data are presented as geometric mean ± SEM (De Lean et al., 1982; Fleming et al., 1972) of at least 3 independent experiments. One- and two-component models were compared using the root mean square error of each fit and the *F*-test. We did simple regression analyses to compare between the pK_D's of the human monoamine transporters and the pK_i's of the rat monoamine transporters for antidepressants (except mirtazapine, (+)-mirtazapine, (−)-mirtazapine, desmethylcitalopram, phenelzine, iproniazid and tranylcypromine) with the use of StatView software (Jandel Co., San Rafael, CA).

3. Results

The data are summarized in Tables 1 and 2. The K_D's for antidepressants and related compounds in this study were derived from competition experiments with varying concentrations of the compounds. Hill coefficients (n_H's) 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 assay 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 ($n = 51$), 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.

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

Among the antidepressants that we tested, paroxetine, which is a serotonin selective re-uptake inhibitor based on animal data, was the most potent for the human serotonin transporter with a $K_D = 0.13 \pm 0.01$ nM. Some tricyclic antidepressants (clomipramine, imipramine and amitriptyline), as well as some other antidepressants (sertraline, fluoxetine, citalopram and fluvoxamine) and some of their metabolites (norfluoxetine, desmethylsertraline and desmethylcitalopram) were also very potent at the human serotonin transporter. The tetracyclic antidepressants (maprotiline, oxaprotiline, mianserin and mirtazapine) and the monoamine oxidase inhibitors (phenelzine, iproniazid and tranylcypromine) were very weak or had no detectable affinity for the human serotonin transporter. For 33 antidepressants there was a significant correlation ($R = 0.93$, $P < 0.0001$) between the log of the K_D's for the human serotonin transporter (pK_D) and the log of the K_i's for the rat serotonin transporter (pK_i) (Bolden-Watson and Richelson, 1993; Richelson and Pfenning, 1984). The equation for the regression line was $pK_i = 0.75\,pK_D + 1.20$.

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

At the human norepinephrine transporter, among the antidepressants desipramine was the most potent with a $K_D = 0.83 \pm 0.05$ nM. All the tetracyclic antidepressants, except mirtazapine, which is a structural analog of mianserin, were more potent at the norepinephrine transporter than at the serotonin transporter. Tomoxetine, considered from animal data to be very selective for the norepinephrine transporter, had high affinity for the human norepinephrine transporter ($K_D = 2.03 \pm 0.06$ nM). However, at the human serotonin transporter, tomoxetine was nearly as potent and close to that for dothiepin and venlafaxine. Venlafaxine, considered a serotonin and norepinephrine re-uptake inhibitor based on animal data, was very weak at the human norepinephrine transporter. Its K_D value was 5× less that than for norepinephrine. All of the serotonin selective re-uptake inhibitors, with the exception of paroxetine, were also weak at the human norepinephrine transporter. The K_D value of paroxetine for the human norepinephrine transporter was 40 ± 2 nM. The correlation between the log of the K_D's for the human serotonin transporter (pK_D) and the log of the K_i's for the rat serotonin transporter (pK_i) (Bolden-Watson and Richelson, 1993; Richelson and Pfenning, 1984) was significant ($R = 0.970$, $P < 0.0001$) for 33 antidepressants. The equation for the regression line was $pK_i = 0.901\,pK_D + 0.999$.

Table 1
Antidepressants: Equilibrium dissociation constants (K_D's) and selectivity for the human serotonin, norepinephrine and dopamine transporters

Antidepressant	Geometric Mean of $K_D \pm$ SEM (nM)			Selectivity factor[a]					
	Serotonin transporter	Norepinephrine transporter	Dopamine transporter	Serotonin transporter over norepinephrine transporter	Norepinephrine transporter over serotonin transporter	Serotonin transporter over dopamine transporter	Norepinephrine transporter over dopamine transporter	Dopamine transporter over serotonin transporter	Dopamine transporter over norepinephrine transporter
Amitriptyline	4.30 ± 0.12	35 ± 2	3250 ± 20	8.1	0.12	760	93	0.0013	0.011
Amoxapine	58 ± 2	16.0 ± 0.3	4310 ± 10	0.28	3.6	74	270	0.013	0.0037
Bupropion	9100 ± 300	52000 ± 1000	520 ± 20	5.7	0.18	0.057	0.01	18	100
Butriptyline	1360 ± 50	5100 ± 400	3940 ± 40	3.7	0.27	2.9	0.78	0.35	1.3
Citalopram	1.16 ± 0.01	4070 ± 80	28100 ± 700	***3500***	0.0003	2400	6.9	0.00004	0.14
Clomipramine	0.28 ± 0.01	38 ± 1	2190 ± 40	130	0.0075	7800	58	0.0001	0.017
Desipramine	17.6 ± 0.7	***0.83 ± 0.05***	3190 ± 40	0.047	21	180	3800	0.0055	0.0003
Desmethylcitalopram	3.6 ± 0.2	1820 ± 40	18300 ± 500	500	0.002	5000	10	0.0002	0.099
Desmethylsertraline	3.0 ± 0.2	390 ± 10	129 ± 2	130	0.0077	43	0.33	0.023	3.0
Dothiepin	8.6 ± 0.4	46 ± 1	5310 ± 30	5.3	0.19	620	120	0.0016	0.0086
Doxepin	68 ± 1	29.5 ± 0.8	12100 ± 400	0.43	2.3	180	410	0.0056	0.0024
Etoperidone	890 ± 40	20000 ± 2000	52000 ± 4000	23	0.044	59	2.6	0.017	0.39
Femoxetine	11.0 ± 0.3	760 ± 40	2050 ± 40	69	0.015	190	2.7	0.0054	0.37
Fluoxetine	0.81 ± 0.02	240 ± 10	3600 ± 100	300	0.0034	4300	15	0.00023	0.068
Fluvoxamine	2.2 ± 0.2	1300 ± 30	9200 ± 200	580	0.0017	4100	7.1	0.00024	0.14
Imipramine	1.40 ± 0.03	37 ± 2	8500 ± 100	27	0.037	6100	230	0.00016	0.0044
Iprindole	1620 ± 40	1262 ± 7	6530 ± 20	0.78	1.3	4.0	5.2	0.25	0.19
Iproniazid	> 100000	> 100000	> 100000	—	—	—	—	—	—
Lofepramine	70 ± 4	5.4 ± 0.4	18000 ± 1000	0.077	13	260	3400	0.0038	0.0003

Maprotiline	5800 ± 200	11.1 ± 0.3	1000 ± 20	0.0019	520	0.17	90	5.8	0.011
Mianserin	4000 ± 300	71 ± 2	9400 ± 200	0.018	56	0.24	13	4.2	0.075
Mirtazapine	> 100000	4600 ± 300	> 100000	—	—	—	—	—	—
(+)-Mirtazapine	> 100000	2900 ± 200	> 100000	—	—	—	—	—	—
(−)-Mirtazapine	> 100000	26000 ± 3000	> 100000	—	—	—	—	—	—
Nefazodone	200 ± 20	360 ± 40	360 ± 10	1.8	0.56	1.8	1.0	0.56	1.0
Nomifensine	1010 ± 30	15.6 ± 0.4	56 ± 3	0.015	65	0.055	3.6	18	0.28
Norfluoxetine	1.47 ± 0.06	1426 ± 9	420 ± 20	970	0.001	290	0.3	0.0035	3.4
Nortriptyline	18 ± 1	4.37 ± 0.07	1140 ± 30	0.24	4.2	62	260	0.016	0.0038
Oxaprotiline	3900 ± 100	4.9 ± 0.2	4340 ± 30	0.0012	***800***	1.1	890	0.91	0.0011
Paroxetine	***0.13 ± 0.01***	40 ± 2	490 ± 20	300	0.0033	3700	12	0.00027	0.081
Phenelzine	> 100000	49000 ± 5000	8400 ± 200	—	—	—	—	—	—
Protriptyline	19.6 ± 0.5	1.41 ± 0.02	2100 ± 60	0.072	14	110	1500	0.0093	0.00067
Sertraline	0.29 ± 0.01	420 ± 20	***25 ± 2***	1400	0.0007	86	0.06	0.012	17
Tomoxetine	8.9 ± 0.3	2.03 ± 0.06	1080 ± 50	0.23	4.4	120	530	0.0083	0.0019
Tranylcypromine	39000 ± 2000	5900 ± 200	5100 ± 200	0.15	6.7	0.13	0.87	7.7	1.2
Trazodone	160 ± 20	8500 ± 300	7400 ± 300	53	0.019	46	0.87	0.022	1.1
Trimipramine	149 ± 6	2450 ± 30	3780 ± 10	16	0.061	25	1.5	0.039	0.65
Venlafaxine	8.9 ± 0.3	1060 ± 40	9300 ± 50	120	0.0084	1000	8.8	0.00096	0.11
Viloxazine	17300 ± 500	155 ± 8	> 100000	0.009	110	—	—	—	—
Zimelidine	152 ± 6	9400 ± 100	11700 ± 400	62	0.016	77	1.2	0.013	0.8

The most potent in each transporter category or the most selective for serotonin transporter over norepinephrine transporter or norepinephrine transporter over serotonin transporter is in bold and italics.

[a] Selectivity factor is calculated from the ration of K_D's for each compound at each transporter.

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

At the human dopamine transporter, sertraline and nomifensine were the most potent with K_D's of 25 ± 2 and 56 ± 3, respectively. Except for these two compounds, most antidepressants were not potent at the human dopamine transporter. The correlation between the log of the K_D's for the human dopamine transporter (pK_D) and the log of the K_i's for the rat dopamine transporter (pK_i) (Richelson and Pfenning, 1984; Bolden-Watson and Richelson, 1993) was significant ($R = 0.871$, $P < 0.0001$) for 33 antidepressants. The equation for the regression line was $pK_i = 0.63\,pK_D + 2.08$.

3.4. Compounds other than antidepressants at the human transporters (Table 2)

3.4.1. Some drugs used for mood disorders

Lithium carbonate, a mood stabilizing drug, had no detectable effect at the respective monoamine transporters. Carbamazepine, an antiepileptic drug used to treat mood disorders, was very weak at the human serotonin transporter, and had no effects on both the human norepinphrine and dopamine transporter. Other antiepileptic drugs and a few benzodiazepines had no detectable affinity for the human monoamine transporters. 3,3′,5-triiodo-*L*-thyronine (thyroid hormone; T_3) and estrogen, which are sometimes used to treat refractory depression as an aug-

Table 2
The related compounds: Equilibrium dissociation constants (K_D's) for the human serotonin, norepinephrine and dopamine transporters (Geometric mean of $K_D \pm$ SEM (nM))

Compounds	Serotonin transporter	Norepinephrine transporter	Dopamine transporter
Benzodiazepines			
Alprazolam	> 100000	> 100000	> 100000
Clonazepam	> 100000	> 100000	> 100000
Mood stabilizers			
Acetazolamide	> 100000	> 100000	> 100000
Carbamazepine	32000 ± 500	> 100000	> 100000
Lithium carbonate	> 100000	> 100000	> 100000
Valproic acid	> 100000	> 100000	> 100000
Hormones			
Estrone	50000 ± 3000	> 100000	13500 ± 600
T_3	> 100000	> 100000	8100 ± 300
Ca^{2+} channel antagonists			
Nifedipine	> 100000	> 100000	> 100000
Verapamil	245 ± 6	48000 ± 1000	20000 ± 300
Antihypertensive			
Reserpine	1460 ± 60	15200 ± 900	41000 ± 2000
Psychostimulants			
(S +)-amphetamine	> 100000	530 ± 40	2900 ± 200
Cocaine	340 ± 20	1420 ± 50	220 ± 9
Mazindol	39 ± 1	0.45 ± 0.03	8.1 ± 0.4
(S +)-methamphetamine	> 100000	660 ± 20	2800 ± 100
Methylphenidate	44000 ± 1000	234 ± 9	24 ± 1
GBR12935	940 ± 20	310 ± 10	27.2 ± 0.2
Antihistamines			
Chlorpheniramine	15.2 ± 0.8	1440 ± 50	1060 ± 4
Diphenhydramine	3800 ± 400	960 ± 30	2200 ± 100
Other monoamines			
Dopamine	> 100000	32000 ± 3000	2400 ± 100
Norepinephrine	> 100000	2200 ± 30	16200 ± 500
Serotonin	2100 ± 100	> 100000	> 100000
Other			
Amfonelic acid	—	—	207 ± 5

mentation strategy, were very weak or had no effects at the human monoamine transporters. The Ca^{2+} channel antagonist verapamil was relatively weak at the human serotonin transporter and very weak for both the human norepinephrine and dopamine transporters.

3.4.2. Psychostimulants and related drugs

Interestingly, mazindol was the most potent drug at the human dopamine transporter with a $K_D = 8.1 \pm 0.4$ nM and at the human norepinephrine transporter with a $K_D = 0.45 \pm 0.03$ nM among all compounds we tested in this study. Mazindol was 1.8 × more potent than desipramine at the human norepinephrine transporter. Cocaine, although relatively weak, was most potent at the human dopamine transporter, compared to its binding at serotonin and norepinephrine transporters, with K_D values of 220 ± 9 nM, 340 ± 20 nM, and 1420 ± 50 nM, respectively. The binding affinities of (S +)-amphetamine, which were similar to those of (S +)-methamphetamine, were weak at all monoamine transporters.

3.4.3. Antihistamines

The histamine H_1 receptor antagonist chlorpheniramine was potent for the human serotonin transporter, with a potency ($K_D = 15.2 \pm 0.8$ nM) similar to that of the secondary amine tricyclic antidepressants desipramine ($K_D = 17.6 \pm 0.7$ nM), nortriptyline ($K_D = 18 \pm 1$ nM), and protriptyline ($K_D = 19.6 \pm 0.5$ nM).

4. Discussion

This study reports the binding potencies of 37 antidepressants, three metabolites of antidepressants, some mood stabilizers including antiepileptic drugs, two Ca^{2+} channel antagonists, two hormones, two benzodiazepines, some psychostimulants, antihistamines, and other monoamines for the human serotonin, norepinephrine and dopamine transporters.

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 the value ($K_D = 3.2$ nM) reported by Barker et al. (1994). However, it was nearly 30 × more potent at the human serotonin transporter, when compared to its inhibitor constant (K_i) for inhibiting [^{3}H]serotonin uptake into rat brain synaptosomal preparations in our previous studies (Richelson and Pfenning, 1984; Bolden-Watson and Richelson, 1993). These species differences for imipramine between human and rat were found by Blakely and colleagues (Barker et al., 1994; Barker and Blakely, 1996) with binding to the molecularly cloned serotonin transporters and by Wielosz et al. (1976) with 5-HT uptake into platelets.

Our affinity 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 2 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.

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. For example, with membranal preparations, Pristupa et al. (1994) describe two-sites, while Eshleman et al. (1995) found one or two sites, depending upon the cell type used for the expression of this protein. In addition, Reith et al. (1996), using an intact cell assay found two binding sites. The low-affinity binding sites for WIN35428 have been in the micromolar concentration range. However, under the conditions of our assay, we identified only one binding site for [^{3}H]WIN35428 and this site was of high affinity.

The pK_D's of 33 antidepressants for binding to the three monoamine transporters were significantly correlated with their respective pK_i's for uptake blockade into synaptosomal preparations of rat brain (Richelson and Pfenning, 1984; Bolden-Watson and Richelson, 1993). These findings for the dopamine transporter are consistent with those of Eshleman et al. (1995) and Pristupa et al. (1994). Although our correlations were significant, the equations for the regression lines did not indicate identity of the numbers derived by these vastly different techniques. There could be many different reasons for this lack of identity, including that inhibiting transport by a drug involves more sites or processes than does binding of the drug to the transporter.

Among the drugs that are used to treat mood disorders as augmentation and maintenance therapy, such as lithium carbonate, most had no detectable affinity for the human monoamine transporters. The Ca^{2+} channel antagonist verapamil, although relatively weak, was most potent among this class of compounds at the human serotonin transporter, while very weak at both the human norepinephrine and dopamine transporters. Previously, McGee and Schneider (1979) and Brown et al. (1986) reported that Ca^{2+} channel blockers had low potencies for inhibiting uptake by monoamine transporters. Estrogen, which is used to treat women with refractory depression, was very weak for the human monoamine transporters. However, it would also be important to investigate interactions between antidepressants and these drugs at the monoamine transporters.

From a perusal of the selectivity of antidepressants for all three human monoamine transporters (Table 1), one finds some interesting results when these are compared to our previously reported data obtained with rat brain synaptosomal preparations (Richelson and Pfenning, 1984; Bolden-Watson and Richelson, 1993). Although we previously reported that all tricyclic antidepressants, except

clomipramine, were selective for norepinephrine over serotonin, we found in the present study that in addition to clomipramine, several other tertiary amine tricyclics, including amitriptyline and imipramine (Table 1), were serotonin selective re-uptake inhibitors. Nonetheless, all secondary amine tricyclic antidepressants remained selective for norepinephrine over serotonin (Table 1). In addition, venlafaxine, based upon animal data, has been called a serotonin and norepinephrine re-uptake inhibitor. However, from the present data obtained with the human transporters, it was potent and selective for serotonin (Table 1). Therefore, it is a serotonin selective re-uptake inhibitor.

Although paroxetine was the most potent compound at the serotonin transporter, as we previously reported for rat brain studies (Bolden-Watson and Richelson, 1993) and as Barker et al. (1994) reported with the molecularly cloned transporter, it was not the most selective. Instead, citalopram was the most selective for the human serotonin transporter. Since selectivity is determined by the ratio of K_d's for each transporter, citalopram was more selective (citalopram K_d for serotonin transporter/K_d for norepinephrine transporter = 1.16 nM/4070 nM = 1/3500) than was paroxetine (K_d for serotonin transporter/K_d for norepinephrine transporter = 0.13 nM/40 nM = 1/300), mostly because citalopram was about 100 × weaker for the human norepinephrine transporter than was paroxetine. Thus, selectivity cannot be equated with potency. In addition to selecivity, potency shoud be considered in predicting potential adverse effects or drug–drug interactions resulting from inhibition of transporters.

Fourteen antidepressants were selective for the human norepinephrine transporter over the serotonin transporter (Table 1). Oxaprotiline was the most selective compound for the human norepinephrine transporter. The rank order of selectivity was the same as that which we previously reported for rat synaptosomal studies (Bolden-Watson and Richelson, 1993). All of the tetracyclic antidepressants such as oxaprotiline, maprotiline, and mianserin, and some secondary amine tricyclic antidepressants such as desipramine, protriptyline, and nortriptyline, were more selective for the human norepinephrine transporter than for the human serotonin transporter. However, some secondary amine tricyclic antidepressants were more potent at the human serotonin transporter than were some tertiary amine drugs, such as trimipramine and butriptyline (Bolden-Watson and Richelson, 1993).

Mazindol, which is used clinically as an anorectic agent, was the most potent drug at the human dopamine transporter among all the compounds we tested. However, it was not selective for or most potent at this transporter, since it was nearly 20 fold more potent at blocking the norepinephrine transporter (Table 2). Both of these transporter blocking properties of mazindol may explain its appetite suppressing effects. Although the rank of mazindol at the dopamine transporter differs from that reported in earlier studies by Giros et al. (1992), Pristupa et al. (1994) and Eshleman et al. (1995), our K_D value was similar to those of Giros et al. (1992) and Eshleman et al. (1995). Possibly, reflecting the differences in our K_D value for WIN35428 (as mentioned above), our K_D for cocaine was about 6, 3, and 3 × less than K_i's reported by Pristupa et al. (1994), Eshleman et al. (1995) and Reith et al. (1996).

The monoamine oxidase inhibitors (iproniazid, phenelzine and tranylcypromine) were very weak or had no detectable binding at the human monoamine transporters. However, monoamine oxidase inhibitors, which inhibit the degradation of serotonin and other biogenic amines, when combined with serotonin selective re-uptake inhibitors or other drugs that potently block the serotonin transporter, can cause a syndrome ('serotonergic syndrome') that can be fatal in patients.

The tricyclic antidepressants were originally synthesized as antihistamines and, therefore, are structurally similar to antihistamines. Accordingly, we determined the K_D's for a few antihistamines. Although antihistamines are known to block monoamine transporters (Carlsson and Lindqvist, 1969; Young et al., 1988), the potency of the antihistamine chlorpheniramine for the human serotonin transporter was greater than expected based on the value reported for rat brain synaptosomal studies. The clinical relevance of this potent blockade by chlorpheniramine is uncertain. However, the possibility exists that chlorpheniramine may cause the 'serotonergic syndrome', if combined with monoamine oxidase inhibitors.

Interestingly, sertraline, nomifensine, and sertraline's metabolite desmethylsertraline were the most potent among the antidepressants that we tested at the human dopamine transporter. In fact, these compounds were more potent than cocaine at blocking this transporter. These results are similar to those reported previously for rat brain synaptosomal re-uptake (Bolden-Watson and Richelson, 1993). The structural similarity between sertraline and nomifensine (Fig. 1) may explain this common property. Some of the common, possible clinical consequences of blocking dopamine re-uptake are decreased appetite, increased alertness, and insomnia. The clinical relevance of sertraline's blockade of the dopamine transporter is uncertain. However, this property may explain sertraline's significant

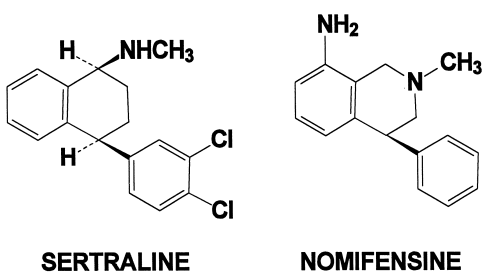

Fig. 1. Structures of the two most potent antidepressants for the human dopamine transporter.

improvement of cognitive function over fluoxetine in a group of depressed older patients (Oxman, 1996). Fluoxetine is about as potent as sertraline at binding to the serotonin and norepinephrine transporters, but over 100 fold weaker at binding to the dopamine transporter (Table 1).

With neurotransmission, monoamines are released into their respective synapses to activate their postsynaptic and presynaptic receptors. In addition, diffusion beyond the synapse leads to activation of cells far distant from the synapse (Agnati et al., 1995). After release these neurotransmitters in the synapse can be taken back into the nerve ending by their respective transporters, thereby reducing synaptic levels of chemical messengers and preventing overstimulation of their receptors. The inhibition by antidepressants of monoamine transporters increases synaptic levels of monoamines available to stimulate receptors in the synapse. This action by antidepressants is one of the cornerstones of so-called biogenic amine (serotonin, norepinephrine, and dopamine) hypothesis of mood disorders (Maas, 1975). Moreover, blockade of neurotransmitter re-uptake at the synapse by antidepressants and related compounds can account for certain adverse effects and drug–drug interactions. These clinical consequences (Richelson, 1994; Richelson, 1996) can be inferred from the effects that selective drugs have in patients.

Blocking the norepinephrine transporter, for example, may explain the antidepressant effects of drugs, their ability to cause tremors and tachycardia, and their ability to block the antihypertensive effects of guanethidine and guanadrel. Blocking the serotonin transporter may also relate to antidepressant effects, as well as gastrointestinal disturbances (e.g., nausea), sexual dysfunction (e.g., anorgasmia), and the development of the 'serotonergic syndrome'. Finally, the likely clinical consequences of blocking the re-uptake of dopamine include amelioration of the signs and symptoms of depression, parkinsonism, and attention deficit hyperactivity disorder; and increased alertness. The possible adverse effects of dopamine uptake blockade include anorexia, insomnia, and aggravation or precipitation of psychosis. Thus, the data presented can lead to a rationale basis for understanding many of the clinical effects of antidepressant drugs.

Acknowledgements

This work is supported by Mayo Foundation for Medical Education and Research and USPHS grant MH27692 from N.I.M.H. We thank Dr. Zdenek B. Pristupa and Dr. H.B. Niznik, University of Toronto for supplying a cDNA encoding the human dopamine transporter.

References

Agnati, L.F., Zoli, M., Stromberg, I., Fuxe, K., 1995. Intercellular communication in the brain: wiring versus volume transmission. Neuroscience 69, 711–726.

Barker, E.L., Blakely, R.D., 1996. Identification of a single amino acid, phenylalanine 586, that is responsible for high affinity interactions of tricyclic antidepressants with the human serotonin transporter. Mol. Pharmacol. 50, 957–965.

Barker, E.L., Kimmel, H.L., Blakely, R.D., 1994. Chimeric human and rat serotonin transporters reveal domains involved in recognition of transporter ligands. Mol. Pharmacol. 46, 799–807.

Bolden-Watson, C., Richelson, E., 1993. Blockade by newly-developed antidepressants of biogenic amine uptake into rat brain synaptosomes. Life Sci. 52, 1023–1029.

Brown, N.L., Sirugue, O., Worcel, M., 1986. The effects of some slow channel blocking drugs on high affinity serotonin uptake by rat brain synaptosomes. Eur. J. Pharmacol. 123, 161–165.

Brust, P., Bergmann, R., Johannsen, B., 1995. Specific binding of [^{3}H]imipramine indicates the presence of a specific serotonin transport system on endothelial cells of porcine brain. Neurosci. Lett. 194, 21–24.

Bylund, D.B., Yamamura, H.I., 1990. Methods for receptor binding. In: Yamamura, H.I., Enna, S.J., Kuhar, M.J. (Eds.), Methods in Neurotransmitter Receptor Analysis. Raven Press, New York, NY, pp. 1–35.

Carlsson, A., Lindqvist, M., 1969. Central and peripheral monoaminergic membrane-pump blockade by some addictive analgesics and antihistamines. J. Pharm. Pharmacol. 21, 460–464.

Chen, C., Okayama, H., 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. 7, 2745–2752.

De Lean, A., Hancock, A.A., Lefkowitz, R.J., 1982. Validation and statistical analysis of a computer modeling method for quantitative analysis of radioligand binding data for mixtures of pharmacological receptor subtypes. Mol. Pharmacol. 21, 5–16.

Eshleman, A.J., Neve, R.L., Janowsky, A., Neve, K.A., 1995. Characterization of a recombinant human dopamine transporter in multiple cell lines. J. Pharmacol. Exp. Ther. 274, 276–283.

Fleming, W.W., Westfall, D.P., De La Lande, I.S., Jellett, L.B., 1972. Log–normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. J. Pharmacol. Exp. Ther. 181, 339–345.

Gehlert, D.R., Schober, D.A., Gackenheimer, S.L., 1995. Comparison of (R)-[^{3}H]tomoxetine and (R/S)-[^{3}H]nisoxetine binding in rat brain. J. Neurochem. 64, 2792–2800.

Giros, B., el Mestikawy, S., Godinot, N., Zheng, K., Han, H., Yang-Feng, T., Caron, M.G., 1992. Cloning, pharmacological characterization and chromosome assignment of the human dopamine transporter. Mol. Pharmacol. 42, 383–390.

Glowinski, J., Axelrod, J., 1964. Inhibition of uptake of tritiated-noradrenaline in the intact rat brain by imipramine and structurally related compounds. Nature 204, 1318–1319.

Jayanthi, L.D., Prasad, P.D., Ramamoorthy, S., Mahesh, V.B., Leibach, F.H., Ganapahy, V., 1993. Sodium- and chloride-dependent, cocaine-sensitive, high-affinity binding of nisoxetine to the human placental norepinephrine transporter. Biochemistry 32, 12178–12185.

Koe, B.K., 1976. Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. J. Pharmacol. Exp. Ther. 199, 649–661.

Kuhn, R., 1958. The treatment of depressive states with G 22355 (imipramine hydrochloride). Am. J. Psychiatry 115, 459–464.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Maas, J.W., 1975. Biogenic amines and depression. Biochemical and pharmacological separation of two types of depression. Arch. Gen. Psychiatry 32, 1357–1361.

Madras, B.K., Fahey, M.A., Bergman, J., Canfield, D.R., Spealman, R.D., 1989. Effects of cocaine and related drugs in nonhuman primates. I. J. Pharmacol. Exp. Ther. 251, 131–141.

McGee, R. Jr., Schneider, J.E., 1979. Inhibition of high affinity synaptosomal uptake systems by verapamil. Mol. Pharmacol. 16, 877–885.

Munson, P.J., Rodbard, D., 1980. LIGAND: A versatile computerized approach for characterization of ligand-binding systems. Analyt. Biochem. 107, 220–239.

O'Riordan, C., Phillips, O.M., Williams, D.C., 1990. Two affinity states for [^{3}H]imipramine binding to the human platelet 5-hydroxytryptamine carrier: an explanation for the allosteric interaction between 5-hydroxytryptamine and imipramine. J. Neurochem. 54, 1275–1280.

Oxman, T.E., 1996. Antidepressants and cognitive impairment in the elderly. J. Clin. Psychiatry 57, 38–44.

Pacholczyk, T., Blakely, R.D., Amara, S.G., 1991. Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. Nature 350, 350–354.

Pfenning, M.A., Richelson, E., 1990. Methods for studying receptors with cultured cells of nervous tissue origin. In: Yamamura, H.I., Enna, S.J., Kuhar, M.J. (Eds.), Methods in Neurotransmitter Receptor Analysis. Raven Press, New York, pp. 147–175.

Pristupa, Z.B., Wilson, J.M., Hoffman, B.J., Kish, S.J., Niznik, H.B., 1994. Pharmacological heterogeneity of the cloned and native human dopamine transporter: disassociation of [^{3}H]WIN 35,428 and [^{3}H]GBR 12,935 binding. Mol. Pharmacol. 45, 125–135.

Ramamoorthy, S., Bauman, A.L., Moore, K.R., Han, H., Yang-Feng, T., Chang, A.S., Ganapathy, V., Blakely, R.D., 1993. Antidepressant- and cocaine-sensitive human serotonin transporter: Molecular cloning, expression and chromosomal localization. Proc. Natl. Acad. Sci. USA 90, 2542–2546.

Reith, M.E., Xu, C., Zhang, L., Coffey, L.L., 1996. Translocation of dopamine and binding of WIN 35,428 measured under identical conditions in cells expressing the cloned human dopamine transporter. Naunyn Schmiedeberg's Arch. Pharmacol. 354, 295–304.

Richelson, E., 1994. Pharmacology of antidepressants: Characteristics of the ideal drug. Mayo Clin. Proc. 69, 1069–1081.

Richelson, E., 1996. Synaptic effects of antidepressants. J. Clin. Psychopharmacol. 16, S1–S7.

Richelson, E., Pfenning, M., 1984. Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: Most antidepressants selectively block norepinephrine uptake. Eur. J. Pharmacol. 104, 277–286.

Ross, S.B., Renyi, A.L., 1969. Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. Eur. J. Pharmacol. 7, 270–277.

Shank, R.P., Gardocki, J.F., Schneider, C.R., Vaught, J.L., Setler, P.E., Maryanoff, B.E., McComsey, D.F., 1987. Preclinical evaluation of McN-5707 as a potential antidepressant. J. Pharmacol. Exp. Ther. 242, 74–84.

Tejani-Butt, S.M., 1992. [^{3}H]nisoxetine: A radioligand for quantitation of norepinephrine uptake sites by autoradiography or by homogenate binding. J. Pharmacol. Exp. Ther. 260, 427–436.

Wielosz, M., Salmona, M., de Gaetano, G., Garattini, S., 1976. Uptake of ^{14}C-5-hydroxytryptamine by human and rat platelets and its pharmacological inhibition. A comparative kinetic analysis. Naunyn Schmiedeberg's Arch. Pharmacol. 296, 59–65.

Young, C.S., Mason, R., Hill, S.J., 1988. Inhibition by H1-antihistamines of the uptake of noradrenaline and 5-HT into rat brain synaptosomes. Biochem. Pharmacol. 37, 976–978.