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Pharmacological properties of the active metabolites of the antidepressants desipramine and citalopram

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Abstract

Although major metabolites of some antidepressant drugs are known to be active, their pharmacological effects are poorly characterized. Two of the most selective antidepressants, desipramine (selectively inhibits norepinephrine reuptake) and citalopram (selectively inhibits serotonin reuptake) are frequently used in animal studies of antidepressant action, as well as being useful therapeutically. The primary aim of this study was to determine the affinity of desmethyl-desipramine, an active metabolite of desipramine, for the rat norepinephrine and serotonin transporters, as well as for the rat α_2 -adrenoceptor. The pharmacological characteristics of desmethyl-desipramine and desmethyl-citalopram, an active metabolite of citalopram, were also determined for various human transporters and neurotransmitter receptors. Competition binding studies using [3 H]nisoxetine and [3 H]citalopram showed desipramine to be 25 times more selective for the rat norepinephrine as compared to serotonin transporter (6.2 nM vs. 158 nM) whereas desmethyl-desipramine is 12 times more selective for the serotonin over the norepinephrine transporter (12.8 nM vs. 153 nM). Interestingly, the affinity of desmethyl-desipramine for the serotonin transporter is similar to the affinity of desipramine for the norepinephrine transporter. Desipramine and desmethyl-desipramine were found to have a lower affinity for the rat $\alpha_{2A(D)}$ -adrenoceptor than the transporters, suggesting that this receptor is not a major site of action for either compound. Thus, the pharmacological effects of desipramine in rats may be attributed not only to the inhibition of the norepinephrine transporter by desipramine but also to the inhibition of serotonin transporter by the active metabolite desmethyl-desipramine.

Keywords

Antidepressants; Active Metabolites; Tricyclic antidepressants; Selective serotonin reuptake inhibitors; norepinephrine reuptake inhibitors

1. Introduction

Two of the main classes of drugs used to treat major depressive disorder in adults are the tricyclic antidepressants and the selective serotonin reuptake inhibitors. Many of the tricyclic antidepressants or their metabolites are selective norepinephrine reuptake inhibitors including desipramine which has a 400-fold higher affinity for the norepinephrine transporter than the serotonin transporter in humans (Owens et al., 1997). By contrast, escitalopram is a selective serotonin reuptake inhibitor which has a 7,000-fold selectivity for the serotonin transporter over the norepinephrine transporter (Owens et al., 2001). Although both of these drugs are

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metabolized to compounds that are pharmacologically active, very little is known about the affinities of the metabolites for the norepinephrine transporter and serotonin transporter or various neurotransmitter receptors.

In the rat, the major metabolite of desipramine is desmethyl-desipramine. Chronic treatment of rats with desmethyl-desipramine has been shown to down-regulate the β -adrenoceptor with a potency similar to desipramine (Argenti and D'Mello, 1994; Deupree et al., 2007). Treatment of rats with desipramine can result in the concentration of desmethyl-desipramine reaching levels similar to that of the parent compound (Kozisek et al., 2007). Thus, desmethyl-desipramine may well contribute significantly to the antidepressant effect of desipramine. However, no studies have reported its affinity for either norepinephrine transporter or serotonin transporter. Escitalopram is metabolized to desmethyl-citalopram and didesmethyl-citalopram. Desmethyl-citalopram has a similar affinity for the human serotonin transporter as citalopram and a 500 fold lower affinity for norepinephrine transporter (Tatsumi et al., 1997), but little else is known about its pharmacological characteristics.

The primary focus of this study was to ascertain whether the some pharmacological effects of desmethyl-desipramine could be attributed to binding to norepinephrine transporter and/or serotonin transporter. Our findings indicate that desmethyl-desipramine has a higher affinity for serotonin transporter than norepinephrine transporter suggesting that part the pharmacological effects of administered desipramine could be due to inhibition of both norepinephrine transporter and serotonin transporter in rats. In addition, we report the pharmacology profile of desmethyl-desipramine and desmethyl-citalopram for various human transporters and neurotransmitter receptors. The affinity of desmethyl-desipramine for the α_2 -adrenoceptor was also determined in order to rule out the possibility that some of the pharmacological effects of this metabolite are due to an interaction with this receptor.

2. Materials and methods

2.1 Materials

Desmethyl-desipramine and desmethyl-citalopram were provided by the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program. Imipramine and desipramine were purchased from Sigma-Aldrich (St. Louis, MO, USA). [^3H]Nisoxetine (86 Ci/mmol) was obtained from Perkin Elmer Life Sciences (Boston, MA, USA). [^3H]Citalopram (84 Ci/mmol) and [^3H]RX821002 (50 Ci/mmol) were purchased from Amersham Biosciences (Piscataway, NJ, USA). Adult male Sprague-Dawley rat brains were obtained frozen from Harlan Industries (Indianapolis, IN). The BCA protein assay kit was purchased from Pierce (Rockford, IL, USA).

2.2 Inhibition studies on norepinephrine transporter and serotonin transporter

Adult rat brains lacking cerebellum and brain stem were homogenized in 20 volumes of 50 mM Tris, pH 7.4 using a TissueMizer Ultra Turrax (IKA Works, Inc., Wilmington, N.C.) at a speed setting of 16,000 rpm for 30 s. The homogenized tissue was centrifuged at $1000 \times g$ for 10 min at 4 °C. The membrane fractions were isolated by centrifugation at $20,000 \times g$ for 20 min. The pellets were resuspended in Tris buffer by gently sucking up and down in a pipet and recentrifuged at $20,000 \times g$ for 20 min. The tissue was immediately used for the norepinephrine transporter assays, and some was frozen at -80°C for the serotonin transporter binding experiments.

Norepinephrine transporter was assayed using published procedures with minor modifications (Gehlert et al., 1995; Tejani-Butt, 1992). Briefly, 0.3 g of membrane pellet was homogenized in 11.5 ml of 50 mM Tris, 300 mM NaCl, and 5 mM KCl at pH 7.4. Each 0.5 ml assay contained 0.35 mg of protein and 0.09 to 9 nM of [^3H]nisoxetine. Non-specific binding was determined

using 0.1 mM desipramine. Binding was allowed to proceed for 4 h at 4 °C. The reaction was stopped by filling the tube with cold 50 mM Tris buffer, pH 8, and immediately filtering over Whatman GF/B filter paper (Brandel Inc). Inhibition experiments were conducted using a similar assay procedure with K_d concentrations of [3 H]nisoxetine and various concentrations of unlabeled drug.

The 0.6 ml saturation binding assay for serotonin transporter (D'Amato et al., 1987) contained 9 μ g of tissue, 50 mM Tris, 120 mM NaCl, 5 mM KCl, and between 0.06 and 6 nM [3 H] citalopram at pH 7.4. Non-specific binding was determined using 6 μ M fluoxetine. The reaction was allowed to proceed for 3 h at room temperature (22 °C) before stopping with cold 50 mM Tris, pH 8 and filtering over Whatman GF/B filter paper. Inhibition experiments were conducted using a similar protocol with K_d concentrations of [3 H]citalopram and increasing concentrations of the unlabeled drug.

2.3 α_2 -Adrenoceptor Binding Studies

Rat cortex samples were prepared as described previously (Deupree et al., 2007). Briefly, adult male Sprague-Dawley cortex samples were homogenized in 50 mM Tris, pH 7.4 and centrifuged at 4 for 12 min. Membrane pellets were washed twice, divided into tubes, and stored at -80°C for future binding assays. Competition binding assays using imipramine, desipramine, and desmethyldesipramine were performed as described previously (O'Rourke et al., 1994). Each 1 ml assay contained various concentrations of drug in the presence of [3 H] RX821002. Binding reactions were incubated at room temperature for 1 h with mixing at 30 min. Reaction mixtures were filtered through GF/B glass-fiber filter strips (Whatman, Clifton, NJ) using a 48-well Brandel Cell Harvester (Biomedical Research and Development, Gaithersburg, MD) to terminate the reactions. The tubes and filters were rinsed three times with 5 ml ice-cold 50 mM Tris HCl pH 8.0. Filters were placed in scintillation vials with 5 ml liquid scintillation cocktail and counted in a liquid scintillation counter the next day. The concentration of [3 H]RX821002 used was $0.8 \times K_d$.

Inhibition studies were analyzed using the global non-linear regression analysis in the Prism program (Graph pad, San Diego, CA). In this analysis, multiple assays are fitted to one equation sharing one or more parameters between data sets. In the inhibition experiments, both the equations for the competitive inhibition and the Cheng-Prusoff equation (Cheng and Prusoff, 1973) were used simultaneously. The K_d for the radioactive ligand was set as a constant for all experiments, the concentrations of radioactive ligand for each experiment were defined as constants and the program analyzed the data from multiple experiments to determine the best-fit K_i for the unlabeled drug for all experiments.

2.4 NIMH Psychoactive Drug Screening Program (PDSP)

Desmethyldesipramine and desmethyldesipramine were submitted to PDSP (NIMH and University of North Carolina, Chapel Hill, NC) for analysis of affinities for various human transmitter reuptake transporters and receptors. In the initial screen, the percent inhibition at various transporters and receptors was determined at a concentration of 10 μ M. Complete inhibition curves were conducted for some of the transporters/receptors for which the metabolite inhibited the binding by greater than 50%.

3. Results

3.1 Affinity of desipramine and desmethyldesipramine for rat norepinephrine transporter and serotonin transporter

The affinity of desipramine and its metabolite, desmethyldesipramine, was determined for both norepinephrine transporter and serotonin transporter in rat brain homogenates. For comparison

purposes the affinity of escitalopram for these transporters was also determined, because escitalopram is one of the most selective of the selective serotonin reuptake inhibitors. [³H] Nisoxetine bound to both a high affinity and a low affinity binding site in rat brain homogenates. The data from five saturation experiments were analyzed using the global fit non-linear regression analysis to determine K_d and B_{max} for both one-site and two-site fit. The data best fit a two-site model ($p < 0.0001$) with a well-defined high affinity site ($K_d = 2.3 \text{ nM} \pm 0.85 \text{ nM}$) and a poorly-defined low affinity binding site ($K_d = 43 \pm 57 \text{ nM}$). [³H]Citalopram also bound to both a high affinity and a low affinity site with K_d of 0.37 ± 0.09 and $37 \text{ nM} \pm 1.4 \text{ nM}$, respectively. Based on the K_d reported by others (Owens et al., 1997; Paczkowski and Bryan-Lluka, 2001; Cheetham et al., 1996), the high affinity binding sites appear to represent binding to the transporter. The K_d values of these radioligands for their high affinity sites were used in the calculations of the K_i values of the drugs for the transporters.

Competition binding experiments were conducted to determine the affinity of desipramine, Desmethyldesipramine and escitalopram for the norepinephrine transporter and serotonin transporter using [³H]nisoxetine and [³H]citalopram as the radioactive ligands. The data from multiple assays were analyzed simultaneously to find the best fit using global fit non-linear regression analysis to determine IC_{50} and the K_i values (Table 1). The radioactive ligand concentrations were defined for each experiment and the $\log K_{i1}$ and $\log K_{i2}$ values were shared among the experiments. An F-test was used to determine whether the data fit a one-site or two-site model better. The data from the desipramine competition for both [³H]nisoxetine and [³H]citalopram fit a two-site model better than a one-site model. Desmethyldesipramine bound to a low and high affinity site with [³H]nisoxetine and a single high affinity site with [³H]citalopram. Escitalopram bound to a single site when competing with [³H]nisoxetine and two sites when competing with [³H]citalopram. The high affinity binding values were taken to reflect the binding to the transporters. The ratio of the high affinity binding sites of the drugs for serotonin transporter/norepinephrine transporter or norepinephrine transporter/serotonin transporter provides an indication of the selectivity of the drugs for the two transporters (Table 1). Desipramine is 25 times more selective for norepinephrine transporter compared to serotonin transporter. The active metabolite desmethyldesipramine on the other hand is 12 times more selective for serotonin transporter over norepinephrine transporter, and escitalopram is 100,000 times more selective for serotonin transporter than norepinephrine transporter. Importantly, the affinity of desmethyldesipramine for the serotonin transporter is similar to the affinity of desipramine for the norepinephrine transporter.

3.2. Affinity of Antidepressants for α_{2D} -Adrenoceptors

The affinity of imipramine, desipramine, and desmethyldesipramine for the α_{2D} -adrenoceptor was determined by competition binding on adult rat cortex using [³H]RX821002. Imipramine is the parent compound of desipramine and was used for comparison purposes. A one-site global nonlinear regression analysis was used to determine EC_{50} and $\log K_i$ values from three experiments. A K_d value of 70.1 nM, obtained from similar previous studies in our laboratory (Deupree et al., 2007), was used to calculate K_i values. The K_i values for imipramine, desipramine, and desmethyldesipramine were found to be 2.4, 3.4, and 2.6 μM , respectively. The R^2 values for each global fit analysis were 0.99 for imipramine, 0.98 for desipramine, and 0.98 for desmethyldesipramine indicating a good fit for the data.

3.2 Pharmacological profile of desmethyldesipramine and desmethyldesipramine in human tissue

The affinity of desmethyldesipramine and desmethyldesipramine for human transporters was determined by the PDSP screening program of NIMH (Table 2). In addition the affinity of these metabolites for other human receptors was also determined (Table 3).

4. Discussion

4.1. Transporter Affinities

The major finding of this study is that desmethyl-desipramine has a 12-fold higher affinity for serotonin transporter than norepinephrine transporter, and that its affinity for serotonin transporter is similar to the affinity of desipramine for norepinephrine transporter. Thus, the inhibition of serotonin transporter by the metabolite desmethyl-desipramine may contribute significantly to the pharmacological effects of desipramine in rats.

In rats the major metabolite of desipramine is desmethyl-desipramine (Breyer-Pfaff et al., 1978; Mancinelli et al., 1987). Recent studies in our laboratory (Kozisek et al., 2007) indicate that in adult rats treated twice a day for four days with desipramine (10 mg/kg, i.p.), the desmethyl-desipramine concentrations are 12 % of the desipramine concentrations 2 h after the last dose, but similar to the desipramine concentrations 12 h after the last dose. This suggests that immediately after desipramine is given, when the concentrations of desipramine are high and desmethyl-desipramine concentrations are low, the pharmacological effect of desipramine may be primarily associated with inhibition of norepinephrine transporter. However, later as the desipramine levels drop and the desmethyl-desipramine levels increase, both serotonin transporter and norepinephrine transporter may be inhibited and serotonin effects may contribute to the pharmacological effects observed.

Behavioral test, such as the forced-swim test, indicate that the effects of desipramine administration are the result of norepinephrine transporter inhibition. This is consistent with the desipramine concentrations being 9-fold higher than the desmethyl-desipramine concentrations at the time of testing (Kozisek et al., 2007) and so the noradrenergic effects would predominate. In more chronic treatment paradigms, the inhibition of norepinephrine transporter by desmethyl-desipramine may also contribute to the pharmacological effects of desipramine administration because desmethyl-desipramine has been shown to down-regulate the β -adrenoceptor with similar potency to desipramine (Argenti and D'Mello, 1994) and the concentration of desmethyl-desipramine 2 h after the last dose of desipramine in a 5.5-day treatment paradigm are sufficiently high (1.4 μ g/gm wet weight tissue) (Kozisek et al., 2007). In addition, there may well be serotonin effects of chronic desipramine administration due to the build-up of desmethyl-desipramine and its relatively high affinity for serotonin transporter. However, inhibition of serotonin transporter by desipramine administration in humans is not likely because desmethyl-desipramine is not produced to any extent in humans (DeVane et al., 1981; Sallee and Pollock, 1990).

The affinity of escitalopram for norepinephrine transporter and serotonin transporter in rat brain has not previously been reported. Not unexpectedly, the affinity of escitalopram is higher than the racemic mixture for serotonin transporter (D'Amato et al., 1987; Owens et al., 1997; Van, 1993) and higher than the K_i for citalopram previously reported for norepinephrine transporter (Gehlert et al., 1995; Owens et al., 1997; Van, 1993) in rat brain. The affinity of escitalopram and desmethyl-citalopram has been reported to be 700- and 500-fold lower for norepinephrine transporter than serotonin transporter in humans, respectively (Owens et al., 2002; Tatsumi et al., 1997), suggesting that the pharmacology of citalopram and its active metabolite is predominately related to inhibition of serotonin transporter with negligible effects from inhibition of norepinephrine transporter.

The K_i (6.2 nM) for desipramine competition with [3 H]nisoxetine for norepinephrine transporter in rat brain reported here are higher than that reported by others (0.05 to 1.6) (Sharpe et al., 2003; Gehlert et al., 1995; Owens et al., 1997; Beique et al., 1998) using rat brain and this is probably due to differences in the tissue used, how it was isolated and/or the buffers employed in the assay.

4.2. α_{2D} -Adrenoceptor Affinities

We considered the possibility that the pharmacological effects of desmethyldesipramine in rats are a result of binding to sites other than norepinephrine transporter and serotonin transporter, such as the α_2 -adrenoceptor. Although the rat and human α_{2A} -adrenoceptor orthologs have a high sequence homology, their pharmacological profiles are distinct. In fact, the rat ortholog has been classified pharmacologically as the α_{2D} -adrenoceptor (O'Rourke et al., 1994). A few atypical antidepressants, such as mianserin and mirtazapine, are antagonists at these presynaptic receptors, but have no effects on neurotransmitter uptake (Baumann and Maitre, 1977; de, 1996). By acting as an antagonist at these receptors, mianserin and mirtazapine attenuate the inhibitory effect on neurotransmitter release, thereby resulting in increased levels of neurotransmitters in the synapse. However, this does not appear to be the an important mechanism of action for desmethyldesipramine as its affinity for rat cortex α_2 -adrenoceptors was found to be 17- and 200-fold lower than its affinity for norepinephrine transporter and serotonin transporter, respectively. The same is true for its parent compounds, imipramine and desipramine. The affinity of desmethyldesipramine for the human α_{2A} -adrenoceptor is similar to that found for the rat α_{2D} . The low affinity of desmethyldesipramine for the α_{2D} indicates that the pharmacological effects of desmethyldesipramine in rat cortex do not result from actions at the α_{2D} adrenoceptor.

4.3 Affinities for Other Neurotransmitter Receptors

At a variety of neurotransmitter receptors desmethyldesipramine generally has low affinity ($> 1 \mu\text{M}$), with a few exceptions such as the muscarinic acetylcholine receptor, several of the serotonin subtypes and the α_{2C} -adrenoceptors (Table 3). For the purposes of comparison, Table 3 also contains the published affinities of desipramine for these receptors. If the metabolite had significantly higher affinity for some of these receptors than the parent compound, then some of the pharmacological action of the administered parent compound could be mediated by the metabolite at these receptors. However, as can be seen from Table 3, with the possible exception of the 5-HT₇ receptor, this is not the case.

Desmethyldesipramine did not have a high affinity for any of the receptors screened, and thus the observed effects of administered desipramine are not likely to be mediated by desmethyldesipramine at these receptors.

4.4. Summary

The presence of active metabolites of a drug must be considered in attempting to understand its mechanism of action, particularly if the concentration of active metabolite approaches that of the parent compound. The pharmacological effects of desipramine in rats may be attributed not only to the inhibition of the norepinephrine transporter by desipramine but also to the inhibition of serotonin transporter by the active metabolite desmethyldesipramine. However, the metabolite of desipramine does not appear to change the pharmacological profile of the parent compound.

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Table 1

Affinity (K_i) of desipramine, desmethyldesipramine and escitalopram for norepinephrine transporter and serotonin transporter in rat brain homogenates

Drug	K_i for Norepinephrine Transporter (nM)	K_i for Serotonin Transporter (nM)	Selectivity for Norepinephrine Transporter	Selectivity for Serotonin Transporter
Desipramine	6.2 ± 0.7	158 ± 9	25	0.0039
Desmethyldesipramine	153 ± 14	12.8 ± 0.9	0.084	12
Escitalopram	$28,200 \pm 2,850$	0.28 ± 0.01	0.00001	100,000

Table 2

Affinity of desipramine, desmethyl-desipramine, escitalopram, and desmethylcitalopram for human transporters. Affinity of the compounds was determined by PDSP for the human transporters using [³H]nisoxetine and [³H]citalopram to assay for affinity for norepinephrine transporter and serotonin transporter, respectively. The values reported by others are also given.

Transporter	K _i (nM)			
	Desipramine	Desmethyl-desipramine	Escitalopram	Desmethylcitalopram
Norepinephrine	0.63 ^a	47	7,841 ^c	1,820 ^e
Serotonin	3.8 ^b			6
	22 ^a	34	1.1 ^c	3.6 ^e
Dopamine				>10,000
	>10,000 ^d	3,280	>100,000 ^b	18,300 ^e

^aOwens et al., 1997

^bBymaster et al., 2002

^cOwens et al., 2002 (¹²⁵I-RTI-55 used for the DAT assays)

^dBymaster et al., 2002 (using [³H]Win 35,428 for DAT assays)

^eTatsumi et al., 1997 (using [³H]imipramine for serotonin transporter assays)

Table 3

Pharmacological characteristics of desmethyldesipramine and desmethylcitalopram. Binding assays were performed by PDSP at human receptors. The values reported by others for the parent compounds are also given to provide a comparison with the metabolites.

Human Receptor	Desmethyl-desipramine, K_i , nM	Desipramine K_i , nM	Desmethyl-citalopram, K_i , nM	Escitalopram K_i , nM
5-HT _{1A}	>10,000	>10,000 ^a	6,760	>10,000 ^e
5-HT _{1B}	8,365		>10,000	>10,000 ^e
5-HT _{2A}	207	106 ^a	>10,000	>10,000 ^e
5-HT _{2B}	2,055		1,636	
5-HT _{2C}	488	748 ^a	3,290	2,531 ^f
5-HT _{5A}	3,060		>10,000	
5-HT ₆	922		>10,000	
5-HT ₇	568	>1,000 ^b	2,040	
α_{1A} -adrenoceptor	1,200	23 ^c	>10,000	3,870 ^f
α_{2A} -adrenoceptor	4,480	1379 ^c	>10,000	>10,000 ^e
α_{2B} -adrenoceptor	285		>10,000	
α_{2C} -adrenoceptor	426		6,710	
Dopamine-1	5,750	5,460 ^a	>10,000	>10,000
Dopamine-3	835		4,900	>10,000 ^e
Histamine-1	333	31 ^c	674	1,970 ^f
Histamine-2	3,110		>10,000	
Muscarinic M1	404	110 ^d	>10,000	1,242 ^f
Muscarinic M2	927	540 ^d	>10,000	
Muscarinic M3	317	210 ^d	>10,000	
Muscarinic M4	629	160 ^d	>10,000	
Muscarinic M5	121	143 ^d	>10,000	
Sigma-2	4,170		2,010	

Desmethyldesipramine at a concentration of 10 μ M inhibited less than 50% of the binding to the following human receptors: 5-HT_{1D}, 5HT_{1E}, 5-HT₃, α_{1B} -adrenoceptor, cannabinoid-2, dopamine-2, dopamine-4, dopamine-5, delta opioid, histamine-4, kappa opioid, multi-drug transporter-1, mu opioid and sigma-1.

Desmethylcitalopram at a concentration of 10 μ M inhibited less than 50% of the binding to the following human receptors: 5-HT_{1E}, 5HT₃, α_{1B} -adrenoceptor, α_{2B} -adrenoceptor, cannabinoid-2, dopamine-1, dopamine-2, dopamine-4, dopamine-5, delta opioid, histamine-3, kappa opioid, multi-drug transporter-1 and mu opioid.

^aToll et al., 1998

^bShen et al., 1993

^cOwens et al., 1997

^dStanton et al., 1993

^eMillan et al., 2001

^fOwens et al., 2002.