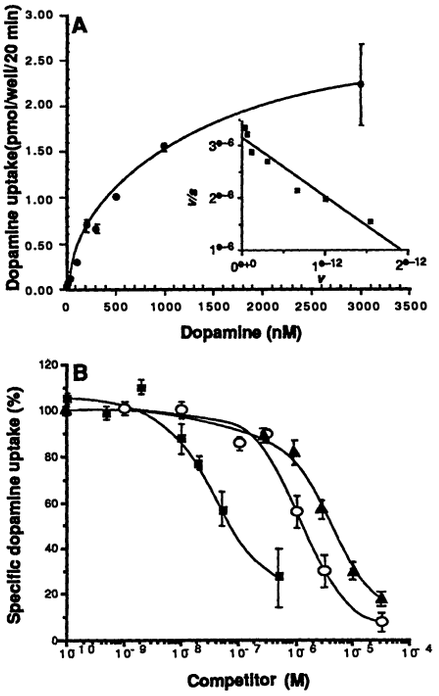

Fig. 2. (**A**) Kinetics of DA uptake into pDAT-transfected HeLa cells. Transfected cells were incubated with 10 nM [^{3}H]DA and increasing concentrations of unlabeled DA for 20 min as described (*8*). (Inset) Eadie-Hofstee transformation. K_m, 885 nM. (**B**) Inhibition of DA transport by mazindol (■), cocaine (○), and desipramine (▲). pDAT-transfected HeLa cells were simultaneously incubated with 50 nM [^{3}H]DA and various concentrations of antagonist as described (*8*). Representative curves are shown; values reflect the mean ± SEM.

strated saturable DA accumulation with a K_m (Michaelis constant) of 885 nM (Fig. 2A), somewhat lower than reported affinities for DA uptake in striatal synaptosomes (*9*). This is similar to results with a cDNA encoding the NE transporter (pNET) (*2*), which exhibits an affinity in HeLa cells two- to fourfold lower than has been reported for synaptosomal studies (*9*).

Pharmacological studies indicate that this carrier is distinct from the NE and 5HT transporters. The pDAT-encoded DA transport activity was sensitive to pharmacologic inhibition by mazindol, cocaine, and desipramine with the relative rank order of inhibition constants (K_i = 70 nM, 2 μM, 4 μM, respectively) (Fig. 2B) characteristic of synaptosomal DA uptake (*9*). The pharmacological sensitivity of pDAT-encoded transport was distinguishable from the inhibition of DA transport in pNET-transfected cells where the rank order was mazindol, desipramine, cocaine (K_i = 2 nM, 4 nM, 200 nM, respectively).

Determining whether DA transporters in mesolimbic areas are distinct from those in the dopaminergic systems such as the nigrostriatal pathway, the retina, the hypothalamus, or the olfactory bulb is important in understanding the neurobiologic basis of cocaine abuse. In addition, the ability to express the cocaine-sensitive DA transporter may provide insights into the neurobiology of drug addiction.

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4. The following degenerate oligonucleotides, which span transmembrane domains II and VI, were used for PCR: Sense, [CCGCTCGAGAAGAACGG(C/T)GG(C/T)GG(C/T)GC(C/T)TTC(C/T)T(G/A)AT(C/T)CC(A/G)TA]; antisense, [GCTCTAGAAA-(G/A)AAGATCTG(G/A)GT(G/T)GC(G/A)GC(G/A)TC(G/A/C)A(G/T)CCA].
5. S. Amara, unpublished data.
6. Complementary DNA libraries were generated as described (*2*). cDNAs were completely sequenced in both strands with Sequenase (U.S. Biochem) with a set of overlapping exonuclease III–digested unidirectional deletions. GenBank accession number M80233.
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Cloning of a Serotonin Transporter Affected by Antidepressants

BETH J. HOFFMAN,* EVA MEZEY, MICHAEL J. BROWNSTEIN

A complementary DNA clone for a serotonin (5HT) transporter has been isolated from rat basophilic leukemia cells. The complementary DNA sequence predicts a 653–amino acid protein with 12 to 13 putative transmembrane domains. The 5HT transporter has significant homology to the γ-aminobutyric acid, dopamine, and norepinephrine transporters. Uptake by CV-1 cells expressing the transporter complementary DNA resembles 5HT uptake by platelets and brain synaptosomes; it is sensitive to antidepressants, amphetamine derivatives, and cocaine.

AFTER ITS RELEASE AND ACTION ON receptors, 5HT is taken up into presynaptic terminals by plasma membrane transporters (*1*), terminating the action of the neurotransmitter. The 5HT transporter also allows platelets and rodent mast cells to concentrate 5HT (*2*). These cells store and secrete large amounts of the amine, but do not synthesize it. 5HT transporters are a site of action for some antidepressants and drugs of abuse such as amphetamine derivatives and cocaine (*2–4*). Antidepressants that block 5HT uptake, such as fluoxetine and clomipramine, are used to treat both depression and obsessive-compulsive disorder, and may be useful for treating panic disorder, bulimia, obesity, and alcoholism (*5*).

We have isolated a cDNA clone for a 5HT transporter from rat basophilic leukemia cells (RBL 2H3, a cognate mast cell) (*6*) by expression of cDNA pools in COS and CV-1 cells (*7*). In parallel with the functional assay of cDNA clones, subdivisions were screened with a degenerate oligonucleotide (*7*) directed at a region conserved in norepinephrine (NE) (residues 78–98) (*8*) and γ-aminobutyric acid (GABA) transporters (residues 66–86) (*9*). A single positive clone was identified from a pool of 100 clones. Sequencing of the 2.8-kb insert revealed an open reading frame of 1,959 bp (Fig. 1), predicting a protein of 653 amino acids with a molecular weight of ~73,000 (*10*). The initiating ATG is 52 bases downstream from a stop codon, and the surrounding sequence conforms to a consensus translation initiation site (*11*). Hydropathy analysis (*12*) indicates 12 to 13 potential transmembrane domains with no apparent signal sequence (*13*). On this basis and by analogy to the GABA transporter (GAT-1) and the NE transporter (NET), the NH_2- and COOH-termini would be intracellular and a large loop with two potential glycosylation sites would be extracellular.

Comparison of the 5HT transporter (5HTT) to other proteins indicates no signifi-

B. J. Hoffman and M. J. Brownstein, Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.
E. Mezey, First Department of Anatomy, Semmelweis University Medical School, Budapest, Hungary.

*To whom correspondence should be addressed.

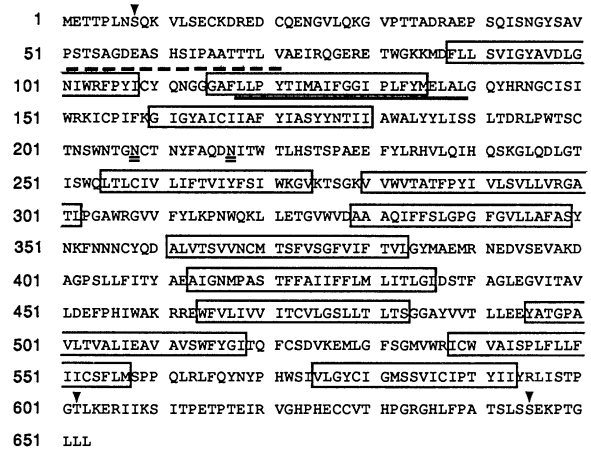

Fig. 1. Amino acid sequence of a 5HT transporter. Boxes, hydrophobic domains predicted by the Kyte-Doolittle algorithm (*12*); double underline, potential glycosylation sites; arrowheads, potential protein kinase C phosphorylation sites; underline, leucine zipper motif (*23*); dashed line, the consensus oligonucleotide (7).

cant homology except to NET (49%), GAT-1 (41%), and the DA transporter (DAT) (47%) (*14*). Accounting for conservative substitutions, the overall levels of similarity increase to 65%, 62%, and 69%, respectively. Comparing the first eight transmembrane domains, 5HTT is more similar to NET (91%) and DAT (90%) than to GAT-1 (66%) but the last four hydrophobic regions show about the same degree of homology (69%, 74%, 66%, respectively). The intracellular NH_2- and COOH-terminal tails are least conserved among the transporters. The region of the consensus oligonucleotide is highly conserved among the three transporter proteins (48 of 61 bases identical).

Table 1. Drug affinities for inhibition of 5HT uptake in CV-1 cells transfected with the 5HT transporter cDNA. CV-1 cells (140,000 per well) were infected with vaccinia virus containing T7 RNA polymerase and transfected with 5HTT cDNA (7). Transfected cells were incubated with [^{3}H]5HT (50 to 100 nM) with or without inhibitors for 15 min. Values are the mean ± SEM from three determinations performed in triplicate. Nonspecific was defined as uptake in the presence of 1 μM paroxetine. Inhibitory constant (K_i) values were calculated according to Cheng and Prusoff (*24*).

Inhibitor	K_i (nM)
Paroxetine	3.1 ± 0.8
Citalopram	6.1 ± 1.0
Clomipramine	7.1 ± 2.0
Fluoxetine	33 ± 1.0
S(+)fenfluramine	129 ± 28
± MDMA	186 ± 27
Imipramine	209 ± 28
Amitriptyline	262 ± 66
Zimelidine	382 ± 100
Mazindol	548 ± 120
Cocaine	1080 ± 150
Desipramine	1680 ± 400
Doxepin	1850 ± 570
D-amphetamine	3180 ± 440
Reserpine	>10,000
Dopamine	>10,000
Norepinephrine	>10,000

In situ hybridization histochemistry with a 5HTT-specific oligonucleotide revealed 5HTT mRNA exclusively in areas in the brain with serotonergic neurons, in the lamina propria of the stomach and the duodenum, and in chromaffin cells of the adrenal (*15*). By Northern (RNA) blot analysis with this oligonucleotide, there are high levels of a single mRNA species of 3.1 kb in both brain (brainstem) and in peripheral tissues (gut, lung). Spleen and adrenal have intermediate levels, and stomach, uterus, and kidney have low levels of mRNA (*16*).

5HT uptake in CV-1 cells transfected with the 5HTT cDNA was saturable, with a Michaelis constant (K_m) of 529 ± 107 nM (mean ± SEM), comparable to that determined in RBL 2H3 cells (*6*), rat synaptosomes (*16*), and platelets (*2, 17*). [^{3}H]5HT uptake was Na^+- and Cl^--dependent (*17–19*) and was potently inhibited by fluoxetine, paroxetine, citalopram, and clomipramine, which are specific for the 5HT transporter (Table 1). Fenfluramine, an anorectic drug, (*20*) also blocked uptake. Antidepressants more selective for NE and DA transporters (*21*), such as mazindol and desipramine, had lower affinity for the 5HT transporter. DA and NE as well as reserpine, an inhibitor of vesicular uptake, do not block 5HT uptake. A single protein appears to be responsible for both antidepressant binding and 5HT uptake.

3,4-Methylenedioxy-methamphetamine (MDMA or "ecstasy") is a potent neurotoxin of serotonergic neurons that causes irreversible cell degeneration (*22*) and exhibits potent inhibition of [^{3}H]5HT uptake in cells transfected with the 5HTT cDNA (Table 1). 5HT transporter is also blocked by amphetamine derivatives and cocaine (*3*) and may contribute to the behavioral effects of these drugs. Additional information about the function of this transporter in cells and intact animals may contribute to a better understanding of affective disorders.

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