CACCTCCGCCGTTCTTGTAGCACAGG-TAGGGGAAGCGCCACACGTT3′, which was based on sequences of NET, GABAT, and PCR products of library amplification derived with these sequences. Capped mRNA was synthesized from plasmids autoexcised from 27 positively hybridizing phage and tested for ability to confer uptake into injected oocytes. Messenger RNA from pDAT1 induced cocaine-blockable [^{3}H]DA uptake and was subjected to restriction mapping, automated sequencing of both strands, and confirmatory manual sequencing.

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10. COS cells were transfected with pcDNADAT1 by electroporation (*13*), cultured for 2 to 3 days, washed with Hank's solution, stored at −70°C, and membranes tested for binding of [^{3}H]CFT (82.7 Ci/mmol, NEN) as described (*11*).
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7 August 1991; accepted 30 September 1991

Cloning and Expression of a Cocaine-Sensitive Rat Dopamine Transporter

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The action of dopamine and other monoamine neurotransmitters at synapses is terminated predominantly by high-affinity reuptake into presynaptic terminals by specific sodium-dependent neurotransmitter transport proteins. A complementary DNA encoding a rat dopamine transporter has been isolated that exhibits high sequence similarity with the previously cloned norepinephrine and γ-aminobutyric acid transporters. Transient expression of the complementary DNA in HeLa cells confirms the cocaine sensitivity of this transporter.

REUPTAKE SYSTEMS FOR THE BIOgenic amines have a central role in determining net synaptic activity and are the initial sites of action for a wide range of drugs with both therapeutic and abuse potential. Psychomotor stimulants such as cocaine and amphetamines act directly on neurotransmitter transporters to inhibit the reuptake of dopamine (DA), serotonin (5HT), and norepinephrine (NE). The reinforcing effects of cocaine, however, have been attributed to inhibition of DA reuptake in the nucleus accumbens and related targets of the mesolimbic DA system (*1*). The significance of the DA transporter to the addictive properties of cocaine provides impetus for understanding more about the structure, cellular physiology, and regulation of the transporter.

Degenerate oligonucleotides corresponding to regions of high sequence identity between the NE (*2*) and γ-amino-butyric acid (GABA) (*3*) transporters were used in polymerase chain reactions (PCR) with mRNA from midbrain (*4*). One PCR product, KW27, hybridized a 3.6-kb mRNA selectively expressed in the midbrain (*5*). A full-length clone (pDAT) was identified by high-stringency screening of midbrain and substantia nigra cDNA libraries with the KW27 probe (*6*). The deduced amino acid sequence (Fig. 1) reveals 12 hydrophobic segments and predicts the same topologic model as that suggested for the GABA and NE transporters with 12 transmembrane domains and both NH_2- and COOH-termini located cytoplasmically. The degree of sequence identity between pDAT and the NE and GABA transporters is 64 and 40%, respectively. One potentially significant difference is the presence of four predicted glycosylation sites in pDAT (Fig. 1), while the other two transporters have only three sites.

In situ hybridization studies provide further evidence that pDAT encodes a DA transporter. Brain regions known to contain dopaminergic neurons show specific hybridization to a KW27 cRNA probe and include the substantia nigra and ventral tegmental area, with less intense signals apparent in the periphery of the olfactory bulb and in discrete regions of the hypothalamus (*5, 7*). In order to confirm that pDAT represents the actual DA transporter and not a related gene product expressed in dopaminergic neurons, the transporter was expressed in HeLa cells with a T7-vaccinia virus transient expression system (*8*). Transfected HeLa cells demon-

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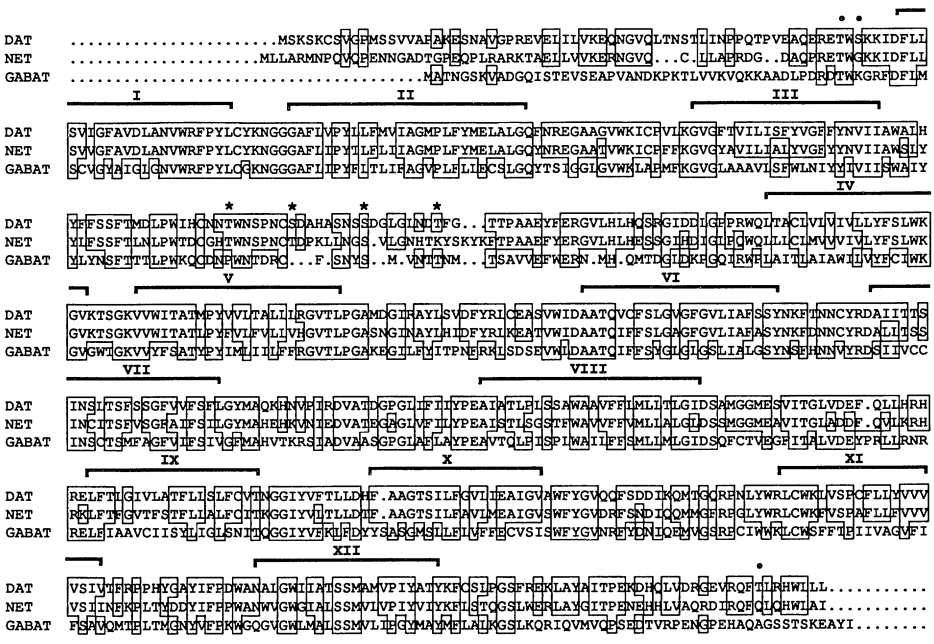

Fig. 1. Alignment of protein sequences of the rat DA transporter with the human NE (*2*) and GABA (*3*) transporters. Boxes, regions of identity; brackets, transmembrane helices; *, glycosylation sites on the putative extracellular loop. Two potential sites for phosphorylation by protein kinase C in the NH_2-terminal domain and one potential site for phosphorylation by either protein kinase C or Ca^{2+}-calmodulin–dependent kinase II in the COOH-terminal domain are indicated by solid dots.

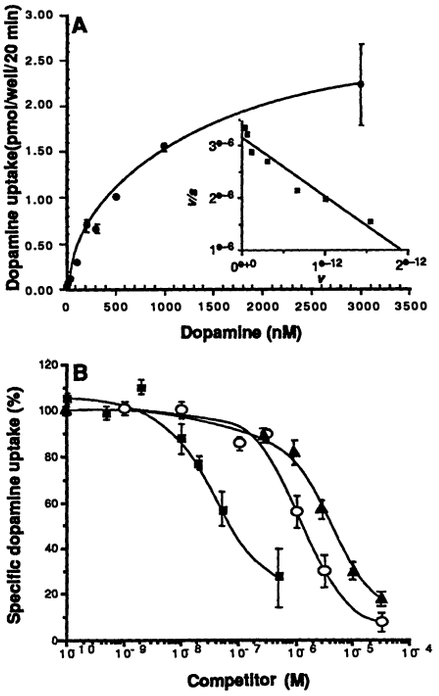

Fig. 2. (**A**) Kinetics of DA uptake into pDAT-transfected HeLa cells. Transfected cells were incubated with 10 nM [^{3}H]DA and increasing concentrations of unlabeled DA for 20 min as described (*8*). (Inset) Eadie-Hofstee transformation. K_m, 885 nM. (**B**) Inhibition of DA transport by mazindol (■), 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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21 August 1991; accepted 30 September 1991

Cloning of a Serotonin Transporter Affected by Antidepressants

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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-

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