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Cloning and functional characterization of a cocaine-sensitive dopamine transporter

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We report the cloning of a rat cDNA encoding a functional dopamine transporter. This cDNA, derived from an intron-containing gene, encodes a protein of 620 amino acids. Hydropathicity analysis of the protein sequence suggests the presence of 12 putative transmembrane domains. The protein displays considerable identity with transporters for noradrenaline and GABA (64 and 30%, respectively). Transient expression of the cDNA in COS7 cells directs the expression of dopamine uptake activity with appropriate pharmacology and in a sodium-dependent fashion. In situ hybridization reveals that the mRNA for this transporter is expressed in the substantia nigra and ventral tegmental area, regions that contain dopaminergic cell bodies.

Dopamine transporter; Transmembrane domain; Substantia nigra pars compacta; Ventral tegmental

1. INTRODUCTION

Neurotransmission is a complex phenomenon involving the interplay of several processes. These various systems include: (i) the synthesis, storage and release of the neurotransmitter; (ii) the interaction of the neurotransmitter with specific receptors located on both pre- and postsynaptic terminals; and (iii) the rapid removal of the transmitter, and termination of its action via enzymatic degradation or via specialized transporter proteins on presynaptic terminals [1].

Whereas many of the protein components and mechanisms involved in the synthesis and receptor-mediated effects of neurotransmitters have been fairly well studied, the biochemical and molecular details of termination processes have been mostly unknown. The uptake of neurotransmitters such as noradrenaline, dopamine and serotonin is mediated by a Na^+-dependent process [2] and seems to represent the most important pathway for cessation of the action of these monoamines. For example, 80–90% of noradrenaline is removed from the synapse into nerve terminals by a specific noradrenaline uptake mechanism [3], as opposed to being degraded by metabolizing enzymes such as monoamine oxidase or catechol-*O*-methyl transferase [3]. The importance of uptake mechanisms in the synaptic function is further highlighted by the large number and dramatic pharmacological effects of drugs that interact with these transporter proteins, such as anti-depressants and substances of abuse such as cocaine and amphetamine. Dopamine uptake is related, for example, to locomotor activity, positive reinforcement and cocaine self-administration [4]. Because of the potential involvement of the dopaminergic neurotransmission system in the etiology of psychiatric disorders or neurological diseases, elucidation of the molecular properties of the dopamine transporter could provide new and powerful means to understand these mechanisms and develop novel therapeutic tools.

The recent biochemical purification of a brain γ-aminobutyric acid (GABA) transporter [5] and the development of expression cloning techniques for these proteins [6,7] has led to the elucidation of the structure of 2 members of this transporter protein family, one for GABA and another selective for the biogenic amine noradrenaline (NA) [8,9]. These proteins are single polypeptides of ~600 amino acids, characterized by the presence within their sequence of 12 hydrophobic stretches of 20–25 amino acids that may represent transmembrane domains [8,9]. In that respect, these proteins resemble the ATP-driven and nutrient families of transporter proteins [10,11]. However, their primary structure clearly indicates that they define a unique family of transmembrane proteins. Within several of their transmembrane domains, the NA and GABA transporters display significant (>46%) amino acid identities. Neurotransmitter transporters are synthesized within the same neurons that synthesize their neurotransmitters. Thus, for the dopaminergic, noradrenergic and serotonergic systems, mRNA for these transporters would be expected to show a distribution essentially restricted to these particular cell bodies. Based on these premises, it was surmised that the use of degenerate oligonucleotide primers from conserved regions of known transporters

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GAATTCCCTCCAGTCTACCC ATG AGT AAG AGC AAA TGC TCC GTG GGA CCA ATG TCT TCA GTG GTG GCC CCG GCT AAA GAG   60
                     MET Ser Lys Ser Lys Cys Ser Val Gly Pro Met Ser Ser Val Val Ala Pro Ala Lys Glu   20
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Ser Asn Ala Val Gly Pro Arg Glu Val Glu Leu Ile Leu Val Lys Glu Gln Asn Gly Val Gln Leu Thr Asn Ser Thr Leu    47
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ATC AAC CCG CCA CAG ACA CCA GTG GAG GCT CAA GAG CGG GAG ACC TGG AGC AAG AAA ATT GAT TTC CTG CTA TCA GTC ATC   222
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                                                             *

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Gly Phe Ala Val Asp Leu Ala Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly Gly Ala Phe Leu Val Pro   101

TAC CTG CTC TTC ATG GTT ATT GCT GGG ATG CCC CTC TTC TAC ATG GAG CTG GCT CTC GGA CAG TTC AAC AGA GAA GGA GCT   384
Tyr Leu Leu Phe Met Val Ile Ala Gly Met Pro Leu Phe Tyr Met Glu Leu Ala Leu Gly Gln Phe Asn Arg Glu Gly Ala   128

GCT GGT GTC TGG AAG ATC TGT CCT GTC CTG AAA GGT GTG GGC TTC ACT GTT ATC CTC ATC TCT TTC TAC GTG GGC TTC TTC   465
Ala Gly Val Trp Lys Ile Cys Pro Val Leu Lys Gly Val Gly Phe Thr Val Ile Leu Ile Ser Phe Tyr Val Gly Phe Phe   155

TAC AAT GTC ATC ATC GCA TGG GCA CTG CAC TAC TTC TTC TCC TCC TTC ACC ATG GAC CTC CCA TGG ATC CAC TGC AAC AAC   546
Tyr Asn Val Ile Ile Ala Trp Ala Leu His Tyr Phe Phe Ser Ser Phe Thr Met Asp Leu Pro Trp Ile His Cys Asn Asn   182

ACC TGG AAT AGC CCC AAC TGC TCC GAT GCC CAT GCC AGC AAC TCT AGC GAC GGC CTG GGC CTC AAT GAC ACC TTT GGG ACC   627
Thr Trp Asn Ser Pro Asn Cys Ser Asp Ala His Ala Ser Asn Ser Ser Asp Gly Leu Gly Leu Asn Asp Thr Phe Gly Thr   209

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Val Val Trp Ile Thr Ala Thr Met Pro Tyr Val Val Leu Thr Ala Leu Leu Leu Arg Gly Val Thr Leu Pro Gly Ala Met   290

GAT GGC ATC AGA GCA TAC CTC AGT GTG GAC TTC TAC CGA CTC TGT GAG GCA TCT GTG TGG ATC GAT GCT GCC ACC CAG GTG   951
Asp Gly Ile Arg Ala Tyr Leu Ser Val Asp Phe Tyr Arg Leu Cys Glu Ala Ser Val Trp Ile Asp Ala Ala Thr Gln Val   317

TGC TTC TCC CTC GGC GTT GGG TTT GGA GTG CTG ATT GCC TTC TCC AGT TAC AAT AAA TTC ACC AAT AAC TGC TAT AGA GAC  1032
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GCA ATC ATC ACC ACC TCC ATT AAC TCC CTG ACA AGC TTC TCC TCT GGC TTC GTC GTC TTC TCC TTC CTG GGG TAT ATG GCA  1113
Ala Ile Ile Thr Thr Ser Ile Asn Ser Leu Thr Ser Phe Ser Ser Gly Phe Val Val Phe Ser Phe Leu Gly Tyr Met Ala   371

CAG AAG CAC AAT GTG CCC ATC AGA GAT GTG GCC ACA GAT GGA CCT GGG CTC ATC TTC ATC ATC TAT CCT GAG GCG ATC GCC  1194
Gln Lys His Asn Val Pro Ile Arg Asp Val Ala Thr Asp Gly Pro Gly Leu Ile Phe Ile Ile Tyr Pro Glu Ala Ile Ala   398

ACA CTC CCG CTG TCT TCT GCC TGG GCT GCT GTC TTC TTC CTC ATG CTG CTC ACT CTG GGT ATC GAC AGT GCA ATG GGG GGC  1275
Thr Leu Pro Leu Ser Ser Ala Trp Ala Ala Val Phe Phe Leu Met Leu Leu Thr Leu Gly Ile Asp Ser Ala Met Gly Gly   425

ATG GAG TCA GTG ATC ACT GGG CTC GTC GAT GAG TTC CAG CTG CTA CAT CGG CAT CGA GAG CTC TTC ACT CTT GGC ATT GTC  1356
Met Glu Ser Val Ile Thr Gly Leu Val Asp Glu Phe Gln Leu Leu His Arg His Arg Glu Leu Phe Thr Leu Gly Ile Val   452

CTG GCT ACT TTC CTG CTG TCT CTC TTC TGC GTC ACC AAC GGC GGC ATC TAC GTC TTC ACA CTG CTG GAC CAC TTT GCA GCT  1437
Leu Ala Thr Phe Leu Leu Ser Leu Phe Cys Val Thr Asn Gly Gly Ile Tyr Val Phe Thr Leu Leu Asp His Phe Ala Ala   479

GGC ACA TCT ATC CTC TTT GGC GTG CTC ATT GAA GCC ATT GGG GTG GCC TGG TTC TAC GGC GTC CAG CAA TTC AGT GAT GAC  1518
Gly Thr Ser Ile Leu Phe Gly Val Leu Ile Glu Ala Ile Gly Val Ala Trp Phe Tyr Gly Val Gln Gln Phe Ser Asp Asp   506

ATC AAG CAA ATG ACA GGG CAG CGA CCC AAC CTG TAC TGG CGG CTA TGC TGG AAG CTG GTC AGC CCC TGC TTC CTC CTG TAT  1599
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GTG GTC GTG GTC AGC ATT GTG ACC TTC AGA CCC CCA CAC TAT GGG GCC TAC ATC TTC CCA GAC TGG GCC AAT GCC CTG GGC  1680
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TGG ATC ATC GCC ACA TCC TCC ATG GCC ATG GTG CCC ATT TAT GCG ACC TAC AAG TTC TGC AGC CTG CCG GGG TCC TTC CGG  1761
Trp Ile Ile Ala Thr Ser Ser Met Ala Met Val Pro Ile Tyr Ala Thr Tyr Lys Phe Cys Ser Leu Pro Gly Ser Phe Arg   587
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GAG AAA CTG GCC TAT GCC ATC ACA CCT AAG AAA GAC CAT CAG CTA GTG GAC AGA GGG GAG GTG CGC CAA TTC ACG CTG CGT  1842
Glu Lys Leu Ala Tyr Ala Ile Thr Pro Lys Lys Asp His Gln Leu Val Asp Arg Gly Glu Val Arg Gln Phe Thr Leu Arg   614
                             *                                                                   *
                    1857
CAC TGG CTG TTG CTG TAA AGTGGAAGGAGACAGCTGCCAGCTGGGCCACCTCACAACAGCGGGGACAGGGAGATCGCAAAGGAAACCCACGAGTCAAGGAA  1937
His Trp Leu Leu Leu Stop                                                                                   619

AGGAAGGAGCGGCCACTTCCATGCTTCTCCTTTGTCGTACGGAAAAATAATCGAAGCAAGGGCTTCAACCTTTGACTGTTCACACCCAAATCATTGCCACAAAGAAGC  2046
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AGGCTGACTCCCCCATCTGTGGGTCACTCTGGGAGAACAGGTCATACTGCCCCCTGAATTC                                                2218
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could be used to amplify by polymerase chain reaction (PCR) partial cDNA clones from specific brain areas to obtain putative related members of this family. In this paper, we report using a partial PCR cDNA clone obtained from the substantia nigra, the cloning and characterization of a full length cDNA which encodes a cocaine-sensitive brain dopamine transporter.

2. MATERIALS AND METHODS

2.1. *Drugs and chemicals*

Enzymes used in recombinant DNA manipulations were obtained from Boehringer and Promega Biotech. Chemicals were from RBI (Natick, MA). Mazindol and GBR 12783 were gifts from Dr. J.-J. Bonnet (Rouen, France), whereas cocaine and l- and d-amphetamine were obtained from Dr. E. Ellinwood (Duke University, NC). The cDNA library was a generous gift from Dr. T.P. Snutch [12].

2.2. *Amplification of mRNA by the polymerase chain reaction*

Total mRNA was extracted from rat substantia nigra using the method of Chomcynsky and Sacchi [13]. First-strand synthesis of cDNA was performed using a cDNA synthesis kit (Invitrogen) according to the supplier's instructions, except that mRNA was primed with a degenerate 21mer oligonucleotide derived from the alignment of the sixth transmembrane (TM 6) segment of the rat GABA transporter and human NA transporter [8,9], ATCTG(A/G)GT(G/T)GCGGC(A/G)TCIA(T/G)C (Primer A) at a final concentration of 400 nM. Double-stranded cDNA was synthesized and amplified using 2.5 U Taq Polymerase (Cetus) and 75 nM primers A and B (AA(C/T)GT(G/A)TGG(A/C)G(G/C)TTCCCITA, derived from the consensus alignment of TM 1 (as above) in 50 μl of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dNTP, 0.5 mM dithiothreitol and 0.01% gelatin, for 30 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 80 s, ended by 12 min at 72°C. The products of this reaction were resolved on a 1% agarose gel and the cDNA ranging from 500–800 bp was extracted (Gene-clean, BIO 101) and an aliquot re-amplified using primers C, located in TM 6 (TACGAATTCTG(A/G)GT(G/T)GCGGC(A/G)TCIATCCA) and D, located in TM 1 (GCCGAATTCAA(C/T)GT(G/A)TGGAGGTTCCCITA) for 30 cycles at 94°C for 1 min, 54°C for 45 s, and 72°C for 80 s, ended by 12 min at 72°C. These primers each contained *Eco*RI restriction sites. The cDNA obtained (~700 bp) was then digested with *Eco*RI and subcloned into pBlueScript (Stratagene) both for sequencing and synthesis of cRNA probes.

2.3. *PCR-based library screening*

Two specific primers, E (TGCCCTACCTGCTCTTCATGG, nucleotides 299–318 on Fig. 1) and F (GCCCAGGTCATCAATGCCA-C, nucleotides 680–699 on Fig. 1) were designed from the sequence of clone PCT2 and used to amplify the DNA from 15 batches comprising each 150 000 individual clones from a high molecular weight-enriched cDNA library from total brain, constructed in λZAP [12]. One of these samples revealed the presence of a specifically amplified material, and the 150 000 starting clones from this batch were split into 25 fractions of ~10 000 pfu, among which one fraction was positive again after PCR amplification. The lambda clones were then plated and lifted on nitrocellulose filters (Schleicher and Schluell), and hybridized with a nick-translated probe obtained from PCT2, as described elsewhere [14]. The pBlueScript was excised from the purified phage, and a 2.2 kb fragment was sequenced and subcloned into pCMV5 [15] for expression. Screening of a *Sau*3A partial rat genomic library (Stratagene) and rat genomic Southern blot analysis with a nick-translated probe from clone PCT2 were performed as described [14].

2.4. *In situ hybridization*

The PCT2 clone which encodes sequences of the transporter protein from the putative TM 1 to TM 6 ligated into the *Eco*RI site of pBlueScript was used to synthesize sense and antisense strand cRNA probes. Uridine 5'-([α-^{35}S]thio)triphosphate-labeled probes were prepared by in vitro transcription of circular templates with T7 and T3 RNA polymerases, to a specific activity of $>10^9$ cpm·μg^{-1}. In situ hybridization was conducted as previously described [16]. Briefly, frozen rat brain sections were thawed and fixed for 10 min in 4% paraformaldehyde with phosphate buffered saline at 4°C. Sections were hybridized with heat-denaturated probes ($2–5\times10^6$ cpm·ml^{-1}) and treated with RNase A at 50 $\mu g \cdot ml^{-1}$. Sections were washed in 4 l of 0.1X standard saline citrate, 14 mM 2-mercaptoethanol, 0.05% sodium pyrophosphate for 3 h at 55–58°C and cooled to room temperature gradually over 12–16 h. Slides were exposed at −80°C to Kodak XAR film for 6 days.

2.5. *^{3}H-Dopamine uptake experiments*

African green monkey kidney (COS-7) cells were transiently transfected with a pCMV5-TS3 construct using the lipofectine method (Gibco BRL) in 12-well tissue culture plates (22-mm diameter) according to the supplier's instructions. In brief, each well containing $4–5\times10^5$ subconfluent COS-7 cells was washed 3-times with 0.5 ml of serum-free DMEM medium before adding 15 μl of a mixture containing 0.5 μg DNA and 5 μg lipofectine. After 5–7 h incubation at 37°C, the wells were supplemented with an additional 0.5 ml DMEM medium containing 20% fetal bovine serum and the antibiotic gentamycin (10 000 U/l) and incubated an additional 48 h at 37°C in a 5% CO_2 incubator.

For uptake experiments, the DMEM medium was replaced by 5 mM Tris-base, 7.5 mM HEPES, 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM ascorbic acid, 5 mM D-glucose, final pH 7.1 (uptake buffer). At the end of the incubation, cells were washed 3-times in the uptake buffer containing 0.5 mM nomifensine, and then resuspended in 0.5 ml of 0.1 N NaOH for 1 h. An aliquot was then taken for scintillation counting. For determination of inhibition of uptake, cells were incubated 2 min with the drug to be tested, and then 15 nM of ^{3}H-dopamine was added in a final volume of 0.5 ml for 5 min. To determine the Na^+ dependency, NaCl was substituted with 140 mM LiCl. The K_i values were determined using the EBDA and LIGAND programs [17].

3. RESULTS AND DISCUSSION

The recent cloning and molecular characterization of specific transporter proteins for the neurotransmitters GABA and NA has revealed the emergence of a novel family of transmembrane proteins with similar putative topography and significant amino acid identity [8,9]. Since NA and dopamine are both catecholamines, it may be expected that a specific dopamine transporter may share significant homology with both the GABA and NA transporters. To test this hypothesis, and attempt to isolate a putative dopamine transporter, a set

Fig. 1. Nucleotide and deduced amino acid sequences of the rat TS3 clone. Nucleotides and amino acids are numbered (right side) consecutively from the putative translation initiation codon. Solid bars represent putative transmembrane domains. Cross-hatched bars indicate consensus sites for *N*-glycosylation. Asterisks indicate potential sites of protein kinase A and C phosphorylation on presumed intracellular domains. Arrows indicate the location of intron-exon junctions mapped from the partial sequence of a rat genomic clone.

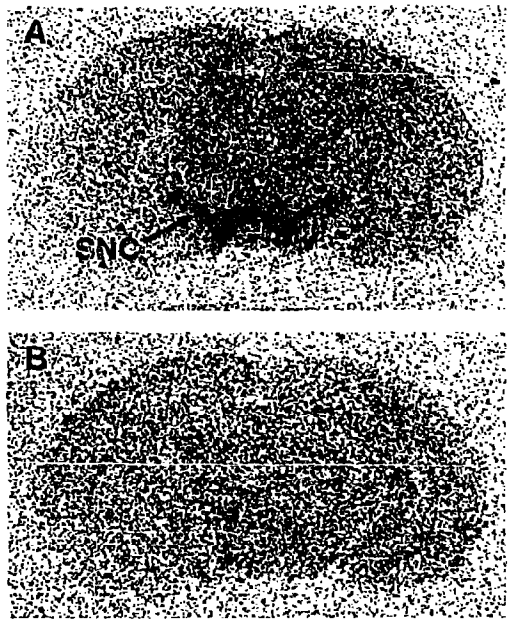

Fig. 2. Localization of the dopamine transporter mRNA in rat brain by in situ hybridization. Consecutive coronal sections (Bregma-5) were incubated with cRNA probes in the antisense (A) and sense (B) orientation as described in section 2. SNC: substantia nigra pars compacta; VTA: ventral tegmental area. No positive signal was observed in other sections at the level of the caudate putamen and nucleus accumbens, areas where functional dopamine uptake is present.

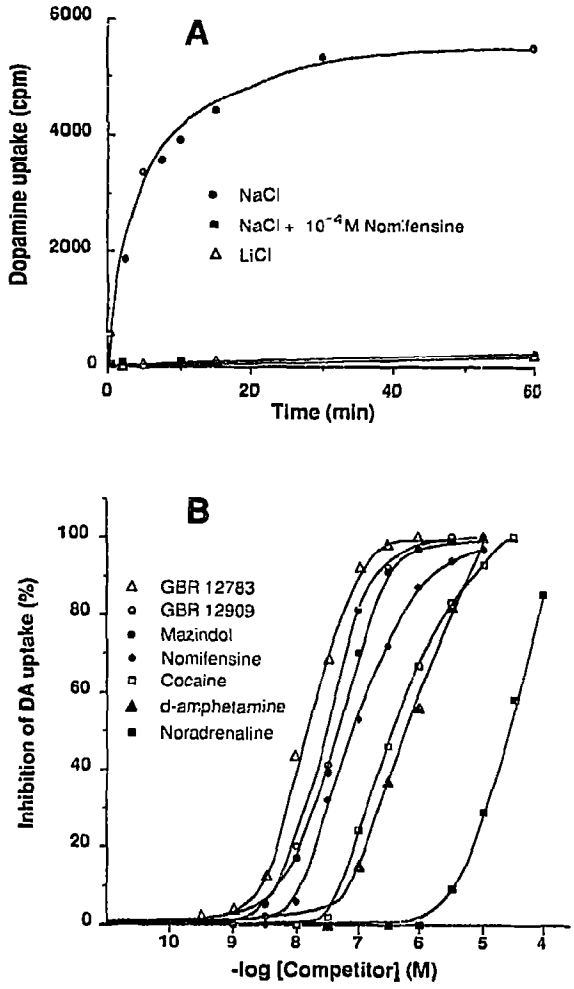

Fig. 3. Pharmacological characterization of the expressed dopamine transporter in COS-7 cells. (A) Time-course of dopamine uptake. Cells were incubated as described in section 2 with 15 nM ^{3}H-dopamine. (B) Competition of ^{3}H-dopamine-uptake by various neurotransmitters and uptake blockers. Results are the mean of triplicate determinations from a single experiment and are representative of 2–3 similar determinations.

of degenerate primers derived from conserved sequences among the GABA and NA transporters in the first (primers B and D, nucleotides 244–264 in Fig. 1) and sixth (primers A and C, nucleotides 948–928 in Fig. 1) TM were synthesized and used for PCR amplification (see Materials and Methods). To increase the probability of amplifying a dopamine transporter, we used as template mRNA from substantia nigra, a brain nucleus which mainly comprises dopaminergic cell bodies. Among the clones obtained, one (PCT2) displayed a high level of identity in its nucleotide sequence both with the human NA (70%) and rat GABA (66%) transporters.

In an attempt to isolate a full length cDNA clone, a PCR-based library screening approach was used. Two specific oligonucleotides (primers E and F) were synthesized based on the sequence of PCT2 clone, and employed to amplify DNA from a cDNA library (see Materials and Methods). Three identical clones (TS1, TS2 and TS3) were finally isolated by plaque hybridization, and their 2.2 kb cDNA insert used for sequencing and expression in mammalian cells. The nucleotide and deduced amino acid sequence of clone TS3 is shown on Fig. 1. An open reading frame for a putative protein of 620 residues (M_r 69 029) starts with methionine at base 1 and extends to base 1860 (Fig. 1) which is followed by a stop codon. The methionine tentatively assigned as the start of translation for this protein was chosen on the basis that it conformed to the Kozak consensus sequence [18]. Although no in-frame stop codon could be identified in the 12 bp 5′ upstream of this putative start site, the protein sequence matches fairly well with that of other members of this protein family. Hydropathicity analysis (not shown) of the deduced amino acid sequence suggests that this protein comprises 12 stretches of 22–24 amino acids (underlined in Fig. 1) that may represent transmembrane spanning domains. The deduced sequence indicates the presence of 4 putative sites for *N*-glycosylation, all contained within the sequence which lies between proposed TM 3 and TM 4. The lack of a readily identifiable signal sequence peptide suggests that the N-terminal segment of the protein resides on the cytoplasmic side of the plasma membrane. These various features of the protein encoded by this TS3 clone strikingly resemble the molecular properties of transporter proteins for GABA and NA [8,9].

To determinate initially whether the isolated PCT2 clone might represent a specific transporter for the biogenic amine dopamine, in situ hybridization was performed on rat brain sections. In the central nervous system (CNS) dopamine is mainly synthesized in the substantia nigra pars compacta (SNC, A9) and in the ventral tegmental area (VTA, A10). Fig. 2A shows that an antisense cRNA probe synthesized from the PCT2 clone hybridizes selectively to both the SNC and the VTA on coronal brain sections. In Fig. 2B the same experiment was repeated on a consecutive section with a sense cRNA-labelled probe. Under these conditions no radiolabelling of the A9 and A10 dopaminergic neurons was observed thus confirming the specificity of the signal observed in Fig. 2A. Thus, the distribution of the mRNA encoding for this protein is consistent with it being a dopamine transporter.

To confirm the identity of this presumed dopamine transporter, transient expression of the protein was examined in COS-7 cells using the 2.2 kb cDNA clone subcloned into pCMV5. As shown in Fig. 3A expression of the cDNA established in these cells a time- and sodium- dependent uptake of dopamine. At that concentration of dopamine (15 nM) a plateau was reached between 20–30 min. The uptake of dopamine was specifically blocked by the uptake blocker, nomifensine, or in the absence of Na^{2+} (i.e. presence of Li^{+}). The K_m for dopamine uptake was ~300 nM. The ability of several neurotransmitters and uptake blockers to compete for the uptake of dopamine by transfected COS-7 cells was determined (Fig. 3B and Table I). The selective dopamine uptake blockers GBR-12783 [19], GBR-12909 [20] and mazindol, were the most potent competitors of uptake. Nomifensine and cocaine showed intermediate levels of potency. D- and L-amphetamine showed about a 10-fold stereoselectivity in their ability to decrease ^{3}H-dopamine-uptake. Several other compounds listed in Table I, like the tricyclic anti-depressants, nortryptiline or desipramine, displayed K_i values greater than 1 μM for inhibition of uptake, as expected [21]. Noteworthy is the fact that the K_i value for cocaine is similar to that which has been reported previously for the cloned human NA transporter (300 vs. 150 nM) [9]. Noradrenaline has a K_i of 10 μM at this cloned transporter, a value significantly greater than that which has been previously reported for the ^{3}H-dopamine transporter in rat brain (1 μM) [22]. Thus, the pharmacological profile of the uptake of dopamine by transfected COS-7 cells confirms the identity of this protein as a dopamine transporter protein.

Comparison of the amino acid sequence of this rat dopamine transporter with the sequence of the previously cloned GABA and NA transporters reveals a significant degree of amino acid conservations. The rat dopamine transporter is 64% identical to the human NA transporter and 30% to the rat GABA transporter. These identities are even more pronounced when comparing sequences within the putative transmembrane domains (74% with NE and 49% with GABA transporters). An alignment (not shown) of the amino acid sequences of this dopamine transporter with those for the NA and GABA transporters shows that these 3 proteins obviously belong to the same family of proteins. Their amino- and carboxyl-terminal domains are of similar lengths. Various intra- and extracellular connecting loops display similar sizes. In addition, specific motifs appear to be conserved, as there are a total of 217 amino acids that are invariant between all 3 proteins.

Table I

Pharmacology of the rat dopamine transporter expressed in COS-7 cells

Compound	K_i(nM)	Hill coefficient
GBR 12783	11	0.83
GBR 12909	12	1.28
Mazindol	27	1.12
Nomifensine	118	0.81
Dopamine	316	1.38
Cocaine	336	0.83
D-Amphetamine	881	0.93
L-Amphetamine	9590	1.29
6-OH-Dopamine	>1000	nd
Reserpine	>1000	nd
Epinephrine	>1000	nd
Norepinephrine	>10,000	nd
Histamine	>10,000	nd
Amitryptiline	>10,000	nd
Desipramine	>10,000	nd
GABA	>10,000	nd
Serotonin	>10,000	nd

COS-7 cells were transiently transfected with a pCMV5-TS3 construct as described in Materials and Methods. Dopamine uptake was measured in the presence of 15 nM ^{3}H-dopamine and at least 6 concentrations of each compound as listed. Each point represents the mean of 3 similar experiments. nd, not determined.

Preliminary experiments indicate that the gene for this rat dopamine transporter will display the usual complex structure of eukaryotic genes. Partial sequencing of the rat genomic clone revealed the presence of at least 2 introns in this gene (indicated by the arrow heads in Fig. 1). The complexity of the gene structure for this transporter is confirmed by the results of genomic Southern blot analysis (Fig. 4). Using several restriction enzymes on rat genomic DNA probed with the PCT2 clone, multiple hybridizing bands were obtained even for restriction enzymes for which no restriction sites exist in the cDNA (all but *Bgl*II) (Fig. 4, legend).

The cloning of a cDNA for a dopamine transporter indicates that a single polypeptide chain is capable, when expressed into a host cell, of recapitulating the function of the CNS dopamine transporter with appropriate pharmacological characteristics and dependence on Na^{+}. The distribution of the mRNA for this transporter protein in the areas of dopaminergic cell bodies suggests that this protein may be the main component

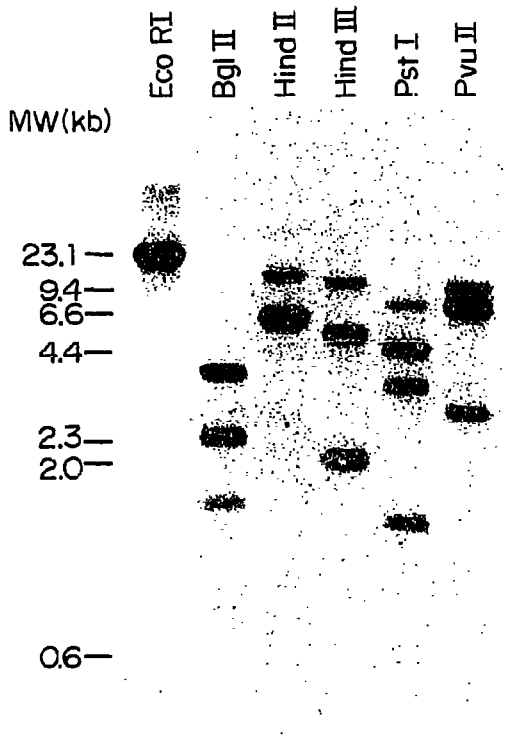

Fig. 4. Southern blot of rat genomic DNA. Rat genomic DNA (10 μg/lane) was digested with the restriction enzymes shown, subjected to agarose gel electrophoresis, blotted onto nitrocellulose and probed with the PCT2 clone. The filter was washed in 2× SSC, 0.1% SDS, two-times for 15 min at 37°C and then 0.1× SSC, 0.1% SDS for 15 min at 37 and 45°C, consecutively. The autoradiogram was developed for 48 h. The position of molecular weight standards are indicated on the right. The PCT2 probe did not contain restriction sites for any enzyme except *Bgl*II (1 site).

for the termination of dopaminergic neurotransmission. In vitro and in vivo pharmacological studies have suggested that this transporter can interact with several classes of neurotransmitter-uptake inhibitors [23]. The most well known example of this would be the interaction of this transporter with the substance of abuse, cocaine. Indeed, the rewarding and addictive effects of cocaine administration seem to be mediated by its interaction with a dopamine transporter in dopaminergic innervated areas of the CNS. Thus, the elucidation of the structure of this transporter should facilitate studies of the structure and function of drugs that interact with this transporter or into the mechanisms mediating reward and addiction to substances of abuse. In addition, since the function of dopamine is impaired in many neuropsychiatric disorders, the availability of probes for a dopamine transporter may help in assessing whether the gene for this transporter might bear relations to the etiology of these disorders.

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