J. exp. Biol. ***196****, 229–236 (1994)*
Printed in Great Britain © The Company of Biologists Limited 1994

NEUROTRANSMITTER TRANSPORTERS: THREE IMPORTANT GENE FAMILIES FOR NEURONAL FUNCTION

GEORGE R. UHL* AND PETER S. JOHNSON

Molecular Neurobiology Branch and Office of the Director, Intramural Research Program, NIDA, and Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MA 21224, USA

Summary

Three distinct gene families encode transporter proteins that aid in temporal and spatial buffering of neurotransmitter and neurotransmitter metabolite concentrations and allow neurons to cycle and recycle transmitter molecules. Analyses of these gene families and their products are likely to enhance understanding of the molecular neurobiology of neuronal function and may elucidate contributors to the genetic etiologies of neurological and psychiatric disease.

Introduction

Neurons and glial cells use transporter molecules that are products of at least three distinct gene families to aid in temporal and spatial buffering of neurotransmitter and neurotransmitter metabolite concentrations and to allow cycling and recycling of transmitter molecules (Iverson and Kelly, 1975; Snyder *et al.* 1970). Neurotransmitters can consist of ubiquitous molecules, such as glutamate, that play multiple metabolic roles in the nervous system. Transporters located on neuronal and glial plasma membranes can serve to maintain local concentrations of glutamate at levels appropriate for amino acidergic neurotransmission, preventing most fluxes of dietary or other origins from overwhelming this system (Pines *et al.* 1992; Storck *et al.* 1992; Kanai and Hediger, 1992). Spatial buffering may also occur for other neurotransmitters. Re-uptake may prevent diffusion of many neurotransmitters away from the synapses of their release, thus minimizing chemical crosstalk between adjacent synapses. Transporters can alter temporal features of neurotransmission as well. Rapid inactivation of released neurotransmitter by transport back into presynaptic or other elements can allow more rapid temporal parameters of neurotransmission than those possible when diffusion away from the site of release is a primary mechanism of termination of action. Transporters allow recycling. Re-uptake of many transmitters into the nerve terminal and then into the vesicular compartment allows the same molecules of neurotransmitter to be utilized again, with presumed savings in synthetic costs. Indeed, vesicular transporters appear to

*To whom reprint requests should be addressed: Molecular Neurobiology, NIDA-IRP, Addiction Research Center, Box 5180, Baltimore, MA 21224, USA.

Key words: cocaine, Parkinson’s disease, symporters, antiporters.

function in the accumulation of even non-recycled or newly synthesized transmitters of many classes into the vesicular compartments from which they are released (Surratt *et al.* 1993*a*,*b*).

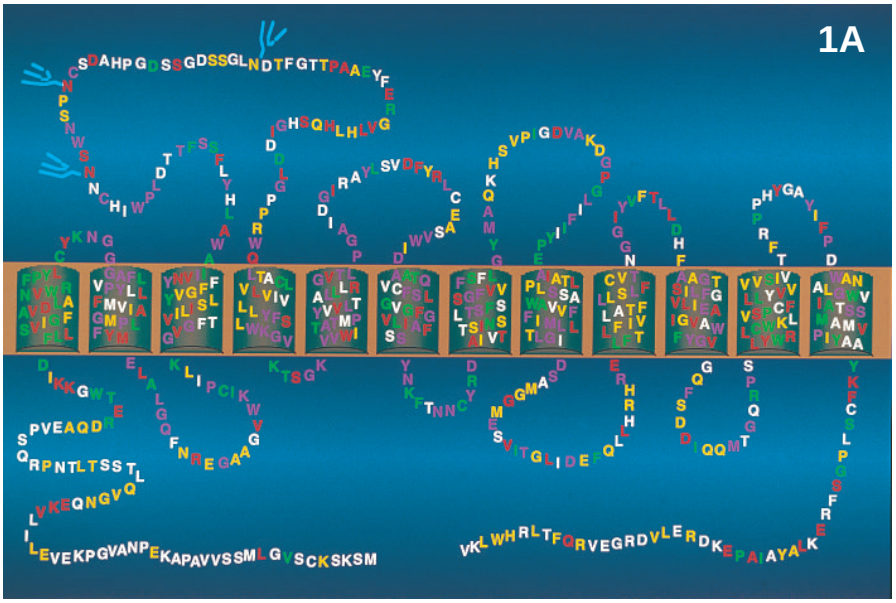
Transporters are central to the actions of numerous drug classes and to the actions of specific toxins that affect the nervous system (Kuhar *et al.* 1991; Rothman *et al.* 1989; Ritz *et al.* 1987; Snyder and D'Amato, 1986). Monoamine transporters, for example, are targeted by major classes of antidepressant, psychostimulant and antihypertensive drugs (Hollister, 1986; Fuller and Wong, 1990). They are also necessary for the actions of several classes of neurotoxins that selectively poison specific neuronal groups (Javitch *et al.* 1985; Snyder and D'Amato, 1986). As a result of these activities, the members of these three gene families become candidate genes for involvement in several neuropsychiatric disorders (Uhl *et al.* 1994).

The sodium- and chloride-dependent neurotransmitter transporter gene family

The genes of the sodium- and chloride-dependent neurotransmitter transporter gene family encode glycoproteins with 12 putative transmembrane regions that mediate sodium-dependent re-accumulation of released neurotransmitters into presynaptic terminals and are the sites of action of several important abused and therapeutic drugs (Uhl, 1992; Uhl and Hartig, 1992; Surratt *et al.* 1993*a*,*b*; Amara and Kuhar, 1993). These genes are largely expressed by neurons, often in highly specific regions of the nervous system. The glycoproteins that they encode are symporters that use transmembrane sodium gradients, and differentially involve chloride and potassium gradients, to drive cellular accumulation of neurotransmitters. The sequence relationships between members of this family suggest evolutionary relatedness into two distinct 'major' subclasses, which share morphological distinctions, and into further 'minor' subfamilies.

The neurotransmitter transporters for which substrates are currently identified fall into one 'major' subclass. The basic properties of neurotransmitter transport by members of this class were elucidated in classical studies of the uptake of radiolabelled monoamines in brain slices and synaptosomal preparations of pinched-off nerve endings by these transporters (see Iverson and Kelly, 1975). These studies first revealed the striking Na^+- and Cl^--dependence of these neurotransmitter/ion symporters that appear to derive their ability to concentrate neurotransmitters largely by concurrent movement of Na^+ down its electrochemical gradient. The affinities of neurotransmitter transporters for their substrates are generally lower than the affinities of the receptors for the corresponding transmitters. The regional distributions of transport activity are often consistent with the distributions of the released neurotransmitters, suggesting that transporters might be expressed in a fashion specific for the neurotransmitter system. Such transporters are chiefly neuronal, although glial subtypes have been proposed (Uhl and Hartig, 1992).

Examination of members of this major subfamily of the Na^+- and $C1^-$-dependent neurotransmitter transporters reveals that they fall into at least two minor subfamilies, each of which is characterized by large second extracellular domains that are likely to be the primary sites for the N-linked glycosylation characteristic of several members of this class. The first minor subfamily includes the dopamine, norepinephrine and serotonin


1A

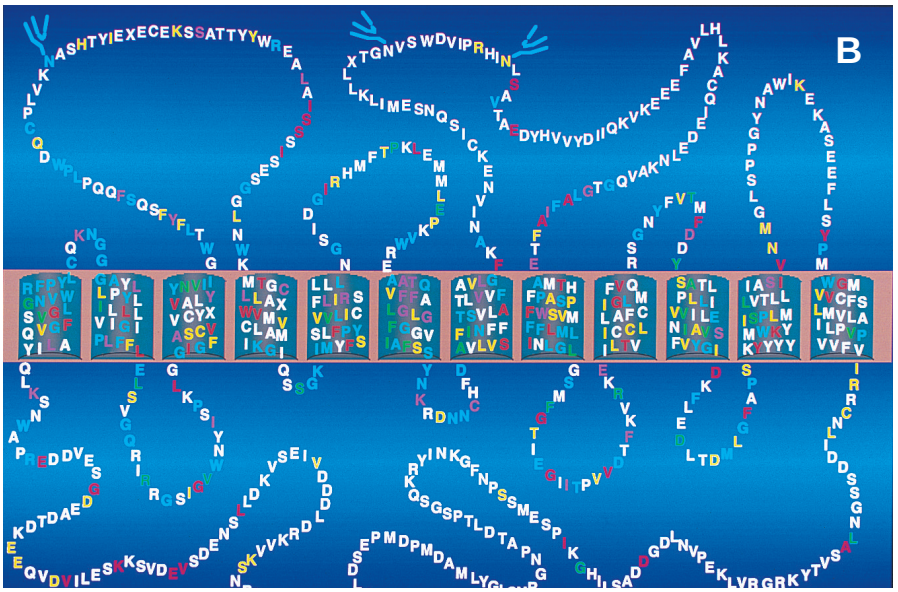

B

Fig. 1. (A,B) Diagrams depicting the amino acid sequence of the (A) human dopamine transporter (Vandenbergh *et al.* 1992) and the (B) rat 'orphan' transporter (Uhl *et al.* 1992) with currrent assignment of sodium- and chloride-dependent neurotransmitter transporter gene subfamily topology and subfamily relatedness. Residues that are positioned uniquely in comparison to other published transporter sequences are indicated in white, those that appear in the same position as in one other published sequence are indicated in yellow, those that are positioned similarly in two others in red, to three others in green and to four others in purple. Transmembrane domains are indicated by helices, although varying α-helical contents are contained in each sequence. Assignment of the exact borders of putative transmembrane domains varies from one publication to the next. Sites for potential N-linked glycosylation are indicated by pale blue branches. Loop sizes and glycosylation site number and position vary among members of each subfamily.

monoamine transporters, which are encoded by cDNAs that are closely related to each other (Shimada *et al.* 1991; Uhl, 1992; Figs 1, 2). The other minor subfamily of the amino acid transporters mediates sodium- and chloride-dependent uptake of amino acids. These relationships can be seen in the sequence relationships documented in Figs 1 and 2). As also noted by Nelson and Lill (1994), the glycine transporters may represent the prototype for an additional minor subfamily that also contains the proline transporter. Relationships can also be noted in studies of gene structure. The serotonin and dopamine transporter genes share a feature not found in the GABA transporter; each monoamine transporter gene encodes the large, second extracellular loop on an exon that also encodes the third transmembrane domain, whereas these domains are split by an intron in the murine $GABA_A$ transporter gene (D. Vandenbergh and G. R. Uhl, in preparation; Lesch *et al.* 1994; Liu *et al.* 1992).

The second 'major' subfamily of this 12-transmembrane domain family consists of products of three genes that appear to encode transporters which differ structurally from the first subfamily; they display large second and fourth extracellular domains with sites for N-linked glycosylation in both large domains. Substrates for neither the initial member of this family that we reported in 1992 nor other members described subsequently have been identified (Uhl *et al.* 1992; Liu *et al.* 1992). Nevertheless, the brain regional distribution patterns of at least the first member of this family suggest that it could play a specific and unique role in brain function (G. Uhl and S. Shimada, in preparation; Nelson and Lill, 1994). These 'orphan transporters' challenge our skills at elucidation of transporter functions.

Sodium-dependent glutamate transporters define a second transporter gene family

Glutamate represents the major excitatory neurotransmitter in the brain. Pharmacological studies in tissue preparations have identified sodium-dependent glutamate transport although no member of the sodium- and chloride-dependent neurotransmitter transporter gene family that transports glutamate has yet been identified. Conversely, nearly simultaneous reports by Pines *et al.* (1992), Kanai and Hediger (1992) and Storck *et al.* (1992) established a new gene family of transporters that displayed substantial functional and structural differences from the first gene family. These genes

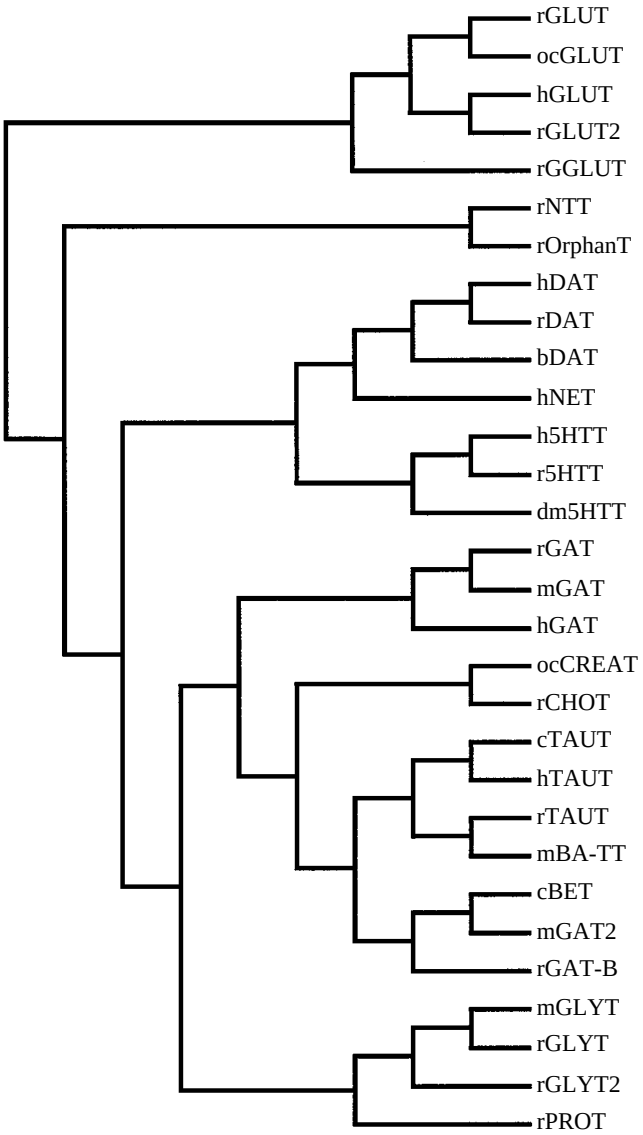


Fig. 2. Dendrogram depicting hierarchies of sequence relatedness of the proteins predicted from sodium-dependent (GLUT) and sodium- and chloride-dependent (others) transporter gene family members, as compiled by the program 'PILEUP' (University of Wisconsin, Genetics Computer Group). The branches indicate distinct subfamilies for glutamate, glycine, other amino acid, monoamine and 'orphan' transporters. The GENBANK sequence accession numbers are: rGLUT (S49854), ocGLUT (L12411), hGLUT (L19158), rGLUT2 (S59158), rGGLUT (X67857), rNTT (S52051), rOrphant (L22022), hDAT (M95167), rDAT (M80570), bDAT (M80234), hNET (M65105), h5HTT (L05568), r5HTT (X63252), dm5HTT (U02296), rGAT (M59742), mGAT (S42126), hGAT (X54673), ocCREAT (X67252), rCHOT (X66494), cTAUT (M95495), hTAUT (Z18956), rTAUT (M96601), mBA-TT (L03292), cBET (M80403), mGAT2 (M97632), rGAT-B (M95738), mGLYT (X67056), rGLYT (L13600), rGLYT2 (L21672) and rPROT (M88111) (P. S. Johnson and G. R. Uhl, unpublished observations).

encode transporters that are sodium-dependent, but only one report describes any chloride-dependence. Furthermore, their topologies are difficult to arrange in the 12-transmembrane domain structure now fashionable for the members of the first gene family: 6–9 transmembrane domains are postulated for most of these proteins. These transporters do exhibit some structural similarity to the H^+/glutamate transporter of *Escherichia coli.*

The cellular localizations of these transporters' expression are also interesting. The transporter cloned by Karai and Hediger (1992), EAAC1, is largely expressed by neurons, whereas the others are largely expressed by glia. Clearly, each type is well-positioned to act in the spatial buffering of glutamate necessary for both proper synaptic function and, possibly, for the prevention of excitotoxicity.

The proton-dependent vesicular monoamine and acetylcholine carriers define a third gene family

Cloning of the tetrabenazine- and reserpine-sensitive vesicular monoamine transporters from brain (VMAT2) and adrenal gland (VMAT1) from rat and human established the paradigm for the (possibly) 12-transmembrane domain molecules that utilize the striking proton gradients maintained across the vesicular membrane by H^+ V-ATPase function to allow accumulation of monoamines into vesicles (Edwards, 1992; Erickson *et al.* 1992; Surratt *et al.* 1993*a*). Studies of a gene whose mutants resulted in an uncoordinated *Caenorhabditis elegans* motor phenotype established the apparent vesicular acetylcholine transporter gene for this species (Alfonso *et al.* 1993). Each protein expresses a single large loop, but the protein sequences are largely hydrophobic and most residues are thus likely to reside within the vesicular membrane. Localization studies have revealed that the *VMAT2* gene is expressed in norepinephrine, serotonin, dopamine and in apparently histaminergic neurons (Erickson *et al.* 1992; Gonzales and Uhl, 1994). These localizations are consistent with its role as the physiological monoamine vesicular transporter.

Transporters as pharmacological targets and possible players in disease processes

The functions of transporters in terminating the synaptic activities of released neurotransmitters can be modulated by several important drug classes. Transporters are key molecular targets for psychostimulants, with several lines of evidence suggesting that cocaine's rewarding and reinforcing actions in the brain are due to activities at dopamine transporters, although the drug also possesses reasonable affinities for the other sodium- and chloride-dependent plasma membrane monoamine transporters and for sodium channels (Ritz *et al.* 1987; Kuhar *et al.* 1991). Effective antidepressants share the ability to inhibit the serotonin transporter, although many also block other monoamine carriers (Fuller and Wong, 1990).

Transporter-mediated accumulation and/or sequestration of neurotoxins play key roles in several current experimental models of the specific dopaminergic neurodegenerative processes found in Parkinson's disease (Snyder and D'Amato, 1986). The substituted

phenylpyridinium dopaminergic neurotoxin MPP+ can be rendered toxic to previously resistant non-neuronal cells if they are allowed to accumulate it through their expression of the plasma membrane dopamine transporter (Kitayama *et al.* 1992). Conversely, cells become resistant to this poison of the mitochondrial electron transport chain if they overexpress the vesicular monoamine transporter, which can function to sequester MPP+ into vesicular compartments and remove it from places where it can intoxicate mitochondria (Edwards, 1992). In brains from patients with Parkinson's disease, certain dopaminergic neuronal cell groups are heavily damaged, while others are relatively spared (Uhl, 1990). Studies of the neuronal levels of expression of the dopamine transporter mRNA in these different cell groups in rat and human brain now document a good fit between transporter expression and this differential dopaminergic pathology (Shimada *et al.* 1992; Cerrutti *et al.* 1993; Uhl *et al.* 1994). The most-depleted cell groups are those that manifest the highest levels of the dopamine transporter's mRNA in normal brains. It will be interesting to see whether parallel studies of cellular expression of the *VMAT2* gene give results that can be interpreted in the light of its 'protective' effect in sequestering toxin in cultured cell systems.

Overactivity of neuronal systems that utilize excitatory amino acid neurotransmitters has been shown to lead to neurodegeneration in many settings. Failure of neuronal or glial transporters adequately to remove released or endogenous glutamate from the vicinities of receptors, where it can exert powerful excitatory influences, would be expected to contribute substantially to epileptogenesis in the short term and to excitatory neurotoxicity in the longer term.

Identification of the genes encoding these transporters, and interindividual variants in these genes in humans, will aid in attributing specific pathological features to transporter gene variants. Study of the human dopamine transporter cDNAs and genes, for example, revealed that a novel, roughly 40 base pair, repetitive element is present in the 3′ untranslated region of the mRNA encoding this transporter (Vandenbergh *et al.* 1992). Further studies revealed that different copy numbers were present in different individuals; this marker represented a VNTR (*v*ariable *n*umber *t*andem *r*epeat). Since 3′ untranslated regions may contribute to mRNA stability or instability in other genes, we are examining the expression of dopamine transporter mRNAs with differing copies of this VNTR. Observation of different levels of transporter expression conferred by different VNTR copy numbers would represent the first example of polymorphism of a transporter gene conferring a functionally significant difference in transporter function, if it also occurs in the brain.

Experimental animal models of altered transporter expression may also suggest that altered functional transporter expression can have consequences, including consequences for the animals' behavior. In several transgenic mouse lines in which the tyrosine hydroxylase promoter sequences that confer catecholaminergic expression of dopamine transporter variants are used to drive expression of the dopamine transporter, higher-order behavioral manifestations of 'learned' 'memory-like' components driven by environmental exposure and psychostimulant drugs are altered (L. Miner, D. Donovan and G. R. Uhl, in preparation). In these transgenic animal lines, when compared with wild-type littermate controls of the same sex, transporter-overexpressing

animals display more rapid habituation to repeated exposure to the same environment and more striking conditioned place preference responses to cocaine administration. Since the levels of overexpression induced by these constructions are relatively modest, these data suggest that interindividual human transporter gene variants could exert substantial and clinically significant effects on important behaviors, including those that contribute to drug abuse. Current studies will assess differences in susceptibility to dopaminergic neurotoxins in some of these same animals; conceivably, such studies may indicate possible means whereby transporter genotype could predispose to cell-selective neurodegeneration.

It is a pleasure to acknowledge the contributions of S. Shimada, S. Kitayama, P. Gregor, B. O'Hara, T. Nishimori, E. Nanthakumor, A. Patel, J. Boja, C. Surratt and C. Sneeringer to the work from this laboratory and numerous helpful discussions with M. Kuhar. The work from this laboratory was supported by the intramural program of the National Institute on Drug Abuse, the Hereditary Disease Foundation and the American Parkinson's Disease Association. P.S.J. is a PRAT Fellow, National Institute of General Medical Sciences.

References

ALFONSO, A., GRUNDAHL, K., DUERR, J. S., HAN, H.-P. AND RAND, J. B. (1993). The *Caenorhabditis elegans unc-17* gene: a putative vesicular acetylcholine transporter. *Science* **261**, 617–619.

AMARA, J. AND KUHAR, M. J. (1993). Neurotransmitter transporters: recent progress. *Annu. Rev. Neurosci.* **16**, 73–93.

CERRUTI, C., PILOTTE, N. S., UHL, G. AND KUHAR, M. J. (1994). Reduction in dopamine transporter mRNA after cessation of repeated cocaine administration. *Molec. Brain Res.* **22**, 132–138.

EDWARDS, R. H. (1992). The transport of neurotransmitter into synaptic vesicles. *Curr. Opin. Neurobiol.* **2**, 586–594.

ERICKSON, J. D., EIDEN, L. E. AND HOFFMAN, B. J. (1992). Expression cloning of a reserpine-sensitive vesicular monoamine transporter. *Proc. natn. Acad. Sci. U.S.A.* **89**, 10993–10997.

FULLER, R. W. AND WONG, D. T. (1990). Serotonin uptake and serotonin uptake inhibition. *Ann. N.Y. Acad. Sci.* **600**, 68–80.

GONZALEZ, A. M. AND UHL, G. R. (1994). 'Choline/orphan V8-2-1/creatine transporter' mRNA is expressed in nervous, renal and gastrointestinal systems. *Molec. Brain Res.* **23**, 266–270.

HOLLISTER, L. (1986). Current antidepressants. *A. Rev. Pharmac. Toxicol*. **26**, 23–37.

IVERSEN, L. L. AND KELLY, J. S. (1975). Uptake and metabolism of gamma-aminobutyric acid by neurones and glial cells. *Biochem. Pharmac.* **24**, 933–938.

JAVITCH, J. A., D'AMATO, R. J., STRITTMATTER, S. M. AND SNYDER, S. H. (1985). Parkinsonism-inducing neurotoxin. *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite *N*-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2173–2177.

KANAI, Y. AND HEDIGER, M. A. (1992). Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature* **360**, 467–471.

KITAYAMA, S., SHIMADA, S., XU, H., MARKHAM, L., DONOVAN, D. M. AND UHL, G. R. (1992). Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding. *Proc. natn. Acad. Sci. U.S.A.* **89**, 7782–7785.

KUHAR, M. J., RITZ, M. C. AND BOJA, J. W. (1991). The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci.* **14**, 299–302.

LESCH, K.-P., BALLING, U., GROSS, J., STRAUSS, K., WOLOZIN, B. L., MURPHY, D. L. AND RIEDER, P. (1994). Organization of the human serotonin transporter gene. *J. neural Transm.* **95**, 157–162.

LIU, Q.-R., MANDIYAN, S., NELSON, H. AND NELSON, N. (1992). A family of genes encoding neurotransmitter transporters. *Proc. natn. Acad. Sci. U.S.A.* **89**, 6639–6643.

NELSON, N, AND LILL, H. (1994). Porters and neurotransmitter transporters. *J. exp. Biol.* **196**, 213–228.

PINES, G., DANBOLT, N. C., BJORAS, M., ZHANG, Y., BENDAHAN, A., EIDE, L., KOEPSELL, H., STORM-MATHISEN, J., SEEBERG, E. AND KANNER, B. I. (1992). Cloning and expression of a rat brain L-glutamate transporter. *Nature* **360**, 464–467.

RITZ, M. C., LAMB, R. J., GOLDBERG, S. R. AND KUHAR, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* **237**, 1219–1223.

ROTHMAN, R. B., MELE, A., REID, A. A., AKUNNE, H., GREIG, N., THURKAUF, A., RICE, K. C. AND PERT, A. (1989). Tight binding dopamine inhibitors as cocaine antagonist. *FEBS Lett.* **257**, 341–344.

SHIMADA, S., KITAYAMA, S., LIN, C. L., NANTHAKUMAR, E., GREGOR, P., PATEL, A., KUHAR, M. J. AND UHL, G. R. (1991). Cloning and expression of a cocaine-sensitive dopamine transporter cDNA. *Science* **254**, 576–578.

SHIMADA, S., KITAYAMA, S., WALTHER, D. AND UHL, G. (1992). Dopamine transporter mRNA: dense expression in ventral midbrain neurons. *Molec. Brain Res.* **13**, 359–362.

SNYDER, S. H. AND D'AMATO, R. J. (1986). MPTP: a neurotoxin relevant to the pathophysiology of Parkinson's disease. *Neurology* **36**, 250–258.

SNYDER, S. H., KUHAR, M. J., GREEN, A. I., COYLE, J. T. AND SHASKAN, E. G. (1970). Uptake and subcellular localization of neurotransmitters in the brain. *Int. Rev. Neurobiol.* **13**, 127–158.

STORCK, T., SCHULTE, S., HOFMANN, K. AND STOFFEL, W. (1992). Structure, expression and functional analysis of a Na^+-dependent glutamate/aspartate transporter from rat brain. *Proc. natn. Acad. Sci. U.S.A.* **89**, 10955–10959.

SURRATT, C. K., PERSICO, A. M., YANG, X.-D., EDGAR, S. R., BIRD, G. S., HAWKINS, A. L., GRIFFIN, C. A., LI, X., JABS, E. W. AND UHL, G. R. (1993*a*). A human synaptic vesicle monoamine transporter cDNA predicts posttranslational modifications, reveals chromosome 10 gene localization and identifies TaqI RFLPs. *FEBS Lett.* **318**, 325–330.

SURRATT, C. K., WANG, J.-B., YUHASZ, S., AMZEL, M., KWON, H. M., HANDLER, J. S. AND UHL, G. R. (1993*b*). Sodium- and chloride-dependent transporters in brain, kidney and gut: lessons from complementary DNA cloning and structure–function studies. *Curr. Opin. Nephrol. Hyperten.* **2**, 744–760.

UHL, G. R. (1990). Parkinson's Disease: neurotransmitter and neurotoxin receptors and their genes. *Eur. J. Neurol.* **30**, 21–30.

UHL, G. R. (1992). Neurotransmitter transporter (plus): a promising new gene family. *Trends Neurosci.* **15**, 265–268.

UHL, G. R. AND HARTIG, P. R. (1992). Transporter explosion: update on uptake. *Trends. Neurosci.* **13**, 421–425.

UHL, G. R., KITAYAMA, S., GREGOR, P., NANTHAKUMAR, E., PERSICO, A. AND SHIMADA, S. (1992). Neurotransmitter transporters family cDNAs in a rat midbrain library: 'orphan transporters' suggest sizable structural variations. *Molec. Brain Res.* **16**, 353–359.

UHL, G. R., WALTHER, D., MASH, D., FAUCHEUX, B. AND JAVOY-AGID, F. (1994). Dopamine transporter messenger RNA in Parkinson's disease and control substantia nigra neurons. *Annls Neurol.* **35**, 494–498.

VANDENBERGH, D. J., PERSICO, A. M. AND UHL, G. R. (1992). A human dopamine transporter cDNA predicts reduced glycosylation, displays a novel repetitive element and provides racially-dimorphic TaqI RFLPs. *Molec. Brain Res.* **15**, 161–166.