Pergamon

Biochemical Pharmacology, Vol. 50, No. 4, pp. 435–442, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00068-2

COMMENTARY

NEUROTRANSPORTERS: REGULATION, INVOLVEMENT IN NEUROTOXICITY, AND THE USEFULNESS OF ANTISENSE NUCLEIC ACIDS

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Key words: uptake; neurotransmitters; neurodegenerative disease; cytotoxicity; depression; antisense oligonucleotides

Growth, proliferation and normal functioning of living cells, prokaryotes as well as eukaryotes, depend upon small molecules that are taken from extracellular fluids. Compounds, such as sugars, certain drugs, ions, amino acids, or other precursors for large molecules, often cross the cell membrane with the aid of membrane-associated glycoproteins, known as transporters. Vast information is available concerning the structure of these transporters [1–5]. Interestingly, transporters of different molecules, or of the same molecule but in different animal species, may possess similar structural features, e.g. human intestinal Na^+/glucose and *Escherichia coli* Na^+/proline cotransporters share a significant degree of sequence similarity [6]. Information about the molecular properties of transporters also has been useful for identifying abnormal expression of these proteins in certain pathological conditions, e.g. underexpression of one type of glucose transporter was observed in noninsulin-dependent diabetes [7]. In another case, the autosomal recessive disease glucose/galactose malabsorption (GGM) was associated with a defect in the Na^+/glucose cotransporter [8]. The major role of the transporter P-glycoprotein [1–3] in multidrug resistance is another example of the medical significance of these proteins.

Although the knowledge about transporters of sugars, amino acids and similar small molecules is well developed, until recently the transport of neurotransmitters in the mammalian nervous system was studied mainly at the pharmacological and biochemical levels. Thus, uptake studies with synaptosomal preparations [9, 10], and binding experiments with ligands selective for serotonin [11–13], noradrenaline [14], and dopamine [15–17] were useful in investigating the selectivity, ion dependence, and neurochemical characteristics of these transporters. Since the isolation of the complementary DNA of the γ-aminobutyric acid (GABA) and the noradrenaline [18–20] transporters in 1990, we have been witnessing an enhanced development in the field of neurotransmitter transporters (neurotransporters). Within 3 years, the cDNAs coding for dopamine [21–25], serotonin (5-HT) [26–29], glycine [30–33], glutamate [34–36] and other plasma membrane transporters were isolated [37–39]. In parallel, a subfamily of vesicular transporters that are involved in uptake of amino acids or amines from the cytoplasm, or in drug transport (see below), have been identified [40–42]; see also [43] for a review. In certain cases, e.g. GABA and glutamate, different cDNAs encode for proteins that transport the same neurotransmitter [18, 34–36, 44–48]. Multiplicity in other neurotransporters, as in receptors, glucose transporters [6–8], or P-glycoprotein [1–3], is therefore very likely. The regulation and functional abnormalities in neurotransporters can now be analyzed at the molecular level, and this may shed light on potential pathological and behavioral disorders related to imperfect transport of neurotransmitters in the central nervous system.

STRUCTURE–FUNCTION CONSIDERATIONS OF PLASMA MEMBRANE NEUROTRANSPORTERS

Three major subfamilies of neurotransmitter transporters have been recognized thus far: (a) monoamine plasma membrane transporters that selectively remove noradrenaline, dopamine or serotonin from the synaptic cleft, (b) amino acid neurotransporters, which include the subgroups of GABA, glutamate, proline and glycine, and (c) the intracellular synaptic vesicle transporters. Several reports about the structural homologies, as well as differences between various members of these groups, were published recently [37–39, 42, 43], and, therefore, will not be repeated here. It is worth mentioning that the three groups share similar molecular features, including twelve putative transmembrane domains, 2–4 glycoslyation sites clustered in one non-membrane loop, and several consensus amino acid sequences that possess serine or threonine phosphorylation sites (see later). The amino acid sequence identity is higher within the three subfamilies than between them. The sequence homology among the monoamine transporter subfamily, for example, is 45–50% considering the entire protein, and about 70% when only conserved, mainly transmembrane regions are compared. The sequence conservation is even stronger between

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different species of the same transporter; hSERT and rSERT* have 92% identical amino acids [28], with differences largely restricted to the N-terminal chain. These observations, therefore, support the notion that a few (if not one) common parent genes are the source of various neurotransporters.

NEUROTRANSPORTERS AS A BACKDOOR TO ENTER NEURONS: NEUROTOXIC IMPLICATIONS

Though selective for their respective neurotransmitters, monoamine plasma membrane transporters can also transport synthetic or natural analogues of the neurotransmitter. This imperfect selectivity implies that such compounds may "abuse" the transporter in order to enter the cell. Should such molecules interact with vital intracellular structures, their penetration into the neuron might have significant consequences. Two examples of such poor selectivity, and the resulting physiological and neurotoxic implications, will be discussed in some detail.

In spite of the vast research effort, and the progress made during the last decade, the mechanisms underlying the death of dopaminergic neurons in Parkinson's disease [49–52], or upon aging [53–57], are still enigmatic. Whether neurotransporters play a role in this cell death will be discussed herein. Early studies have indicated that, when injected into the brain, 6-OHDA is neurotoxic to catecholaminergic neurons, and this effect can be blocked by catecholamine uptake inhibitors [58–61]. Thus, dopaminergic and noradrenergic cells take up 6-OHDA via the plasma membrane transporters. The damaging interaction of this, and possibly other dopamine derivatives such as 2-hydroxydopamine and 4-hydroxydopamine [62], with mitochondrial structures participating in energy metabolism depends, therefore, on active dopamine transporters. This notion that neurotoxic agents can selectively enter certain neurons via the plasma membrane transporter was further established recently, with MPTP [63–66]. It is now ascertained that MPTP is taken up by glial cells, oxidized to MPP^+, released, and then transported to dopaminergic neurons, with the aid of the dopamine transporters ([67] and references included). The selective neurotoxicity of MPTP to dopaminergic neurons is, therefore, determined by dopamine transporters.

A second example of cell-selective neurotoxin penetration via a plasma membrane transporter is the group of amphetamine derivatives [68]. Studied extensively by Rudnick and Wall [69] and Molliver and associates [70], drugs such as MDMA ("ecstasy") and PCA (*p*-chloroamphetamine) enter serotonergic neurons in a relatively selective fashion [69, 70]. This involves direct binding to the plasma membrane serotonin transporter, enhancement of serotonin efflux, and intracellular interaction with vesicular monoamine transporters [38, 69, 70]. Similarly, noradrenaline plasma membrane transporters are involved with the neurotoxic activity of DSP-4 to noradrenergic neurons [71]. Several less-studied neurotoxins, such as tyramine and amiflamine, are also suspected substrates of transporters [72]. Interestingly, both DSP-4 and amphetamine derivatives are neurotoxic to a subgroup of noradrenergic [71] or serotonergic [70] neurons, respectively. This may result from heterogeneity in either the transporters interacting with them or in intracellular target components such as monoamine oxidases or vesicular transporters. Experiments by Edwards [42] and Schuldiner [43] have shown that vesicular transporters can sequester toxic agents (such as MPP^+), and thus prevent harmful interaction with intracellular targets, such as mitochondria. Overall, it is clear that plasma membrane transporters may be considered as a "backdoor" for neurotoxic agents to enter neurons in a relatively selective fashion [72].

ROLE OF PROTEIN KINASES AND STEROID HORMONES IN CONTROLLING THE ACTIVITY OF NEUROTRANSPORTERS

The amino acid sequences of the cloned neurotransporters have indicated that these glycoproteins possess consensus sites for protein kinase A, protein kinase C, and Ca^{2+}-calmodulin-dependent protein kinase ([37–39] and references included). In a recent detailed study, Kanner and associates [73] examined the role of protein kinase C in phosphorylation of glutamate transporters. Purified pig brain and recombinant rat brain glutamate transporters were analyzed upon treatment with TPA. This report shows that a single amino acid, serine-113, located in the intracellular N-terminal chain of the transporter, plays a major role in controlling the activity of a neurotransporter [73].

Experiments with endothelial [74], placental [75–77], and pheochromocytoma [76] cells, platelets [78], and basophilic leukemia cells [79] observed that both cyclic AMP and phorbol esters (such as TPA) control the expression of serotonin transporters. Further support for this notion was obtained in a recent set of experiments in our laboratory.† It was found that in addition to the short-term effect of TPA, which is inhibitory in the systems mentioned above [74, 78, 79], TPA can also increase serotonin transport. Long-term treatment of the human placental JAR cells, which express the transporter constitutively, has a profound stimulatory effect on serotonin uptake. Interestingly, a subtle increase in uptake was also observed after a short (30 min) treatment of COS-7 cells, which were transiently transfected with a pCMV vector (pCMV-rSERT-1) expressing the rat serotonin cDNA†. This immediate increase in serotonin uptake in the transfected cells may involve a direct effect on the transporter protein, whereas in the case of the slow response of JAR cells (which takes 24–72 hr), TPA may affect the expression or biosynthesis of the transporter. Indeed,

* Abbreviations: hSERT and rSERT, human and rat serotonin transporters; MDMA, 3,4-methylenedioxymethamphetamine ("ecstasy"); DAT, dopamine transporter; pCMV-rSERT-1, a new pCMV vector expressing rSERT; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TPA, phorbol 12-myristate 13-acetate; and 6-OHDA, 6-hydroxydopamine.

† Dus L, Ratovitski T, Tauber M, Pantaleoni G and Simantov R, Manuscript submitted for publication.

in JAR cells, TPA increased both the V_{max} of serotonin uptake and the amount of the transporter protein, which was detected with newly prepared antibodies.* It is likely that this long exposure to TPA induces down-regulation of the protein kinase C, as often observed in other systems. On the other hand, immediate changes in serotonin transport upon TPA treatment ([74, 78, 79], and in the transfected COS-7 cells) could reflect phosphorylation of the transporter, changes in its compartmentation, or alterations in the sodium gradient driving the uptake. These possibilities, or an enhanced release of endogenous serotonin that may affect the uptake rate, cannot be excluded at this stage. The recent studies with transfected COS-7 cells indicate, therefore, that immediate changes in transport efficacy can be analyzed, even when genomic regulatory elements that control the expression of the transporter are inactive.

GENE REGULATION: POSSIBLE INVOLVEMENT OF EARLY-RESPONSE REGULATORY GENES

Regarding the role of protein kinase A in controlling the serotonin transporter, cyclic AMP and cholera toxin also increase serotonin uptake in JAR cells [75–77]. This effect is much slower than the induction of cyclic AMP synthesis, which peaks within 2 hr [76, 77]. It has been suggested, therefore, that cyclic AMP stimulation may increase biosynthesis of the transporter, possibly at the translational or transcriptional levels. Indeed, cholera toxin increases the mRNA level of the transporter and the apparent number of transporter molecules [77].

PC12 pheochromocytoma cells, or transgenic mouse L-M fibroblasts containing an 11 kb piece of genomic DNA coding for the serotonin transporter, have different responses than JAR cells to cholera toxin [76]. Yet, serotonin uptake was not affected by cholera toxin in COS-7 cells transfected with pCMV-rSERT-1.* One may hypothesize, therefore, that TPA and cholera toxin activate different regulatory pathways. Yet, we have found that the long-term stimulatory effects of cholera toxin and TPA in JAR cells were not additive, suggesting that the two agents may activate some common systems. As the long-term effects of cholera toxin and TPA coincide with increased mRNA or protein level, respectively, it will be of interest to test whether early-response regulatory genes, such as the AP-1 complex of Jun/Fos, participate in the control of serotonin transport. In various experimental conditions, TPA activates AP-1 ([80] and references included) and NFκB [81, 82] regulatory genes. It is also known that c-*fos* and *Jun*-B respond to cyclic AMP activation [83, 84]. Recently, it has been found that TPA, as in many other cells, activates the c-*fos* in JAR cells (T. Ratovitski and R. Simantov, unpublished results).

In line with the possibility that regulatory early-response genes control the expression of the serotonin transporter, and the previous reports about functional antagonism between AP-1 factors and the glucocorticoid receptor [85, 86], Dus *et al.* found that in placental JAR cells dexamethasone decreased serotonin transport.* Overall, these recent findings suggest that the serotonin transporter, in these and possibly other cells as well, may represent another case of cross-talk between AP-1 and glucocorticoid transcription pathways. The interaction between glucocorticoid hormones and the serotonergic system is apparently even more complicated, as antidepressant transporter blockers stimulate the activity of the glucocorticoid receptor gene promoter [87].

USING THE ANTISENSE KNOCKOUT APPROACH TO RESTRAIN TRANSPORT

The availability of many transport inhibitors (natural or synthetic) enables the widespread use of these agents for medical purposes. It is clear, however, that many of the uptake blockers are not selective for a single transporter. Thus, cocaine interacts with dopamine, serotonin and noradrenaline transporters. Likewise, tricyclic antidepressants are not selective for noradrenaline or other amine transporters. Moreover, abuse of compounds interacting with neurotransporters, cocaine and amphetamine-derivatives, in particular, is well known. It seems essential, therefore, to design molecules that will selectively inhibit a single type of neurotransporter, and will not be easily available for abuse.

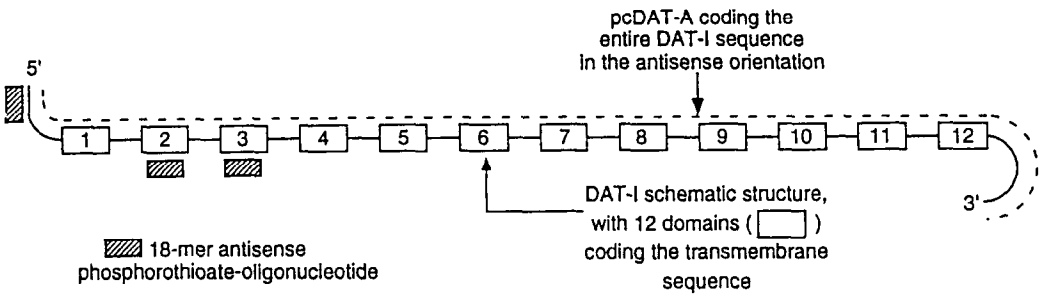
During the last decade or so, antisense oligonucleotides and cDNAs have been used to restrain the activity of various genes, and it is apparent that this approach has wide-range pharmacological and medical applications [88, 89]. The advantage of these molecules, as highly selective inhibitors, is especially important in the case of neurotransporters, as discussed above. We have found recently that this approach can be applied to partially restrain the activity of the DAT.† Cultured human neuroblastoma cells that possess dopamine transporters have been used to test the activity of a newly prepared vector, named pcDAT-A, that expresses the entire rat dopamine transporter rDAT-1 in the reverse orientation (Scheme 1). Three synthetic 18-mer antisense phosphorothioate-oligonucleotides, corresponding to different portions of DAT-1 (Scheme 1), were tested as well. The pcDAT-A vector, and one of the three oligonucleotides, OLIGO-3 (antisense to the 5′-region coding the third transmembrane domain), blocked [^{3}H]dopamine uptake, and were also effective in partially blocking dopamine toxicity.† Furthermore, OLIGO-3 also blocked 6-OHDA neurotoxicity in these cultured cells. Though the stability of these antisense nucleic acids has yet to be improved, this approach should provide a useful way to block transport of neurotransmitters *in vivo* in a highly selective fashion.

FUTURE PROSPECTS

The entire implication of the vast development in

* Dus L, Ratovitski T, Tauber M, Pantaleoni G and Simantov R, Manuscript submitted for publication.

† Simantov R, Blinder H, Ratovitski T, Porat S, Tauber M and Gabbay M, Manuscript submitted for publication.



Scheme 1. Schematic representation of the antisense nucleic acids used to inhibit dopamine transport and neurotoxicity in human neuroblastoma cells.

this field regarding the control of neurotransporters in the brain, under physiological, pharmacological or possibly drug-abuse conditions, is yet premature to predict. It is likely that endogenous inducers, hormones, neurotransmitters, or other factors that control protein kinases (of the A, C or other types) can modulate the activity of neurotransmitter transport and the synaptic level of the neurotransmitter. Such endogenous compounds, therefore, may play an important role in the normal neurotransmission, as well as in pathological conditions. For example, early neuroendocrine studies have shown that the ACTH-CRH-cortisol axis and steroid sex hormones play a role in depressive behavior [90–92], and that depression is gender-linked [93, 94]. Furthermore, an early report showed that uptake of serotonin, but not dopamine, was suppressed upon treatment with progesterone [95]. Further studies are certainly required to determine whether the finding that dexamethasone modulates the expression of the serotonin transporter* is related to these early studies.

Other important issues that also need further analysis are discussed briefly below:

(a) The functional significance of multiple forms of a neurotransporter is yet unclear. The molecular basis for this could be multiple genes [34–36, 44–48], posttranslational modifications [30], or glycosylation [96, 97]. There are pharmacological data confirming that multiple transporters may exist in other cases as well, suggesting that less abundant transporters are likely to be isolated in the future. Different transporters of the same neurotransmitter may be localized in various cell types. Thus, the GABA transporter GAT-1 was observed mainly in glial cells, whereas a β-alanine-sensitive GABA transporter is more abundant in neuronal cells [44]. One cannot also exclude the possibility that multiplicity in neurotransporters may reflect selective cross-talk with other neurochemical pathways. We have observed recently, for example, that glutamate has a different influence on transport of dopamine in two different neuronal cultures (R. Simantov and M. Tauber, manuscript in preparation). Studies with transgenic cells and animals should be useful to shed light on such issues, as was observed with MDR-transgenic mice [98].

(b) Compartmentation of the plasma membrane glucose transporter in various intracellular structures and the role of other membrane-associated proteins, such as Ras, have been studied extensively [99, 100]. Whether neurotransporters are similarly dynamic, and move from one intracellular compartment to another, the mechanisms that may be involved in this translocation, and the functional significance of recompartmentation, are yet to be studied. Differential compartmentation of the transporter in the cell may have an additional facet; transporters of monoamines, for example, are not restricted to the neuronal terminals, as an efficient uptake can be detected in brain regions containing the cell bodies. The implications of such compartmentation of transporters and the role of presynaptic receptors in controlling neurotransmitter release and uptake were studied, in some detail, in the case of serotonin [101–103]. Whether intracellular or cell–cell interactions modulate the localization of neurotransporters is, therefore, a subject of prime importance.

(c) Reverse-transport of neurotransmitters plays an important role in neurotransmitter release under experimental conditions [104], as well as *in vivo* (see Ref. 105 for a review). It is established that this cytoplasmic release depends upon the concentration of the neurotransmitter in the cytoplasm and/or on the magnitude of the Na^+ gradient. Additionally, the presence of transported analogues outside the neuron affects reverse-transport. No data are available, however, regarding the possibility that reverse-transport may change following molecular alterations in the protein, such as phosphorylation.

(d) Unraveling the role of neurotransporters under pathological conditions could certainly be a major outcome of the recent developments in this field. It has already been found that amyotrophic lateral sclerosis (ALS) is associated with decreased glutamate uptake in the brain and spinal cord [106], and that aging is accompanied by a robust decrease in the expression of dopamine transporters [57]. The interaction of abused drugs, such as cocaine and amphetamine-derivatives, with dopamine and serotonin transporters, respectively, is also well

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established. It is assumed that molecular dissection of the transporter's elements involved in transport of the neurotransmitter, or binding of blockers, as has been initiated with the dopamine transporter [107, 108], should contribute to the development of selective and possibly not addictive drugs. The involvement of other neurotransporters in pathological conditions, and the possibility that abnormal transport of catecholamine neurotransmitters contributes to drug abuse, can be studied now at the molecular and genetic levels.

Acknowledgements—The studies conducted in this laboratory were supported by the Israel Antidrug Authority and the Israel Ministry of Health. I wish also to thank my colleagues T. Ratovitski, Dr. S. Porat, H. Blinder, M. Gabbay, L. Dus, G. Pantaleoni and M. Tauber.

REFERENCES

1. Griffith J, Baker M, Rouch D, Page M, Skurray R, Paulsen I, Charter K, Baldwin S and Henderson P, Evolution of transmembrane transport: Relationship between transport proteins for sugars, carboxylate compounds, antibiotics and antiseptics. *Curr Opin Cell Biol* **4**: 684–695, 1992.
2. Juranka PF, Zastaway RL and Ling V, P-Glycoprotein: Multidrug resistance and a superfamily of membrane-associated transport proteins. *FASEB J* **3**: 2583–2592, 1989.
3. White MF, The transport of cationic amino acids across the plasma membrane of mammalian cells. *Biochim Biophys Acta* **822**: 355–374, 1985.
4. Ames GF-L, Bacterial periplasmic transport systems: Structure, mechanism, and evolution. *Annu Rev Biochem* **55**: 397–425, 1986.
5. Higgins CF, Hiles ID, Salmond GPC, Gill DR, Downie JA, Evans IJ, Holland IB, Gray L, Buckel SD, Bell AW and Hermondson MA, A family of related ATP-binding subunits coupled to many biological processes in bacteria. *Nature* **323**: 448–450, 1986.
6. Hediger MA, Turk E and Wright EM, Homology of the human intestinal Na^+/glucose and *Escherichia coli* Na^+/proline cotransporters. *Proc Natl Acad Sci USA* **86**: 5748–5752, 1989.
7. Johnson JH, Ogawa A, Chen L, Orci L, Newgard CB, Alam T and Unger RH, Underexpression of β cell high K_m glucose transporters in noninsulin-dependent diabetes. *Science* **250**: 546–549, 1990.
8. Turk E, Zabel B, Mundlos S, Dyer J and Wright EM, Glucose/galactose malabsorption caused by a defect in the Na^+/glucose cotransporter. *Nature* **350**: 354–356, 1991.
9. Iversen LL, Uptake processes for biogenic amines. In: *Handbook of Psychopharmacology* (Eds. Iversen LL, Iversen S and Snyder SH), Vol. 3, pp. 381–442. Plenum Press, New York, 1976.
10. Horn AS, Dopamine uptake: A review of progress in the last decade. *Prog Neurobiol* **34**: 387–400, 1990.
11. Langer SZ, Moret C, Raisman R, Dubocovich ML and Briley MS, High-affinity [^{3}H]imipramine binding in rat hypothalamus: Association with uptake of serotonin but not of norepinephrine. *Science* **210**: 1133–1135, 1980.
12. Mellerup ET, Plenge P and Englestoft M, High affinity binding of [^{3}H]paroxetine and [^{3}H]imipramine to human platelet membranes. *Eur J Pharmacol* **96**: 303–309, 1983.
13. D'Amato RJ, Largent BL, Snowman AM and Snyder SH, Selective labeling of serotonin uptake sites in rat brain by [^{3}H]citalopram contrasted to labeling of multiple sites by [^{3}H]imipramine. *J Pharmacol Exp Ther* **242**: 364–371, 1987.
14. Lee C-M and Snyder SH, Norepinephrine neuronal uptake binding sites in rat brain membranes labeled with [^{3}H]desipramine. *Proc Natl Acad Sci USA* **78**: 5250–5254, 1981.
15. Berger P, Janowsky A, Vocci F, Skolnick P, Schweri MM and Paul SM, [^{3}H]GBR-12935: A specific high affinity ligand for labeling the dopamine transport complex. *Eur J Pharmacol* **107**: 289–290, 1985.
16. Madras BK, Spealman RD, Fahey MA, Neumeyer JL, Saha JK and Milius RA, Cocaine receptors labeled by [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane. *Mol Pharmacol* **36**: 518–524, 1989.
17. Boja JW, Rahman MA, Philip A, Lewin AH, Carroll FI and Kuhar MJ, Isothiocyanate derivative of cocaine: Irreversible inhibition of ligand binding at the dopamine transporter. *Mol Pharmacol* **39**: 339–345, 1990.
18. Guastella J, Nelson N, Nelson H, Czyzyk L, Keynan S, Miedel MC, Davidson N, Lester HA and Kanner BI, Cloning and expression of a rat brain GABA transporter. *Science* **249**: 1303–1306, 1990.
19. Nelson H, Mandiyan S and Nelson N, Cloning of the human GABA transporter. *FEBS Lett* **269**: 181–184, 1990.
20. Pacholczyk TR, Blakely R and Amara SG, Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. *Nature* **350**: 350–354, 1991.
21. Shimada S, Kitayama S, Lin C-L, Patel A, Nanthakumar E, Gregor P, Kuhar MJ and Uhl G, Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science* **254**: 576–578, 1991.
22. Kilty JE, Lorang D and Amara SG, Cloning and expression of a cocaine-sensitive rat dopamine transporter. *Science* **254**: 578–579, 1991.
23. Usdin TB, Mezey E, Brownstein MJ and Hoffman BJ, Cloning of the cocaine-sensitive bovine dopamine transporter. *Proc Natl Acad Sci USA* **88**: 11168–11171, 1991.
24. Giros B, El Mestikawy S, Bertrand L and Caron MG, Cloning and functional characterization of a cocaine-sensitive dopamine transporter. *FEBS Lett* **295**: 149–154, 1991.
25. Giros B, El Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T and Caron MG, Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol Pharmacol* **42**: 283–290, 1992.
26. Blakely RD, Berson HE, Fremeau RT Jr, Caron MG, Peek MM, Prince HK and Bradley CC, Cloning and expression of a functional serotonin transporter from rat brain. *Nature* **354**: 66–70, 1991.
27. Hoffman BJ, Mezey E and Brownstein MJ, Cloning of a serotonin transporter affected by antidepressants. *Science* **254**: 579–580, 1991.
28. Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V and Blakely RD, Antidepressant- and cocaine-sensitive human serotonin transporter: Molecular cloning, expression and chromosomal localization. *Proc Natl Acad Sci USA* **90**: 2542–2546, 1993.
29. Lesch KP, Wolozin BL, Murphy DL and Reiderer P, Primary structure of human platelet serotonin uptake site: Identity with the brain serotonin transporter. *J Neurochem* **60**: 2319–2322, 1993.
30. Borowsky B, Mezey E and Hoffman BJ, Two glycine transporter variants with distinct localization in the CNS and peripheral tissues are encoded by a common gene. *Neuron* **10**: 851–863, 1992.

31. Smith KE, Borden LA, Hartig PR, Branchek T and Weinshank RL, Cloning and expression of a glycine transporter reveals colocalisation with NMDA receptors. *Neuron* **8**: 927–935, 1992.
32. Liu Q-R, Nelson H, Madiyan S, Lopez-Corcuera B and Nelson N, Cloning and expression of a glycine transporter from mouse brain. *FEBS Lett* **305**: 110–114, 1992.
33. Guastella J, Brecha N, Weigmann C, Lester HA and Davidson N, Cloning, expression and localization of a rat brain high-affinity glycine transporter. *Proc Natl Acad Sci USA* **89**: 7189–7193, 1992.
34. Storck T, Schulte S, Hoffman K and Stoffel W, Structure, expression and functional analysis of a Na^+-dependent glutamate/aspartate transporter from rat brain. *Proc Natl Acad Sci USA* **89**: 10855–10859, 1992.
35. Pines, G, Danbolt NC, Bjørås M, Zhang Y, Bendahan A, Eide L, Koepsell H, Storm-Mathisen J, Seeberg E and Kanner BI, Cloning and expression of a rat brain L-glutamate transporter. *Nature* **360**: 464–466, 1993.
36. Kanai Y and Heidiger MA, Structure and functional characterization of a high-affinity glutamate transporter. *Nature* **360**: 467–471, 1993.
37. Uhl GR and Hartig PR, Transporter explosion: Update on uptake. *Trends Pharmacol Sci* **13**: 421–425, 1992.
38. Rudnick G and Clark J, From synapse to vesicle: The reuptake and storage of biogenic amine neurotransmitters. *Biochim Biophys Acta* **1144**: 249–263, 1993.
39. Amara SG and Arriza JL, Neurotransmitter transporters: Three distinct gene families. *Curr Opin Neurobiol* **3**: 337–344, 1993.
40. Liu Y, Peter D, Roghani A, Schuldiner S, Prive G, Eisenberg D, Brech N and Edwards R, A cDNA clone that suppresses MPP^+ toxicity encodes a vesicular amine transporter. *Cell* **70**: 539–551, 1992.
41. Erickson JD, Eiden LE and Hoffman BJ, Expression cloning of a reserpine-sensitive vesicular monoamine transporter. *Proc Natl Acad Sci USA* **89**: 10993–10997, 1992.
42. Edwards RH, The transport of neurotransmitters into synaptic vesicles. *Curr Opin Neurobiol* **2**: 586–594, 1992.
43. Schuldiner S, A molecular glimpse of vesicular monoamine transporters. *J Neurochem* **62**: 2067–2078, 1994.
44. Clark JA, Deutch AY, Gallipoli PZ and Amara SG, Functional expression of and CNS distribution of a β-alanine sensitive neuronal GABA transporter. *Neuron* **9**: 337–348, 1992.
45. Borden LA, Smith KE, Hartig PR, Banchek TA and Weishank RL, Molecular heterogeneity of the γ-aminobutyric acid (GABA) transporter system. *J Biol Chem* **267**: 21096–21104, 1992.
46. Lopez-Corcuera B, Liu Q-R, Mandiyan S, Nelson H and Nelson N, Expression of a mouse brain cDNA encoding novel γ-aminobutyric acid transporter. *J Biol Chem* **267**: 17491–17493, 1992.
47. Liu Q-R, López-Corcuera B, Mandiyan S, Nelson H and Nelson N, Molecular characterization of four pharmacologically distinct α-aminobutyric acid transporters in mouse brain. *J Biol Chem* **268**: 2106–2112, 1993.
48. Liu Q-R, Mandiyan S, Nelson H and Nelson N, A family of genes encoding neurotransmitter transporters. *Proc Natl Acad Sci USA* **89**: 6639–6643, 1992.
49. Hornykiewicz O, Parkinson's disease: From brain homogenate to treatment. *Fedn Proc* **32**: 183–190, 1973.
50. Seeman P and Niznik HB, Dopamine receptors and transporters in Parkinson's disease and schizophrenia. *FASEB J* **4**: 2737–2744, 1990.
51. Gerlach M, Riederer P, Przuntek H and Youdim MBH, MPTP mechanisms of neurotoxicity and their implications for Parkinson's disease. *Eur J Pharmacol* **208**: 273–286, 1991.
52. Kopin IJ, Parkinson's disease: Past, present, and future. *Neuropsychopharmacology* **9**: 1–12, 1993.
53. Carlsson A and Winblad B, Influence of age and time interval between death and autopsy on dopamine and 3-methoxytyramine levels in human basal ganglia. *J Neural Transm* **38**: 271–276, 1976.
54. McGeer PL, McGeer EG and Suzuki JS, Aging and extrapyramidal function. *Arch Neurol* **34**: 33–35, 1977.
55. De Keyser J, Ebinger G and Vauquelin G, Age-related changes in the human nigrostriatal dopaminergic system. *Ann Neurol* **27**: 157–161, 1990.
56. Zelnik N, Angel I, Paul SM and Kleinman JE, Decreased density of human striatal dopamine uptake sites with age. *Eur J Pharmacol* **126**: 175–176, 1986.
57. Bannon MJ, Poosch MS, Xia Y, Goebel DJ, Cassin B and Kapatos G, Dopamine transporter mRNA content in human substantia nigra decreases precipitously with age. *Proc Natl Acad Sci USA* **89**: 7095–7099, 1992.
58. Ungerstedt U, 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *Eur J Pharmacol* **5**: 107–110, 1968.
59. Bloom FE, Algeri S, Groppetti A, Revuelta A and Costa E, Lesions of central norepinephrine terminals with 6-hydroxydopamine: Biochemistry and fine structure. *Science* **166**: 1284–1286, 1969.
60. Breese GR and Taylor TD, Effects of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence of selective degeneration of catecholamine neurons. *J Pharmacol Exp Ther* **174**: 413–420, 1970.
61. Uretsky NJ and Iversen LL, Effects of 6-hydroxydopamine on catecholamine containing neurones in the rat brain. *J Neurochem* **17**: 269–278, 1970.
62. Slivka A and Cohen G, Hydroxyl radical attack on dopamine. *J Biol Chem* **260**: 15466–15472, 1985.
63. Langston LW, Ballard P, Tetrud JW and Irwin I, Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219**: 979–980, 1983.
64. Burns RS, Chiueh CC, Markey SP, Herbert MH, Jacobowitz DM and Kopin IJ, A primate model of Parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci USA* **80**: 4546–4550, 1983.
65. Heikkila RE, Hess A and Duvoisin RV, Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. *Science* **224**: 1451–1453, 1984.
66. Javitch JA, D'Amato RJ, Strittmatter SM and Snyder SH, 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 Natl Acad Sci USA* **82**: 2173–2177, 1985.
67. Tipton KF and Singer TP, Advances in our understanding of the mechanisms of the neurotoxicity of MPTP and related compounds. *J Neurochem* **61**: 1191–1206, 1993.
68. Fuller RW, Biochemical pharmacology of the serotonin system. *Adv Neurol* **43**: 469–480, 1986.
69. Rudnick G and Wall SC, The molecular mechanism of "ecstasy" [3,4-methylenedioxymethamphetamine (MDMA)]: Serotonin transporters are targets for MDMA-induced serotonin release. *Proc Natl Acad Sci USA* **89**: 1817–1821, 1992.
70. Mamounas LA, Mullen CA, O'Hearn E and Molliver ME, Dual serotonergic projections to forebrain in

the rat: Morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. *J Comp Neurol* **314**: 558–586, 1991.
71. Zaczek R, Fritschy J-M, Culp S, De Souza EB and Grzanna R, Differential effects of DSP-4 on noradrenaline axons in cerebral cortex and hypothalamus may reflect heterogeneity of noradrenaline uptake sites. *Brain Res* **522**: 308–314, 1990.
72. Ross SB, Pharmacological and toxicological exploitation of amine transporters. *Trends Pharmacol Sci* **8**: 227–231, 1987.
73. Casado M, Bendahan A, Zafra F, Danbolt NC, Aragón C, Giménez C and Kanner BI, Phosphorylation and modulation of brain glutamate transporters by protein kinase C. *J Biol Chem* **268**: 27313–27317, 1993.
74. Myers CL, Lazo JS and Pitt BR, Translocation of protein kinase C is associated with inhibition of 5-HT uptake by cultured endothelial cells. *Am J Physiol* **257**: L253–L258, 1989.
75. Cool DR, Leibach FH, Bhalla VK, Mahesh VB and Ganapathy V, Expression and cyclic AMP-dependent regulation of a high affinity serotonin transporter in the human placental choriocarcinoma cells line (JAR). *J Biol Chem* **266**: 15750–15757, 1991.
76. King SG, Tiller AA, Chang AS-S and Lam DM-K, Differential regulation of the imipramine-sensitive serotonin transporter by cAMP in human JAR choriocarcinoma cells, rat PC12 pheochromocytoma cells, and C-33-14-B1 transgenic mouse fibroblast cells. *Biochem Biophys Res Commun* **183**: 487–491, 1992.
77. Ramamoorthy S, Cool DR, Mahesh VB, Leibach FH, Melikian HE, Blakely RD and Ganapathy V, Regulation of the human placental choriocarcinoma cells is accompanied by increased serotonin transporter mRNA levels and serotonin transporter-specific ligand binding. *J Biol Chem* **268**: 21626–21631, 1992.
78. Anderson GM and Horne WC, Activators of protein kinase C decrease serotonin transport in human platelets. *Biochim Biophys Acta* **1137**: 331–337, 1992.
79. Miller KJ and Hoffman BJ, Regulation of the serotonin transporter by PKC and adenosine receptor activation. *Soc Neurosci Abstr* **19**: 221, 1993.
80. Karin M and Smeal T, Control of transcription factors by signal transduction pathways: The beginning of the end. *Trends Biochem Sci* **17**: 418–422, 1992.
81. Sen R and Baltimore D, Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* **46**: 705–716, 1986.
82. Shirakawa F and Mizel SB, *In vitro* activation and nuclear translocation of NF-κB catalyzed by cyclic AMP-dependent protein kinase and protein kinase C. *Mol Cell Biol* **9**: 2424–2430, 1989.
83. Sassone-Corsi P, Visvader J, Ferland L, Mellon PL and Verma IM, Induction of proto-oncogene *fos* transcription through the adenylate cyclase pathway: Characterization of a cAMP-responsive element. *Genes Dev* **2**: 1529–1538, 1988.
84. Berkowitz LA, Riabowol KT and Gilman MZ, Multiple sequence elements of a single functional class are required for cyclic AMP responsiveness of the mouse c-*fos* promoter. *Mol Cell Biol* **9**: 4272–4281, 1989.
85. Yang-Yen H-F, Chambard J-C, Sun Y-L, Smeal T, Schmidt TJ, Drouin J and Karin M, Transcriptional interference between c-Jun and the glucocorticoid receptor: Mutual inhibition of DNA binding due to direct protein–protein interaction. *Cell* **62**: 1205–1215, 1990.
86. Schüle R, Rangarajan P, Kliewer S, Ransone LJ, Bolado J, Yang N, Verma IM and Evans RM, Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* **62**: 1217–1226, 1990.
87. Pepin M-C, Govindan MV and Barden N, Increased glucocorticoid receptor gene promoter activity after antidepressant treatment. *Mol Pharmacol* **41**: 1016–1022, 1992.
88. Green PJ, Pines O and Inouye M, The role of antisense RNA in gene regulation. *Annu Rev Biochem* **55**: 569–597, 1986.
89. Wahlestedt C, Antisense oligonucleotide strategies in neuropharmacology. *Trends Pharmacol Sci* **15**: 42–46, 1994.
90. Prange AJ, Lipton MA, Nemeroff CB and Wilson IC, The role of hormones in depression. *Life Sci* **20**: 1305–1318, 1977.
91. Van der Kar LD, Neuroendocrine aspects of the serotonergic hypothesis of depression. *Neurosci Biobehav Rev* **13**: 237–246, 1989.
92. Murphy BEP, Steroids and depression. *J Steroid Biochem Mol Biol* **38**: 537–559, 1991.
93. Maggi A and Perez J, Role of female gonadal hormones in the CNS: Clinical and experimental aspects. *Life Sci* **37**: 893–906, 1985.
94. Kennett GA, Chaouloff F, Marcou M and Curzon G, Female rats are more vulnerable than males in an animal model of depression: The possible role of serotonin. *Brain Res* **382**: 416–421, 1986.
95. Hackman E, Wirz-Justice A and Lichtsteiner M, The uptake of dopamine and serotonin in rat brain during progesterone decline. *Psychopharmacologia* **32**: 183–191, 1973.
96. Lew R, Vaughan R, Simantov R, Wilson A and Kuhar MJ, Dopamine transporters in the nucleus accumbens and the striatum have different apparent molecular weights. *Synapse* **8**: 152–153, 1991.
97. Patel A, Uhl G and Kuhar MJ, Species differences in dopamine transporters: Postmortem changes and glycosylation differences. *J Neurochem* **61**: 496–500, 1993.
98. Pastan I, Willingham MC and Gottesman M, Molecular manipulations of the multidrug transporter: A new role for transgenic mice. *FASEB J* **5**: 2523–2528, 1991.
99. Czech MP, Clancy BM, Pessino A, Woon C-W and Harrison SA, Complex regulation of simple sugar transport in insulin-responsive cells. *Trends Biochem Sci* **17**: 197–201, 1992.
100. Kozma L, Baltensperger K, Klarlund J, Porras A, Santos E and Czech MP, The Ras signaling pathway mimics insulin action on glucose transporter translocation. *Proc Natl Acad Sci USA* **90**: 4460–4464, 1993.
101. Artigas F, 5-HT and antidepressants: New views from microdialysis studies. *Trends Pharmacol Sci* **14**: 262, 1993.
102. Deakin JFW, Graeff FG and Guimaraes FS, Clinical implication of microdialysis findings. *Trends Pharmacol Sci* **14**: 263, 1993.
103. Fuller RW, Microdialysis studies with 5-HT reuptake inhibitors. *Trends Pharmacol Sci* **14**: 397, 1993.
104. Lonart G and Zigmond MJ, High glutamate concentrations evoke Ca^{++}-independent dopamine release from striatal slices: A possible role of reverse dopamine transport. *J Pharmacol Exp Ther* **256**: 1132–1138, 1991.
105. Levi G and Raiteri M, Carrier-mediated release of neurotransmitters. *Trends Neurosci* **16**: 415–419, 1993.
106. Rothstein JD, Martin LJ and Kuncl RW, Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N Engl J Med* **326**: 1464–1468, 1992.

107. Kitayama S, Shimada S, Xu H, Markham L, Donovan DM and Uhl GR, Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding. *Proc Natl Acad Sci USA* **89**: 7782–7785, 1992.

108. Kitayama S, Wang JB and Uhl GR, Dopamine transporter mutations selectively enhance MPP^+ transport. *Synapse* **15**: 58–62, 1993.