Volume 307, number 1, 76–80 FEBS 11245 July 1992

Minireview

Neurotransmitter transporters

A novel family of integral plasma membrane proteins

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Received 20 May 1992

The re-uptake of neurotransmitters into the nerve terminal terminates synaptic transmission at most central synapses and constitutes a key step in the modulation of synaptic efficacy. Recently, the cloning of several Na^+-driven neurotransmitter transporters has resulted in the description of a novel family of homologous membrane proteins, each with 12 transmembrane segments. These transporters constitute major targets of widely used drugs, and modulation of transporter gene expression and/or activity may represent an important substrate for plasticity in the nervous system.

Neurotransmitter; Transporter; Nervous system

1. INTRODUCTION

Plasma membranes are practically impermeable to polar molecules and build a tight barrier between the inner and outer milieu of the cell. Specialized transmembrane proteins are therefore required to translocate ions and metabolites across the lipid bilayer. Channel proteins allow the passage of different ions and/or small molecules along their electrochemical gradients, whereas carrier proteins transport bound substrates, even against steep concentration gradients. The latter process requires metabolic energy and/or coupling for the co-transport of ions. In this review, we discuss a special type of carrier system, the Na^+-driven neurotransmitter transporters, which recently emerged as a novel family of integral membrane proteins.

During neurotransmission, neurotransmitters undergo the following 'life cycle' at the synapse: (i) exocytotic release from plasma membrane attached synaptic vesicles into the nerve terminal; (ii) binding to their target receptors, thereby mediating signal transduction through the postsynaptic membrane; (iii) dissociation from the receptor followed by re-uptake into the presynaptic terminal or surrounding glial cells; and (iv) transport from the presynaptic cytosol into synaptic vesicles.

Transmitter uptake through the plasma membrane (iii) is driven by Na^+, whereas transport into synaptic vesicles (iv) is driven by a vesicular proton pump. In this cycle, efficient re-uptake of the transmitter from the synaptic cleft constitutes the crucial termination step of neurotransmission, as is amply documented for the neurotransmitters norepinephrine, dopamine, serotonin, L-glutamate, γ-aminobutyric acid (GABA) and glycine. Another mechanism of inactivation is found for acetylcholine, which, after dissociation from the receptor, is hydrolyzed into choline and acetate. Choline then is recovered by Na^+-dependent transport, as described above, and re-utilized for the de novo synthesis of acetylcholine in the nerve terminal.

The regulation of transporter activity is thought to constitute an important mechanism for the control of neurotransmitter action. Short-term changes of glutamate and choline transport in brain have been demonstrated to result from activation of the second messengers, Ca^{2+} and arachidonic acid, and/or membrane depolarization [1,2]. As arachidonic acid is released upon induction of long-term potentiation in the hippocampus [3], the modulation of transporter activity may have an important role in synaptic plasticity and learning processes. Moreover, permanent changes in transporter gene expression may contribute to persistent modifications of synaptic efficacy.

Transmitter uptake systems are also of great medical interest, since these membrane proteins represent the target sites of many clinically important drugs and may be implicated in neurological and psychiatric disorders. For example, tricyclic antidepressants inhibit both norepinephrine and serotonin transport, amphetamines block dopamine and norepinephrine uptake, and cocaine inhibits all these transport systems. Most of the

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aforementioned compounds cause profound changes in behaviour, and have high abuse potential. Interestingly, the concentration of serotonin and its metabolites is lowered in the cerebrospinal fluid of alcohol abusers, and administration of specific serotonin uptake inhibitors decreases ethanol intake [4]. Also, high-affinity choline uptake may be important for the selective vulnerability of cholinergic neurons in Alzheimer's disease [5]. The detailed functional and pharmacological analysis of different transmitter uptake systems thus may help to elucidate pathogenic mechanisms and to develop novel, therapeutically useful compounds.

2. ANALYSIS OF NEUROTRANSMITTER TRANSPORTER FUNCTION

Several methodologies have been used to to analyse the functional and pharmacological characteristics of neurotransmitter transporters. Determination of the uptake of radioactive transmitters into synaptosomes, cultured cells, and plasma membrane fractions displaying transmembrane ion gradients allowed evaluation of transport kinetics, and revealed a strict dependence on extracellular Na^+. Often, Cl^- ions are, in addition, required. This Cl^- dependence discriminates the neurotransmitter transporters from other Na^+-driven transport proteins. In the case of the rat brain glutamate and glycine uptake systems, 2–3 Na^+ and 1 Cl^- ions are co-transported per substrate molecule translocated [6,7]. Similar results were also obtained after reconstitution of the solubilized transporters into liposomes. In fact, rapid reconstitution, followed by determination of tracer uptake, served as an assay for the purification of functional GABA transporter protein [8]. Since the transport process is electrogenic, electrophysiological methods may also be employed. Indeed, in a recent report the whole-cell patch-clamp method was used to monitor the kinetics of glutamate uptake [6,9]. More recently, heterologous expression in *Xenopus* oocytes and mammalian cells has been employed to analyse transporters encoded by brain RNA fractions or isolated cDNAs. In fact, all transporter cDNAs cloned to date (see below) have been verified using this approach [11–21]. As heterologous expression allows analysis of individual functional proteins, this technique will have an important place in future studies of neurotransmitter uptake.

3. PROTEIN STRUCTURE

Biochemical purification of glutamate, GABA and choline transporters resulted in the identification of proteins of approximately 80 kDa [10]. Microsequencing of cyanogen bromide fragments of the purified rat GABA transporter (GAT1) then allowed cDNA cloning of the first neurotransmitter transporter [11]. Using expression cloning strategies and amplification of related sequences by the polymerase chain reaction (PCR), cDNAs encoding Na^+-dependent transporters for norepinephrine [12], serotonin [13–15], dopamine [16–19] and choline [20] have been isolated. Comparison of the deduced polypeptide sequences reveals that all these proteins share significant amino acid identity (30–65%) and a common predicted transmembrane topology, each with 12 putative membrane spanning segments (Figs. 1 and 2). As a hydrophobic N-terminus displaying characteristics of a signal peptide is lacking, the extended N- and C-termini are assigned to the cytoplasm. In most transporters, these cytoplasmic tail regions contain consensus phosphorylation sites which may be important for the regulation of transporter activity. However, reconstitution experiments with proteolytically cleaved GAT1 indicate that neither the N- nor the C-terminal regions are essential for transport function [22]. A long putative extracellular domain containing *N*-glycosylation sites is conserved between transmembrane segments III and IV (Fig. 2). In the case of GAT1, glycosylation at these sites appears to be required for incorporation into the plasma membrane [23]. The structural design shown in Fig. 2 is highly conserved between all neurotransmitter transporters sequenced to date. Interestingly, 11–12 putative transmembrane segments are also found in many, otherwise unrelated, Na^+-driven symporter proteins including the hexose transporters [24]. This may be indicative of convergent evolution, resulting in a common transmembrane architecture for diverse members of a super-family of Na^+-driven plasma membrane transport proteins.

Some conserved sequence features of the neurotransmitter transporter proteins allow further deductions about the possible structure of these membrane proteins. First, the putative transmembrane segment I shows a very high degree of conservation (Fig. 1), but only a low content of hydrophobic residues. This segment may thus participate in ion translocation and probably is centrally positioned in the assembled transporter. Segment IX, in contrast, is not well conserved and may contribute to substrate binding. At least two segments, VI and VII, display a highly amphipathic character and are thus potentially suited to line a transport path. Regions involved in Na^+ translocation have not been identified, however, a glutamate residue is absolutely conserved in a central position of transmembrane segment X. This side chain might constitute an intramembrane binding site for Na^+; alternatively, it may stabilize transporter conformation by pairing with other intramembrane charged residues, e.g. a conserved arginine in segment I. A sequence reminiscent of a degenerate leucine zipper motif overlaps transmembrane segment II and has been discussed as a possible dimerization signal [25]. This raises the interesting possibility that, in common with the intestinal glucose transporter, which functions as a homotetramer [26], neurotransmitter transporters may be oligomeric proteins.

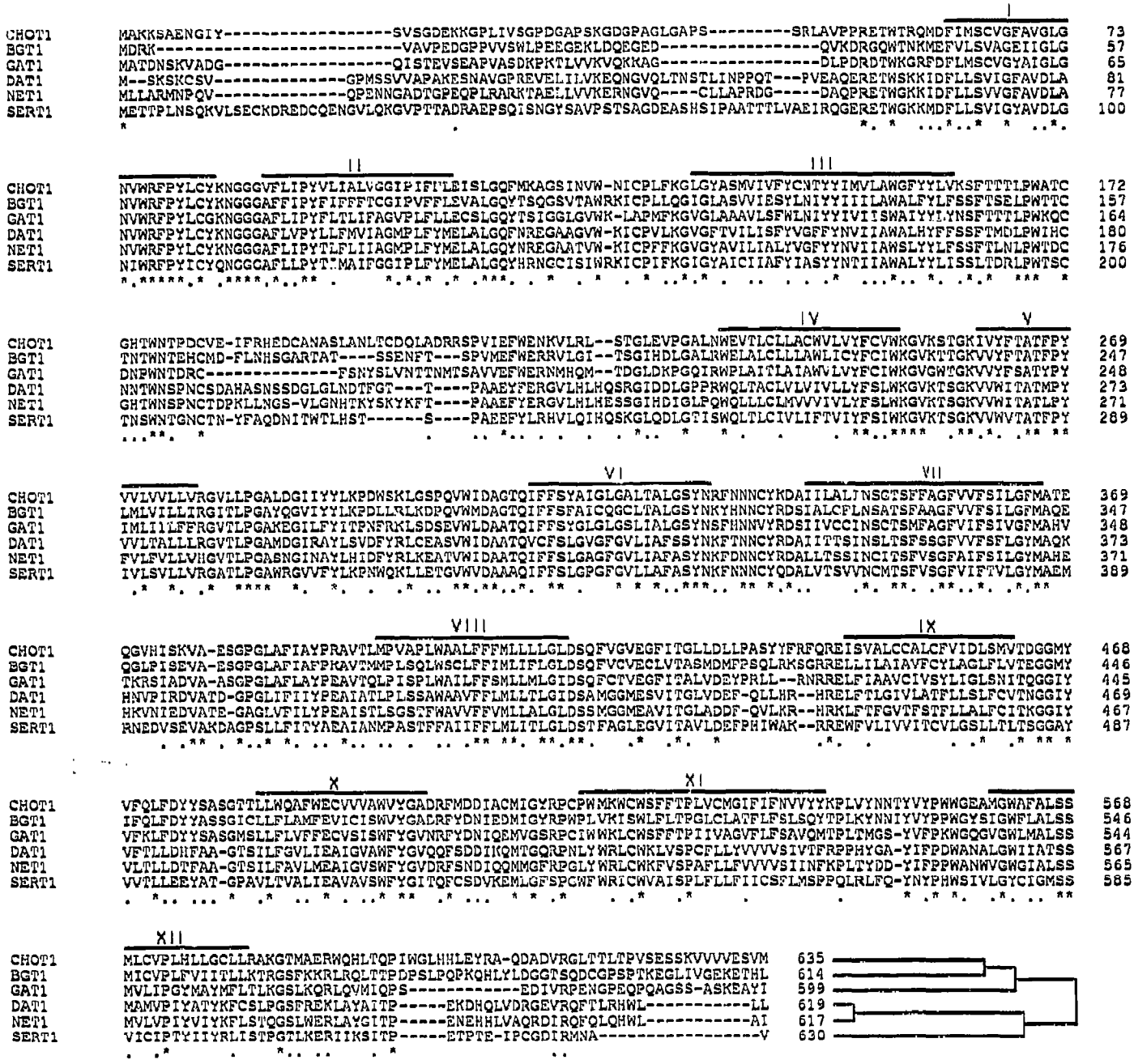

Fig. 1. Alignment of Na^+-dependent transporter proteins. Sequences are taken from the following references: BGT1 [27]; GAT1 [11]; NET1 [12]; DAT1 [16–19]; SERT1 [13–15]; and CHOT1 [20]. Amino acids identical in all transporters are marked by asterisks, and isofunctional residues shared by at least four proteins by a dot. Putative transmembrane regions are indicated by bars. Gaps are introduced to optimize identical sequence positions. A dendrogram at the C-terminal end of the sequences symbolizes the homology relationships between the individual transporters.

4. HETEROGENEITY OF TRANSPORTERS

Presently, transporters for four different neurotransmitters and for choline have been cloned. In addition, the isolation of a cDNA encoding a Na^+-dependent betaine transporter (BGT1) has been reported [27]. Its high amino acid sequence homology (see Fig. 1) identifies this osmolyte carrier as a member of the Na^+-driven neurotransmitter transporter family. However, corresponding transcripts are only found in kidney. Interestingly, BGT1 also transports GABA with an even higher affinity than betaine. Thus, for GABA transport two different proteins have been identified at the molecular level: GAT1, which is neurally expressed and BGT1,

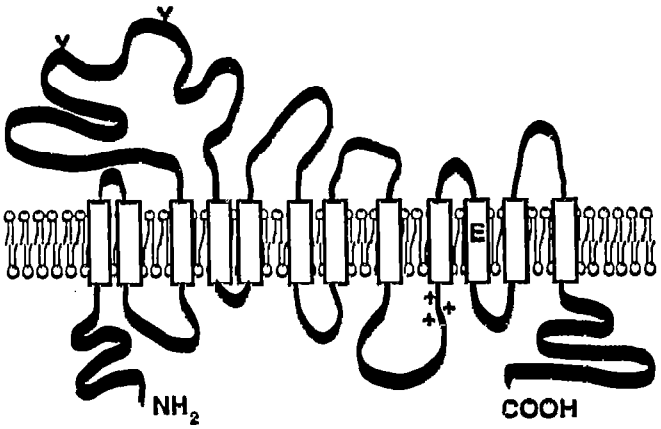

Fig. 2. Proposed transmembrane topology of transporter proteins. Putative transmembrane segments are shown as boxes; N- and C-termini are localized in the cytoplasm, and putative glycosylation sites (Y) are indicated on the second extracellular loop. E, glutamate residue in transmembrane segment X; +++, conserved cluster of positively charged residues preceeding transmembrane segment IX.

which is renally expressed. However, a much higher diversity of GABA transporters is predicted from uptake studies. Na^+-driven transport of taurine, β-alanine and GABA by a single transport system has been demonstrated in cultured glioma cells [28], and two pharmacologically distinct high-affinity GABA transporters have been described in plasma membrane vesicles from rat brain [29]. One of these transporters is expressed only in neurons and recognized by a GAT1 specific antiserum, whereas the other is localized in glial cells and is immunologically unrelated to GAT1 [30]. In addition, low-affinity GABA uptake is observed in brain, and pharmacological evidence indicates that different glial GABA uptake systems may exist [31,32]. Similarly, heterogeneity of dopamine transporters has been predicted, based on the observation that striatal membrane preparations reveal two different binding sites for [^{3}H]cocaine, both of which are associated with the dopamine uptake system [33,34]. Thus, considerable diversity of neurotransmitter transporters may exist in brain and contribute to the functional heterogeneity of synapses. However, such heterogeneity may originate not only from expression of multiple transporter genes, but may also result from post-translational modification and/or the state of oligomerization of these membrane proteins. Moreover, different binding sites and/or affinity states for the substrate may exist on a single transporter. A recent report indeed shows that expression of a single dopamine transporter cDNA in COS cells generates two cocaine binding sites of different affinity [35]. In other words, functional diversity of neurotransmitter transporters may originate by both transcriptional and post-transcriptional mechanisms.

5. CONCLUSIONS

Recent cDNA sequence data have established that neurotransmitter transporters and related carrier proteins like BGT1 constitute a novel family of integral membrane proteins. Their number is likely to increase rapidly within the next few years due to the cloning of other homologous transporters and transporter isoforms. Heterologous expression and site-directed mutagenesis of these proteins will unravel functional domains and sites important for transport activity and drug action. Moreover, analysis of transporter gene expression and regulation might shed light on processes underlying brain plasticity.

Acknowledgements: We thank Drs. M. Duggan and V. O'Connor for critical reading of this manuscript and S. Wartha for secretarial assistance. Work on the authors' laboratory was supported by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

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NOTE ADDED IN PROOF

After acceptance of this manusript, cDNA cloning and functional expression of proline (Fremeau Jr., R.T., Caron, M.G. and Blakely, R.D. (1992) Neuron 8, 915–926) and glycine (Smith, K.E., Borden, L.A., Hartig, P.R., Brancheck, T. and Weinshank, R.L. (1992) Neuron 8, 927–935) transporters from rat brain was reported. The deduced proteins display considerable homology to the GAT1, CHOT1 and BGT1 transporter superfamily (see dendrogram in Fig. 1).