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A family of genes encoding neurotransmitter transporters

QING-RONG LIU, SREEKALA MANDIYAN, HANNAH NELSON, AND NATHAN NELSON*

Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

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ABSTRACT **The genomic and cDNA clones of the mouse γ-aminobutyric acid transporter were sequenced and analyzed. The genomic clone contains 12 introns including 1 intron prior to the initiator methionine. The second intron comes immediately after the stretch of amino acids that is most conserved among the neurotransmitter transporters sequenced so far. By using a probe constructed according to this conserved region, several partial genomic clones were isolated. Sequence analysis of those clones reveals not only homology to the family of neurotransmitter transporters within the reading frame but also an identical location of an exon–intron junction after the conserved region. A search of the GenBank data base (April 1991) revealed that two invertebrate genes exhibit homology to the conserved sequence of the above family. One, a *Drosophila melanogaster* gene, encoded the N-terminal part of a protein homologous to neurotransmitter transporters and the second was in *Caenorhabditis elegans*. The *Drosophila* gene contains an intron that starts at a position identical to the corresponding positions of all the mammalian genes of the family.**

Synaptic transmission involves the release of a neurotransmitter into the synaptic cleft, interaction with a postsynaptic receptor, and subsequent removal of the transmitter from the cleft (1–6). In most synapses the signal is terminated by a rapid reaccumulation of the neurotransmitter into presynaptic terminals. This process is catalyzed by specific neurotransmitter transporters that are energized by the electrochemical gradient of sodium across the plasma membrane of the presynaptic cells (5). The pharmacology and biochemistry of some of these transporters have been studied in the last two decades but only recently have a few of the genes encoding neurotransmitter transporters been cloned and expressed (7–15). We have cloned (7, 8) cDNAs encoding the γ-aminobutyric acid (GABA) transporter in rat and human brains. Expression of the cDNAs in *Xenopus* oocytes yielded GABA uptake activity with properties similar to the isolated high-affinity GABA transporter. Recently, cDNA clones encoding noradrenaline, serotonin, and dopamine transporters were cloned from human and rat brains (9–13). The amino acid sequences of these transporters are very similar to each other and are related to the GABA transporter. The sequence similarity among these transporters established a transporter gene family with ≈12 transmembrane helices but with no homology to the other transporters with similar structure, such as the glucose transporters (16–18). To learn more about the gene family encoding neurotransmitter transporters, we cloned and studied the structure of the genomic DNA encoding the mouse GABA transporter.† Cloning of genomic DNAs that potentially encode several other transporters gave insight into the relationship of the various transporters in the family.

EXPERIMENTAL PROCEDURES

Cloning Procedures. Published procedures were used for screening libraries, dot blot, and Southern blot hybridizations, and gene manipulations (19). Mouse brain libraries obtained from Stratagene were used for cloning the cDNA and genomic clones in this study. The cDNA λZap library was screened for the GABA transporter by hybridizing the cDNA library with ^{32}P-labeled cDNA of the human transporter (8). The Bluescript plasmid was excised from the positive clones and the double-stranded cDNA inserts were sequenced by the dideoxynucleotide termination method after serial deletions by exonuclease III (20, 21). One of the clones of ≈4 kilobases (kb) containing the full-size cDNA encoding the GABA transporter was used for current studies.

The genomic clone encoding the GABA transporter was obtained by screening a genomic cosmid library, constructed in pWE15 vector (Stratagene), with ^{32}P-labeled *Bam*HI fragments of the mouse cDNA clone. A positive cosmid with an insert of ≈35 kb was identified. An *Eco*RI subclone of ≈14 kb was further digested with *Bam*HI and the resulting DNA fragments were subcloned into Bluescript plasmid. This fragment includes 3.4 kb of promoter sequence and a transcribed region of ≈10 kb to the middle of intron 11. The 3′ end of the gene was cloned by a PCR using oligonucleotides synthesized according to the cDNA sequence flanking intron 12. It was verified by cloning and sequencing of a corresponding DNA fragment from a EMBL3 mouse genomic library (Clontech). The DNA fragments were sequenced (19–21) and analyzed using DNASTAR or GCG softwares.

Genomic Clones of Various Members of the Neurotransmitter Transporter Family. A general oligonucleotide probe was designed according to a conserved sequence in the end of the first transmembrane helix of GABA and noradrenaline transporters (8, 9). Its sequence was AAT GTC TGG AGG TTC CCA TAC CTG TGC TAC AAG AAC GGC GGC GGC GCC TTC CTG ATC CCA TA. The pWE15 mouse genomic library was screened with the ^{32}P-labeled oligonucleotide and ≈100 positive clones were further analyzed on dot blots. About 40 of the clones were subjected to restriction endonuclease digestion with *Sau*3A and analyzed by Southern blot hybridization with the ^{32}P-labeled oligonucleotide. Positive fragments of 0.3–0.8 kb were subcloned into the *Bam*HI site of pBluescript and sequenced using T3 and T7 primers. Five clones of the neurotransmitter transporters family were identified. One of them was highly homologous to the human noradrenaline transporter (9). It is likely to encode the equivalent mouse noradrenaline transporter and it was denoted as NET. The unidentified transporters were named NTT and numbered according to the order of their discovery.

Expression in *Xenopus* Oocytes. The synthetic RNA was obtained by transcribing the pBluescript containing the mouse GABA transporter cDNA (GABAT) with an RNA synthesis and capping kit from Stratagene. Oocytes were surgically removed from frogs and defolliculated by collagenase treatment. After recovery for 24 h, the oocytes were

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Abbreviation: GABA, γ-aminobutyric acid.

*To whom reprint requests should be addressed.

†The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M92378 for the cDNA (GABTM) and M92377 for the genomic clone (GABATMG) of the mouse GABA transporter].

injected with 50 nl containing 2–10 ng of synthetic RNA. After 2 or 3 days, these oocytes were assayed for GABA transport. Prior to uptake assay, the oocytes were preincubated for 15 min in 1 ml of a solution containing 100 mM KCl and 10 mM Hepes (pH 7.5). The transport reaction was initiated by the addition of a solution containing 100 mM NaCl, 10 mM Hepes (pH 7.5), ≈0.1 μCi of [^{3}H]GABA (1 Ci = 37 GBq), and the specified amounts of unlabeled GABA. At the end of a 45-min incubation period, the oocytes were washed three times with 1.5 ml of the same buffer in which the radioactive GABA was omitted. Individual oocytes were solubilized in 50 μl of 10% (wt/vol) SDS, and the radioactivity was measured by scintillation counting. Five oocytes were used for each experimental point and the data are expressed as the average uptake per hour.

RESULTS

The possibility of studying the biogenesis of the GABA transporter in transgenic mice prompted us to clone the cDNA and genomic DNA encoding the transporter. Fig. 1 depicts the nucleotide and predicted amino acid sequences of the cDNA encoding the mouse GABA transporter. As expected, the sequences are highly homologous to those of rat and human brain GABA transporters (7, 8). To ensure that the mouse gene encodes the high-affinity GABA transporter, the cDNA was expressed in *Xenopus* oocytes and the K_m for GABA uptake was determined (Fig. 2). The uptake under various GABA concentrations gave a K_m value of ≈6 μM, which is close to the reported K_m value of the rat brain transporter (7). The cDNA encoding the GABA transporter was also expressed in transfected COS cells and resulted in a high activity of GABA uptake (data not shown).

The genomic clone encoding the GABA transporter contains several introns of various sizes. Fig. 3 depicts a schematic presentation of the intron–exon structure of the gene. The exact location of the introns is indicated in Fig. 1. Table 1 shows the sizes of the various introns and the sequences of the exon–intron junctions. The sequence of the gene revealed that the first intron is located prior to the initiator methionine and the coding sequence is divided among 12 exons. The introns are located in hydrophilic amino acid sequences in the coding sequence and none of them is situated inside a potential transmembrane helix. Exon I is an untranslated mRNA sequence and exon II contains the extension of this sequence, the initiator methionine and the N-terminal amino acid sequence including the first potential transmembrane helix. Exons III, IV, VI, VII, VIII, IX, X, and XI each contain a single potential transmembrane segment and exon V contains the hydrophilic glycosylated loop between transmembrane segments 3 and 4. Thus the structure of the gene is a fine example of functional domains divided by introns that allows rapid evolution of the gene family by an "exon shuffling" mechanism (25).

The first intron after the initiator methionine is located in the most conserved region of the family of neurotransmitter transporters sequenced so far (7–15). Using a universal oligonucleotide synthesized according to the amino acid sequence of this region in the GABA transporter, we have cloned several genomic clones encoding neurotransmitter transporters, including expressed cDNAs encoding the glycine, low-affinity GABA, and taurine transporters (ref. 26 and unpublished observations). The five genomic clones that were sequenced around the conserved sequence revealed that the first intron in the coding sequence starts at an identical position in the amino acid and DNA sequences (Fig. 4). Moreover, a search of GenBank (April 1991) with the oligonucleotide sequence identified a homologous sequence in *Drosophila melanogaster* (27). The DNA fragment y28c that contains the predicted neurotransmitter transporter also

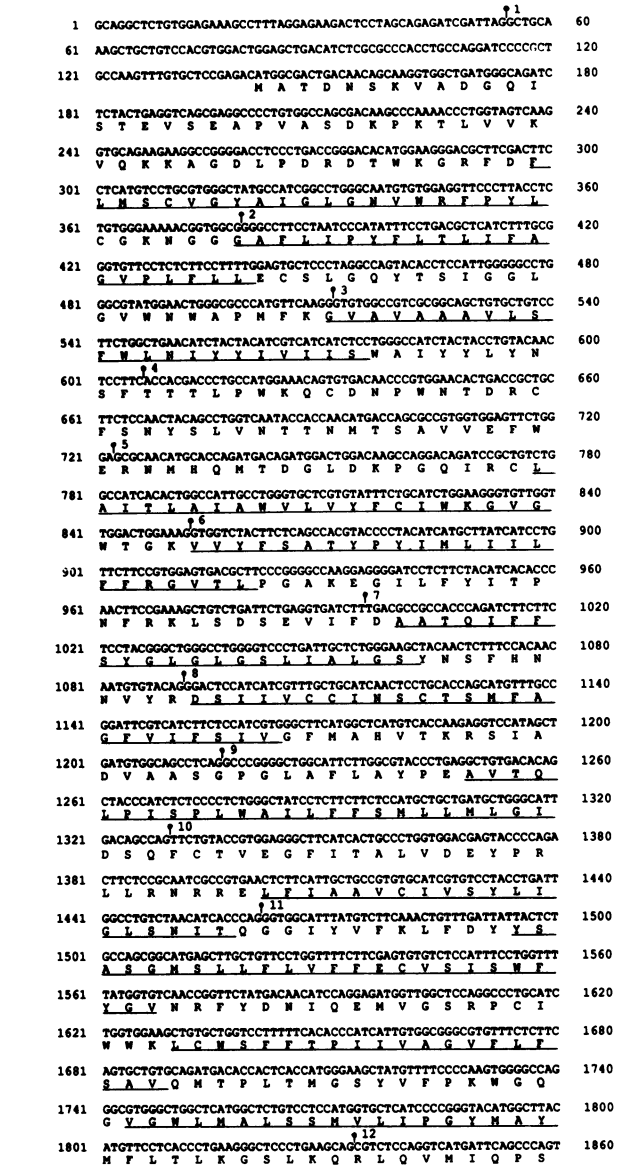

FIG. 1. Nucleotide and deduced amino acid sequences of GABAT cDNA encoding the GABA transporter of mouse brain. The cDNA was cloned and sequenced. The positions of the introns are indicated by ▼. The positions were deduced by sequencing a genomic clone, GABATMG, encoding the GABA transporter. The introns are numbered starting with the intron prior to the initiator methionine.

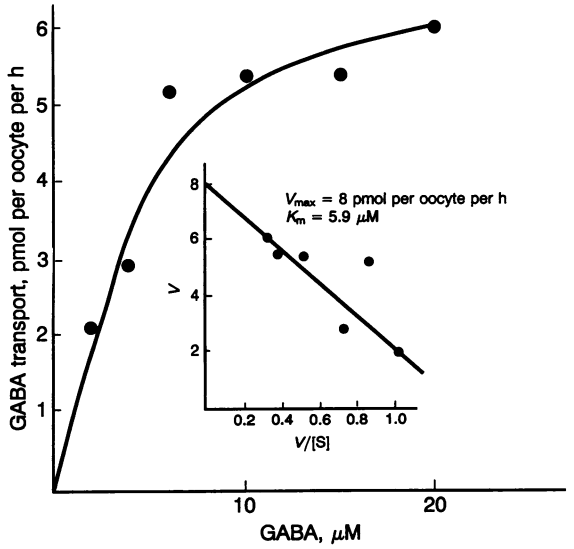

FIG. 2. Effect of GABA concentrations on the rate of GABA uptake into *Xenopus* oocytes injected with mRNA transcribed from GABAT cDNA. The values obtained with uninjected oocytes were subtracted from the corresponding values from injected oocytes. (*Inset*) Eadie–Hofstee analysis. *V*, velocity; S, substrate.

contains a gene encoding one of the tyrosine tRNAs from *Drosophila*. Analysis of the sequenced DNA fragment revealed that the gene encoding the tyrosine tRNA is situated in the first intron of a gene that potentially encodes a neurotransmitter transporter protein. The most striking feature of the intron structure is that it starts in a position identical to that of the corresponding introns in the mammalian genes (see Fig. 4). Fig. 5 shows the predicted DNA and amino acid sequences of the first part of the *Drosophila* neurotransmitter transporter. The predicted amino acid sequence has high degree of sequence similarity with known mammalian transporters. The *Drosophila* amino acid sequence is 42% identical to the noradrenaline transporter (9), 44% identical to the GABA, serotonin, and dopamine transporters (7, 8, 10–12), and 47% identical to a glycine transporter that was recently cloned, sequenced, and expressed in our laboratory (26). *In situ* hybridization, using the labeled DNA fragment as shown in Fig. 4, detected transcripts in a few cells of the developing brain in 8-h *Drosophila* embryos (K. Howard, H.N., and N.N., unpublished results).

A further GenBank search (April 1991) identified a gene from *Caenorhabditis elegans* that is related to mammalian neurotransmitter transporters. This gene is present downstream of a gene encoding an unusually large protein implicated in the regulation of myosin activity (28). Fig. 6 depicts the predicted nucleotide and amino acid sequences of the first part of the gene. The predicted amino acid sequence is ≈30% identical in a 125-amino acid overlap with the published sequences of neurotransmitter transporters (7–15) and the recently cloned glycine transporter (26). The predicted initiator methionine is located at nucleotide 38,386, which is 4943 base pairs downstream from the published poly(A) site identified for the *unc-22* gene (28). The part of the gene identified in this work encodes a hydrophobic protein potentially with four transmembrane helices. By using the consensus donor and acceptor sequences typical to the *C. elegans* introns (29), a highly hydrophobic protein with ≈12 transmembrane helices could be constructed from the downstream DNA sequence (data not shown) and alternative splicing could yield several related neurotransmitter transporters, but a cDNA encoding these transporters in *C. elegans* would be required to show the validity of this suggestion.

Table 1. Size and junction sequences of the introns in the mouse GABAT gene

Intron	5′ junction sequence	Size, bp	3′ junction sequence
1	GATTAG**gt**aaga	746	t**ag**GCTGCA
2	GTGGCG**gt**aggt	300	ct**ag**GGGCCT
3	TCAAGG**gt**gagt	103	ct**ag**GTGTGG
4	ACCACG**gt**gagt	1089	tt**ag**ACCCTG
5	CTGGGA**gt**gagt	705	ct**ag**GCGCAA
6	GGAAAG**gt**aggg	2759	tt**ag**GTGGTC
7	GATCTT**ct**gcct	178	gtgg**ct**TGACGC
8	GTACAG**gt**gcga	416	ctt**ag**GGACTC
9	CCTCAG**gt**cggt	1351	cac**ag**GCCCGG
10	AGCCAG**gt**gagg	198	aac**aag**TTCTGT
11	ACCCAG**gt**aggc	1195	ttt**ag**GGTGGC
12	AAGCAG**gt**aagc	832	cca**ag**CGTCTC

Positions of the introns are indicated in Fig. 1. Uppercase type represents an exon sequence; lowercase type represents an intron sequence. All but one of the splice sites (underlined) followed the "GT/AG" rule (22, 23). In intron 7, the GT and AG presumably were replaced by CT. Unusual splice sites have been reported (24). bp, Base pairs.

DISCUSSION

Cloning and sequencing of the gene encoding the GABA transporter (7, 8) made it apparent that only further cloning of genes encoding other transporters would reveal the extent and nature of this family of genes. Cloning of a cDNA encoding the noradrenaline transporter (9) opened up the possibility for rapid advancement of the field. Genomic cloning may provide some advantages and insights that are not available from the information obtained by cDNA clones. Genes that are expressed at very low levels are difficult to obtain from cDNA libraries, and the structure of the genes encoding neurotransmitter transporters may advance our understanding of their functional domains and evolution. Indeed, the genomic clone of mouse GABA transporter and the partial sequences of related transporters provided the expected information. It was not surprising that introns are positioned at identical places in related genes. Most of the introns of the human gastric H^+/K^+-ATPase gene are positioned at an identical place in the Na^+/K^+-ATPase; these ATPases have ≈60% amino acid identity (30). However, it is not usual to maintain intron positions between distant animals such as *Drosophila* and mice. The observation that the position of the first intron in the reading frame of genes encoding neurotransmitter transporters had been preserved in *Drosophila* and mammals suggests a specific function for this gene organization. The proposal that introns may play a role in RNA processing and differential RNA expression in nuclei of various cells (31) suggests the possibility that introns in genes encoding neurotransmitter transporters function in

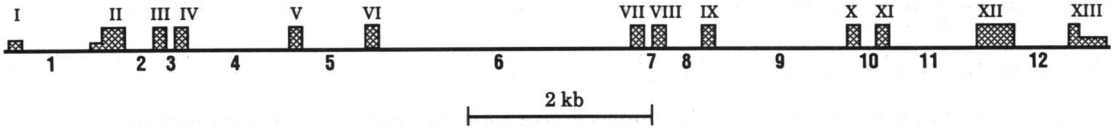

FIG. 3. Schematic presentation of the exon–intron structure of the gene encoding the mouse GABA transporter. Exons are represented as cross-hatched boxes; introns are the lines between the boxes.

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GABAT  TGGAAGGGACGCTTCGACTTCCTCATGTCCTGCGTGGGCTATGCCATCGGCCTGGGCAATGTGTGGAGGTTCCCTTACCTCTGTGGGAAAAACGGTGGCGGT
       W  K  G  R  F  D  F  L  M  S  C  V  G  Y  A  I  G  L  G  N  V  W  R  F  P  Y  L  C  G  K  N  G  G

NET    TGGGGCAAGAAGATTGATTTCCTGCTGTCCGTGGTGGCGTTCGCTGTGGACCTGGCCAACGTGTGGCGGTTCCCCTATCTCTGCTACAAGAATGGTGGTGGT
       W  G  K  K  I  D  F  L  L  S  V  V  A  F  A  V  D  L  A  N  V  W  R  F  P  Y  L  C  Y  K  N  G  G

NTT2   GGGAGGAGATGGGAAGGCAGGGATGAGGACAAAGGCCAGCGGATCATCGGACTGGGCAACGTGTGGCGCTTTCCCTACCTGTGCTACAAAAACGGCGGAGGT
       G  R  R  W  E  G  R  D  E  D  K  G  Q  R  I  I  G  L  G  N  V  W  R  F  P  Y  L  C  Y  K  N  G  G

NTT4   TGGAATAGCAAGCTGCAGTACATCCTGGCCCAGATTGGCTTCTCTGTGGGCCTGGGCAACATCTGGAGGTTCCCCTACCTGTGCCAGAAAAATGGAGGAGGT
       W  N  S  K  L  Q  Y  I  L  A  Q  I  G  F  S  V  G  L  G  N  I  W  R  F  P  Y  L  C  Q  K  N  G  G

NTT5   TGGACCAACAAGATGGAGTTTGTGCTGTCAGTGGCTGGAGAGATCATTGGCTTAGGCAACGTCTGGAGGTTTCCCTATCTCTGCTACAAGAATGGAGGTGGT
       W  T  N  K  M  E  F  V  L  S  V  A  G  E  I  I  G  L  G  N  V  W  R  F  P  Y  L  C  Y  K  N  G  G

NTT7   TGGGGCAACCAGATCGAGTTTGTACTGACGAGCGTGGGCTATGGCGTGGGCCTGGGCAATGTCTGGCGTTTCCCATACCTCTGCTATCGCAACGGGGGAGGT
       W  G  N  Q  I  E  F  V  L  T  S  V  G  Y  G  V  G  L  G  N  V  W  R  F  P  Y  L  C  Y  R  N  G  G

NTT3   TGGAAGAGCAAGTCGGAGTTTATCCTCTCGCTCCTTGGATATGCCATTGGCATTGGCAATGTGTGGCGATTTCCCTATCTCTGCTACCGCAGTGGCGGCGGT
       W  K  S  K  S  E  F  I  L  S  L  L  G  Y  A  I  G  I  G  N  V  W  R  F  P  Y  L  C  Y  R  S  G  G
```

FIG. 4. Comparison of the nucleotide and amino acid sequences of genomic clones of neurotransmitter transporters at the conserved region prior to the first intron in the reading frame. Genomic clones were cloned and sequenced. The GT signal for the beginning of the introns is underlined. GABAT is a partial sequence of the gene encoding the mouse GABA transporter. NET is a mouse genomic clone that potentially encodes the noradrenaline transporter that was cloned and sequenced in this study. NTT5 was identified as a potential low-affinity GABA transporter. NTT7 was identified as one of the genes encoding a glycine transporter. NTT2 and NTT4 are mouse genomic clones of unidentified neurotransmitter transporters. NTT3 is the *Drosophila* gene that potentially encodes the neurotransmitter transporter (see text and Fig. 5).

the differential expression of these proteins. The location of the first intron in the most conserved segment of these genes supports the assumption that it has a specific function in the expression of these genes. The homologous gene identified in *C. elegans* does not have an intron in this position. This gene is quite remote from the *Drosophila* and the mammalian genes. Further studies of this gene family in other organisms may shed light on the function of the gene structure in the expression of neurotransmitter transporters.

Sequence homology among the members of the neurotransmitter transporters family gave a clear indication that they evolved from a common ancestral gene. It is interesting that the percentage identity in the amino acid sequences among most of the members of the family is 42–48%. This includes the partial sequence of the *Drosophila* gene, the GABA transporter, the glycine transporter, the subfamily of catecholamine transporters, and partial sequences of >10 genes and cDNA cloned in our laboratory. The amino acid identity among the catecholamine transporter subfamily is 70–80%. We also identified genes that are similarly related to the GABA or the glycine transporters (data not shown). Therefore, the gene family of neurotransmitter transporters is diverse and several subfamilies of transporters, such as catecholamines and amino acid transporters, are emerging.

Why so many? In addition to the six genomic clones encoding proteins of the neurotransmitter transporters family, we also cloned eight cDNA clones that are homologous to the published transporters. We estimate that >30 transporters of this family function in mammalian brain. This suggests that members of the family may function outside the synaptic cleft in transport of substances that are not directly involved in neurotransmission. Some of these transporters may function in glial cells. For example, a specific GABA transporter was identified in glial cells (32), and it is anticipated that glial transporters may function in the uptake of other neurotransmitters (33). Recently, we cloned and expressed a cDNA of the low-affinity GABA transporter from mouse brain (unpublished data) that is highly homologous to the betaine transporter of canine kidney (34). Amino acids also function in neurotransmission, and the recent cloning of the brain glycine transporter suggests that amino acid transport in brain cells may involve carriers of the neurotransmitter transporters family. The glycine transporter is highly specific and it transports glycine exclusively (26). On the

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GAGTGCTTTAAACTTGACAGAAACAAACTCATGTTCACTCGCTCGTTTATTTTTGACAGCATCAAATAAAAAGTGTAAAAATAGATCTATTATTTCTCAA   100

ATCCCTGTTGGCTTTAGTTCAGTATGGTAAATTCGAAGTTCAGGCTTGAGTGACAATGGCGAATAATCAGCCCCCGACAACGAATGCTACGCGTAAAGAT   200
                                                                  M  A  N  N  Q  P  P  T  T  N  A  T  R  K  D

AAGCGCATAGAGCGGGACGAGAACCGTGGCCAATGGAAGAGCAAGTCGGAGTTTATCCTCTCGCTCCTTGGATATGCCATTGGCATTGGCAATGTGTGGC   300
K  R  I  E  R  D  E  N  R  G  Q  W  K  S  K  S  E  F  I  L  S  L  L  G  Y  A  I  G  I  G  N  V  W  R

GATTTCCCTATCTCTGCTACCGCAGTGGCGGCGCCGCCTTTGTGATTCCCTACCTGTTGATGGTAATTTTAGCCGGCATACCCCTGTTTTATATGGAAAT   400
  F  P  Y  L  C  Y  R  S  G  G  A  A  F  L  I  P  Y  L  L  M  V  I  L  A  G  I  P  I  F  Y  M  E  I

TCTGATCGGTCAGTTCTCGAGCACCGGATGCACTGGCATGTTTCGCATGACGCCTCTGCTGAAGGGAACGGGAATCGCTCAGGTGGTGGTCAATGCCTAC   500
 L  I  G  Q  F  S  S  T  G  C  T  G  M  F  R  M  T  P  L  L  K  G  T  G  I  A  Q  V  V  V  N  A  Y

TGCGTGTGCTACTACTCGGTGATCATATCGTATCCCATTCGGATGATCTTCTACTGTTTCTTCAAGAAGGTGCCCTGGGAGGACTGTTCCAATTCATGGA   600
C  V  C  Y  Y  S  V  I  I  S  Y  P  I  R  M  I  F  Y  C  F  F  K  K  V  P  W  E  D  C  S  N  S  W  N

ATACCGACGACTGTGTAACCGCGTCTGACGTAAGCATACTTATTACAATAAGCATATGTATAAATCAGTATTTTCGATTCTTAGATGGGAAAGCAAAATA   700
  T  D  D  C  V  T  A  S  D

GTAGTGATGTGTTCAAAACTTCCGCCGATGAATTC
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FIG. 5. Predicted DNA and amino acid sequences of a *Drosophila* gene encoding a protein that is highly homologous to mammalian genes encoding neurotransmitter transporters. The gene was constructed from the published sequence of the DNA fragment y28c, which contains the gene encoding one of the tyrosine tRNAs of *Drosophila melanogaster* (27). The potential neurotransmitter transporter gene was constructed by searching for consensus exon–intron junction sequences. The position of the intron containing the tyrosine tRNA is indicated by ▼.

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ATTCAAGCGTGAAAATGCTGGCTCCCCGTTTTCACAAAAATTCTGAACTTTAAAATATTTTTCGAACGAAAAAAAGTCAAACAAGATGAAACCGAAAAAA   100
                                                                                          M  K  P  K  K

GAAGTTCGTAAAGATGCCCTACCGCGTCCCGAATTCAAAAGCTGGGTAGGTATTTTACTTCTTTTTTTCTTACCTATAACTAAAGTTCCATTAAAATTTC   200
E  V  R  K  D  A  L  P  R  P  E  F  K  S  W  V  G  I  L  L  L  F  F  L  P  I  T  K  V  P  L  K  F  L

TGAAATTGCAGTATGACCTACTGTTTTCTGTCGTGAATCTGTGCATTGGATTAAGCAATTTTCTGATATTTCTTGCCAAAGTTCACGAGTACCGTGGTGG   300
  K  L  Q  Y  D  L  L  F  S  V  V  N  L  C  I  G  L  S  N  F  L  I  F  L  A  K  V  H  E  Y  R  G  G

AGCCTTCATTCTCGCCTACGGACTTATCCTTATTATGCTCGGCTACCCGGTTTTATACCTCGAATTGATAATAGGACAATTTCATAGATGCTCCCCGTGG   400
 A  F  I  L  A  Y  G  L  I  L  I  M  L  G  Y  P  V  L  Y  L  E  L  I  I  G  Q  F  H  R  C  S  P  W

ATTTTTATCAGAAGATGTGCTCCGATTCTTCAAGGTTTTGGTTTCATGGCATTAGTATCCGCTGTGACTATCCTGTATCCTTATCAGTACTCAGTGGCAC   500
I  F  I  R  R  C  A  P  I  L  Q  G  F  G  F  M  A  L  V  S  A  V  T  I  L  Y  P  Y  Q  Y  S  V  A  R

GTGCATTCAAATTTTTATTATCTCTAGCCAGATATCGATCACAAGACATGCCATGGTCAACGTGTGGTAATTGGTGGAATACAGAAAGTGATTAATAATT   600
  A  F  K  F  L  L  S  L  A  R  Y  R  S  Q  D  M  P  W  S  T  C  G  N  W  W  N  T  E  S  D
```

FIG. 6. Predicted DNA and amino acid sequences of a *C. elegans* gene encoding a protein homologous to mammalian neurotransmitter transporters. The identification of the gene is described in text. The DNA sequence was obtained from Benian *et al.* (28). Potential splicing sites for the continuation of the gene are underlined.

other hand, amino acid transporters outside the brain are rather unspecific and they can transport a wide variety of amino acids. Recently, a cDNA encoding a Na^+-independent neutral amino acid transporter was cloned from a rat kidney library (35). *Xenopus* oocytes injected with synthetic RNA of this gene took up several neutral amino acids. It may be that brain cells are utilizing highly specific transporters not only for neurotransmitters but also for amino acids and other components. This may explain the requirement of a large number of these transporters. We propose that the utilization of these highly substrate-specific transporters gives higher specificity to transport activities in the brain.

Note Added in Proof. Recently we cloned a mouse genomic DNA fragment encoding a GABA transporter that contains two additional introns at nucleotide positions 1567 and 1664 in Fig. 1.

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