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Cloning of the human brain GABA transporter

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A cDNA clone encoding a transporter for the neurotransmitter γ-aminobutyric acid in human brain was cloned and sequenced. The cDNA contains an open reading frame encoding a hydrophobic protein of 599 amino acids with a calculated molecular weight of 67022 Da. Hydropathy analysis revealed twelve potential transmembrane segments. The human protein is highly homologous to the protein from rat brain. Northern hybridization demonstrated a ubiquitous distribution of the transporter in various parts of the brain.

Human; Transport; Neurotransmitter; γ-Aminobutric acid; cDNA, sequence

1. INTRODUCTION

Synaptic transmission involves the release of a transmitter into the synaptic cleft, interaction with a post-synaptic receptor, and subsequent removal of the transmitter from the cleft [1–3]. The removal of the transmitter can be achieved by enzymatic degradation or by uptake into the presynaptic space. Acetylcholine is degraded by the enzyme acetylcholinesterase; however, the majority of other transmitters such as γ-aminobutric acid (GABA) and glutamic acid are taken up via sodium coupled transport systems [4,5]. GABA is the predominant inhibitory neurotransmitter in the brain and is widely distributed throughout the nervous system [2,3]. In addition, there exist kinetically and pharmacologically distinct GABA transport systems [2–4]. Cloning and sequencing the genes encoding the various transporters will reveal if specific genes are responsible for the distinct transport activities. However, it would not be surprising if a single gene product could function in more than one kinetically distinct transport system.

A glycoprotein exhibiting sodium and chloride dependent GABA transport activity has been purified from rat brain synaptosomes [6]. We isolated cyanogen bromide fragments from the purified GABA transporter, and used their amino acid sequences to clone and sequence the cDNA encoding the transporter [7]. Expression of the cDNA in *Xenopus oocytes* yielded GABA uptake activity with properties similar to the isolated high affinity GABA transporter. This gene was termed GAT1. We used a portion of the GAT1 cDNA of rat to clone the cDNA encoding the GABA transporter in human brain.

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2. MATERIALS AND METHODS

Two human brain cDNA libraries obtained from Stratagene were used for this study: an oligo dT and random primed library of human fetal brain in lambda ZAP II, and an oligo dT human cerebellum library in lambda ZAP. Each libary contained approximately 10^6 primary plaques with an average insert size of 1.0 kb. The libraries were screened with nick translated ^{32}P-labeled GABA cDNA of rat brain using published procedures ([8] and Stratagene protocol). The Bluescript plasmid was excised and the double stranded cDNA inserts were sequenced by the dideoxy termination method following serial deletions by exonuclease III [9,10]. Published procedures were used for dot blot and Southern hybridizations [8]. Nucleotide sequences were aligned and analyzed using DNA star software on an IBM PCAT computer.

3. RESULTS AND DISCUSSION

The majority of the mRNA in the brain contain long extensions from the 3′ ends of the reading frames. Therefore, it is difficult to obtain full size cDNAs. Random priming is the best method to obtain a library containing a high proportion of reading frames. After screening the two human libraries with the ^{32}P labeled rat cDNA encoding the GABA transporter, only four positive clones were obtained. Sequence analysis revealed that their overlapping sequences were identical. It is worth noting that the hybridized filters were washed at fairly low stringency (42°C in 1×SSC). Fig. 1 depicts the scheme of three cDNA clones com-

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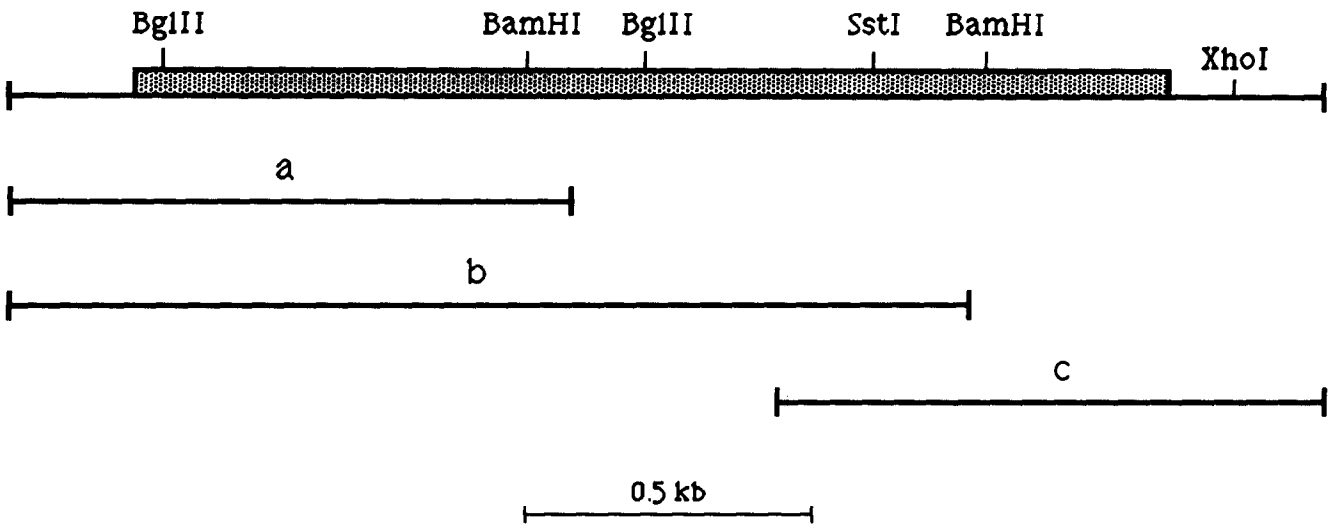

Fig. 1. Alignment of the various cDNA segments used for the construction of the cDNA encoding the GABA transporter from human brain. The open reading frame is marked by a shaded bar and the three cDNA segments are also indicated. Segments 'a' and 'c' were isolated from the human cerebellum library and segment 'b' was isolated from the fetal brain library.

prising the entire human GABA transporter. Some relevant restriction sites are also indicated. The whole gene was reconstructed by cutting segment b with *Sst*I and *Eco*RI (in the plasmid's polylinker) and segment c with *Sst*I and *Hin*dIII (in the plasmid's polylinker). A triple ligation was performed with a *Hin*dIII-*Eco*RI cut pGEM4Z plasmid.

Fig. 2 shows the cDNA and the deduced amino acid sequences of the human GABA transporter in comparison to the amino acid sequence of the corresponding rat gene. Both polypeptides contain 599 amino acids and their sequences are highly homologous. They differ at 17 positions; most of these are located in the N- and C-terminal regions of the proteins. Three potential glycosylation sites, located between helices 3 and 4 [7], are conserved, although in this region a methionine for leucine substitution occurred in the human gene. This is the sole difference in the entire central portion of the two proteins. Three putative kinase C phosphorylation sites (Ser^{24}, Thr^{46}, and Ser^{562}) were identified in N- and C-terminal regions of the rat GAT1[7]. In the human GAT1 Ser^{24} is substituted by asparagine, suggesting that this position may not be an important regulatory site. On the other hand, it is worth noting that serines 591, 592 and 594 were conserved in the two transporters. The potential twelve transmembrane segments were also preserved and hydropathy plots of the two proteins were almost identical (not shown).

A search of the gene bank revealed no significant homology with any other protein, including several transporters that contain twelve transmembrane segments [11,12]. The GAT1 is the first gene of the sodium-dependent neurotransmitter re-uptake system that has been sequenced. An important question is whether this gene is one member of a large family of transporter genes that may also include other transporters such as the glutamate transporter. The fact that low stringency screening rendered no other gene may argue against this possibility. However, other transporters may have slightly modified sequences and thus might escape detection by hybridization. To further elucidate this point, an mRNA prepared from various parts of rat brain was probed by ^{32}P-labeled GAT1 cDNA. At fairly high stringency (65°C), a strong band of about 4.4 kb is seen (Fig. 3). At longer exposure a faint band near 6.5 kb was addionally detected (not shown). This suggests, if a highly homologous family of transporter genes exists, that each should transcribe into mRNA of the same or at most two sizes. An alternate possibility is that poor homology exists among the various transporter genes. The distribution of the GAT1 mRNA in the various parts of the brain supports the functional and pharmacological studies that demonstrated a ubiquitous distribution of GABA activity [5,13].

The availability of the GAT1 cDNA should open a new avenue to the study of neurotransmitters re-uptake and their possible connection to functional brain disorders such as epilepsy. By expressing GAT1 in convenient expression systems such as cell cultures, *E. coli* and yeast cells, the mechanism of neurotransmitter uptake and the function of sodium and chloride in this process could be elucidated.

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GAATTCCGCTCCGGCCGCAGGATCTCCCCAAGGTGGCAGAAGGAGGCCTTCTGGAGCTGACCCACCCCCGACGACCATCAGGGTGAGGCAACTCCAAGGT   100

CCTACTCTCTTTCTGTGCCTGTTACCCACCCCGTCCTCCTAGGGTGCCCTTGAGCCGCAAAACTGCTGTCCACGTGGACCGGGGGTGACATCGCACGTCC   200

ATCTGCCAGGACCCCTGCGTCCAAATTCCGAGACATGGCGACCAACGGCAGCAAGGTGGCCGACGGGCAGATCTCCACCGAGGTCAGCGAGGCCCCTGTG   300
                                  M  A  T  N  G  S  K  V  A  D  G  Q  I  S  T  E  V  S  E  A  P  V
                                     D  N

GCCAATGACAAGCCCAAAACCTTGGTGGTCAAGGTGCAGAAGAAGGCGGCAGACCTCCCCGACCGGGACACGTGGAAGGGCCGCTTCGACTTCCTCATGT   400
A  N  D  K  P  K  T  L  V  V  K  V  Q  K  K  A  A  D  L  P  D  R  D  T  W  K  G  R  F  D  F  L  M  S
   S                                                 G

CCTGTGTGGGCTATGCCATCGGCCTGGGCAACGTCTGGAGGTTCCCCTATCTCTGCGGGAAAAATGGTGGGGGAGCCTTCCTGATCCCCTATTTCCTGAC   500
C  V  G  Y  A  I  G  L  G  N  V  W  R  F  P  Y  L  C  G  K  N  G  G  G  A  F  L  I  P  Y  F  L  T

ACTCATCTTTGCGGGGGTCCCACTCTTCCTGCTGGAGTGCTCCCTGGGCCAGTACACCTCCATCGGGGGGCTAGGGGTATGGAAGCTGGCTCCTATGTTC   600
L  I  F  A  G  V  P  L  F  L  L  E  C  S  L  G  Q  Y  T  S  I  G  G  L  G  V  W  K  L  A  P  M  F

AAGGGCGTGGGCCTTGCGGCTGCTGTGCTATCATTCTGGCTGAACATCTACTACATCGTCATCATCTCCTGGGCCATTTACTACCTGTACAACTCCTTCA   700
K  G  V  G  L  A  A  A  V  L  S  F  W  L  N  I  Y  Y  I  V  I  I  S  W  A  I  Y  Y  L  Y  N  S  F  T

CCACGACACTGCCGTGGAAACAGTGCGACAACCCCCTGGAACACAGACCGCTGCTTCTCCAACTACAGCATGGTCAACACTACCAACATGACCAGCGCTGT   800
  T  T  L  P  W  K  Q  C  D  N  P  W  N  T  D  R  C  F  S  N  Y  S  M  V  N  T  T  N  M  T  S  A  V
                                                  *        L     *        *

GGTGGAGTTCTGGGAGCGCAACATGCATCAGATGACGGACGGGCTGGATAAGCCAGGTCAGATCCGCTGGCCACTGGCCATCACGCTGGCCATCGCCTGG   900
 V  E  F  W  E  R  N  M  H  Q  M  T  D  G  L  D  K  P  G  Q  I  R  W  P  L  A  I  T  L  A  I  A  W

ATCCTTGTGTATTTCTGTATCTGGAAGGGTGTTGGCTGGACTGGAAAGGTGGTCTACTTTTCAGCCACATACCCCTACATCATGCTGATCATCCTGTTCT   1000
I  L  V  Y  F  C  I  W  K  G  V  G  W  T  G  K  V  V  Y  F  S  A  T  Y  P  Y  I  M  L  I  I  L  F  F

TCCGTGGAGTGACGCTGCCCGGGGCCAAGGAGGGCATCCTCTTCTACATCACACCCAACTTCCGCAAGCTGTCTGACTCCGAGGTGTGGCTGGATGCGGC   1100
 R  G  V  T  L  P  G  A  K  E  G  I  L  F  Y  I  T  P  N  F  R  K  L  S  D  S  E  V  W  L  D  A  A

AACCCAGATCTTCTTCTCATACGGGCTGGGCCTGGGGTCCCTGATCGCTCTCGGGAGCTACAACTCTTTCCACAACAATGTCTACAGGGACTCCATCATC   1200
T  Q  I  F  F  S  Y  G  L  G  L  G  S  L  I  A  L  G  S  Y  N  S  F  H  N  N  V  Y  R  D  S  I  I

GTCTGCTGCATCAATTCGTGCACCAGCATGTTCGCAGGATTCGTCATCTTCTCCATCGTGGGCTTCATGGCCCATGTCACCAAGAGGTCCATTGCTGATG   1300
V  C  C  I  N  S  C  T  S  M  F  A  G  F  V  I  F  S  I  V  G  F  M  A  H  V  T  K  R  S  I  A  D  V

TGGCCGCCTCAGGCCCCGGGCTGGCGTTCCTGGCATACCCAGAGGCGGTGACCCAGCTGCCTATCTCCCCACTCTGGGCCATCCTCTTCTTCTCCATGCT   1400
  A  A  S  G  P  G  L  A  F  L  A  Y  P  E  A  V  T  Q  L  P  I  S  P  L  W  A  I  L  F  F  S  M  L

GTTGATGCTGGGCATTGACAGCCAGTTCTGCACTGTGGAGGGCTTCATCACAGCCCTGGTGGATGAGTACCCCAGGCTCCTCCGCAACCGCAGAGAGCTC   1500
L  M  L  G  I  D  S  Q  F  C  T  V  E  G  F  I  T  A  L  V  D  E  Y  P  R  L  L  R  N  R  R  E  L

TTCATTGCTGCTGTCTGCATCATCTCCTACCTGATCGGTCTCTCTAACATCACTCAGGGGGGTATTTATGTCTTCAAACTCTTTGACTACTACTCTGCCA   1600
F  I  A  A  V  C  I  I  S  Y  L  I  G  L  S  N  I  T  Q  G  G  I  Y  V  F  K  L  F  D  Y  Y  S  A  S

GTGGCATGAGCCTGCTGTTCCTCGTGTTCTTTGAATGTGTCTCTATTTCCTGGTTTTACGGTGTCAACCGATTCTATGACAATATCCAAGAGATGGTTGG   1700
G  M  S  L  L  F  L  V  F  F  E  C  V  S  I  S  W  F  Y  G  V  N  R  F  Y  D  N  I  Q  E  M  V  G

ATCCAGGCCCTGCATCTGGTGGAAACTCTGCTGGTCTTTCTTCACACCAATCATTGTGGCGGGCGTGTTCATTTTCAGTGCTGTGCAGATGACGCCACTC   1800
 S  R  P  C  I  W  W  K  L  C  W  S  F  F  T  P  I  I  V  A  G  V  F  I  F  S  A  V  Q  M  T  P  L
                                                                 L

ACCATGGGAAACTATGTTTTCCCCAAGTGGGGCCAGGGTGTGGGCTGGCTGATGGCTCTGTCTTCCATGGTCCTCATCCCCGGGTACATGGCCTACATGT   1900
T  M  G  N  Y  V  F  P  K  W  G  Q  G  V  G  W  L  M  A  L  S  S  M  V  L  I  P  G  Y  M  A  Y  M  F
      S

TCCTCGCCCTAAAGGGCTCCCTGAAGCAGCGCATCCAAGTCATGGTCCAGCCCAGCGAAGACACTGTTCGCCCAGAGAATGGTCCTGAGCACGCCCAGGC   2000
  L  A  L  K  G  S  L  K  Q  R  I  Q  V  M  V  Q  P  S  E  D  T  V  R  P  E  N  G  P  E  H  A  Q  A
     T                        L     I                        I                          Q  P  H  G

GGGCAGCTCCACCAGCAAGGAGGCCTACATCTAGGGTGGGGGGCCACTCACCGACCCGACACTCTCACCCCCCGACCTGGCTGAGTGCGACCACCACTTGA   2100
 G  S  S  T  S  K  E  A  Y  I
 C        A

TGTCTGAGGATACCTTCCATCTCAACCTACCTCGAGTGGTGATCCAGACACCATCACCACGCAGAGAGGGGAGGTGGGAGGACAGTTAGACCCCTGGGTG   2200

GGCCCTGCCGTGGGCAAGGATACCCGGTGGCTTCTGGCACTGGCGGGCTGGTGACCTTTTTAATCCAGGCCCCATCAGCATCCCACTCCTGGCGGGAT   2298
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Fig. 2. Nucleotide and deduced amino acid sequences of the human GAT1. The amino acids that are different in the GAT1 are given below the amino acid sequence of the human GAT1. Potential glycosylation sites are marked by asterisks. The potential transmembrane segments are underlined.

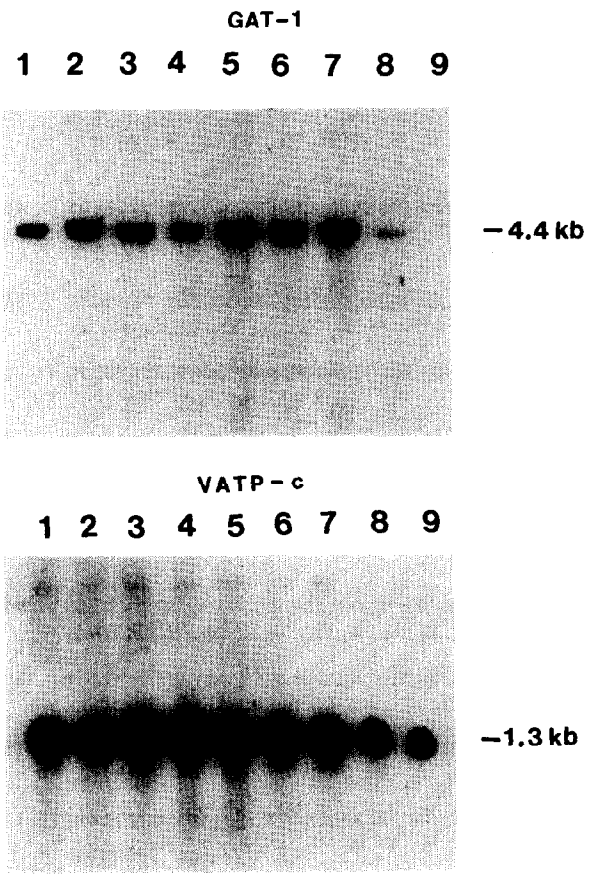

Fig. 3. Northern hybridization of RNA obtained from various parts of the rat brain. Rat brain was dissected to its various parts and RNA was prepared from them as previously described [8]. About 10 μg of total RNA were applied onto RNA denaturing agarose gel, and following electrophoresis and transfer onto a nylon filter it was probed by nick translated ^{32}P labeled GAT1 cDNA. The filter was washed at 65°C as previously described [8]. The same filter was reprobed with ^{32}P-labeled cDNA encoding the proteolipid (VATP-c) of the H^+-ATPase from chromaffin granules [14] to quantitate mRNA. 1, Whole brain; 2, olfactory bulb, 3, cerebral cortex, 4, hippocampus, 5, thalamus plus hypothalamus and midbrain, 6, brainstem, 7, cerebellum, 8, retina, 9, pituitary gland.

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