Molecular Characterization of Four Pharmacologically Distinct α-Aminobutyric Acid Transporters in Mouse Brain*

(Received for publication, August 5, 1992)
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Two novel γ-aminobutyric acid (GABA) transporters, GAT3 and GAT4, were cloned from the mouse neonatal brain cDNA library and expressed in Xenopus oocytes. Sequence analysis indicated they were members of the Na⁺-dependent neurotransmitter transporter family. The GABA uptake activities were measured in cRNA injected Xenopus oocytes. The K_m for GABA uptake by GAT3 was 18 μM and by GAT4 was 0.8 μM. GAT3 also transports β-alanine and taurine with K_m of 28 and 540 μM, respectively. Similarly, GAT4 transports β-alanine with K_m of 99 μM and taurine with a K_m of 1.4 mM. The newly cloned GABA transporters were compared with two previously cloned GABA transporters, GAT1 and GAT2, in terms of molecular and pharmacological properties. While GAT1 and GAT2 gene expression were neural specific, GAT3 and GAT4 mRNAs were detected in other tissues such as liver and kidney, in which GAT3 mRNA was especially abundant. The expression of GAT3 mRNA in mouse brain is developmentally regulated, and its mRNA is abundant in neonatal brain but not in adult brain. High affinity GABA transporters GAT1 and GAT4 were more sensitive to inhibition by nipecotic acid. Low affinity GABA transporters GAT2 and GAT3 were inhibited most effectively by betaine and β-alanine, respectively. The differential tissue distribution and distinct pharmacological properties of these four GABA transporters suggest functional specialization in the mechanisms of GABA transmission termination.

The amino acid γ-aminobutyric acid (GABA) is the predominant inhibitory neurotransmitter in brain and is widely distributed throughout the nervous system (1, 2). The uptake of GABA was extensively studied in various brain tissue preparations such as slices, homogenates, synaptosomal-enriched fractions, and reconstituted transporters (3–10). A multitude of sodium-dependent GABA-uptake systems with K_m ranging from 1 μM to 4 mM were reported. In general, the uptake systems were divided into two groups of neuronal and glial transporters according to their sensitivity to nipecotic acid or β-alanine (11, 12). However, a closer look at the distribution of one of the GABA transporters (GAT1) revealed a much more complicated picture suggesting the presence of more GABA transporters localized in specific parts of the brain (10–13). Moreover, different types of GABA transporters could be present not only in different brain areas but also in different stages during nervous system development. Such a versatility has already been proven for GABA receptors (14), and it would not be surprising if the same phenomenon would be observed for GABA transporters, whose activity is physiologically coupled with that of the receptors in the synapses.

The cDNA encoding two different brain GABA transporters (GAT1 and GAT2) were cloned, sequenced, and expressed in Xenopus oocytes (15, 16). These genes are part of a family of neurotransmitter transporters sharing similar structures and amino acid sequences but different pharmacological properties (15–24). In this paper we report on the cloning and expression of two new cDNAs encoding GABA transporters (GAT3 and GAT4). They also encode highly hydrophobic proteins of about 70 kDa with 12 potential transmembrane segments. All four GABA transporters differ in their expression, substrate specificity, and sensitivity to inhibitors.

**EXPERIMENTAL PROCEDURES**

Expression of the GABA Transporters in Xenopus Oocytes—Following linearization of the plasmid by XhoI, RNA was synthesized using T3-RNA polymerase with RNA synthesis and capping kit from Stratagene. The synthetic RNA was injected into Xenopus oocytes, and the GABA uptake was assayed as previously described (15). Briefly, oocytes were surgically removed from frogs and defolliculated by collagenase treatment. After recovery for 24 h, the oocytes were injected with 50 nl containing 2–10 ng of synthetic RNA. After 2–3 days they were assayed for GABA transport. Prior to the uptake assay, the oocytes were washed three times with 1 ml of a solution containing 102 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, and 10 mM HEPES (pH 7.5) and preincubated in 0.5 ml of this solution for 15 min. After removal of the preincubation medium the transport reaction was started by the addition of 0.5 ml of solution containing 100 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES (pH 7.5), about 0.1 μCi of [³H]GABA and 1 μM or the specified amounts of cold GABA. After incubation for 30 min at room temperature, the oocytes were washed three times with 1 ml of the incubation solution in which the substrate was omitted. Individual oocytes were solubilized in 500 μl of 1% SDS and the radioactivity measured by scintillation counting. Six to 12 oocytes were used for each experimental point, and the data are expressed as the average uptake/hour. The effect of various chemicals on GABA uptake was assayed by including them at the indicated concentrations during the preincubation and assay periods.

Screening, Cloning, and Sequencing—A neonatal mouse brain cDNA library, cloned into the EcoRI-XhoI site of the Uni ZAP XR cloning vector, was obtained from Stratagene. This library was screened with a ³²P-labeled cDNA encoding the taurine transporter (28). The clone NTT12 that encodes a GABA transporter GAT3 was isolated from this library. This library also yielded the clone NTT13

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L04663 and L04662.

† Recipient of a North Atlantic Treaty Organization fellowship.

1. The abbreviations used are: GABA, γ-aminobutyric acid; DABA, 1,2,4-diamino-n-butric acid; βGPA, β-guadinopropionic acid; DAPA, 1,2,3-diaminopropionic acid; PEG, polyethylene glycol; MOPS, (3-[N-morpholino]propanesulfonic acid).
that encodes about two-thirds of the GABA transporter GAT4. To obtain the full-length clone of GAT4, an adult mouse brain 5'-STRETCH cDNA library (Agt10, Clontech) was screened with NTT13. A cDNA of 4 kilobases was isolated and identified as a full-length GABA transporter by expression in Xenopus oocytes. The clone was sequenced as follows: it contained the insert in plasmid pBluescript SKⅡ+ (Stratagene). The insert was subcloned in pUC18 with EcoRI restriction enzyme and the plasmid was used to infect the E. coli host cells. The secondary screening was performed using a cDNA probe that encodes about 300 pg/ml ampicillin and incubated at 37 °C with shaking. The cell debris were spun down at 6,000 × g for 10 min. The supernatant was transferred to a 250-ml centrifuge tube and half volume (50 ml) of 15% PEG (8,000), 1.5 M NaCl was added. The Agt10 phage particles were precipitated by storage at 4 °C overnight and then centrifuged at 12,000 × g for 30 min at 4 °C. The supernatant was washed in 3 ml of SM by pipetting up and down. The suspension was transferred to a 15-ml polypropylene tube containing an equal volume of chloroform. The mixture was vortexed for 30 s and centrifuged at 6,000 × g for 10 min at 4 °C. The aqueous phase was transferred to a fresh 15-ml tube. DNase I and RNase A digestion was carried out by adding 15 μl of 1 mg/ml DNase I and 30 μl of 1 mg/ml RNase A and incubated at 37 °C for 30 min. Proteinase K digestion was carried out by adding 30 μl of 0.5 M EDTA (pH 8.0), 8 μl of 20 mg/ml proteinase K, and 30 μl of 10% SDS sequentially and incubated at 68 °C for 15 min. The reaction mixture was extracted twice with an equal volume of phenol-chloroform. Sodium acetate (pH 6.0) was added to give a final concentration of 0.2 M and the mixture was precipitated with equal volumes of ethanol. Sodium acetate was dissolved in 480 μl of 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA (TE) buffer containing 20 μg/ml RNase A and incubated at 37 °C for 30 min. The solution was mixed with 120 μl of 5 M NaCl and 600 μl of 15% PEG (8,000). The mixture was set on ice for 30 min and centrifuged at 10,000 × g for 15 min at 4 °C. The pellet was rinsed once with liquid nitrogen, ethanol, lyophilized, and dissolved in 50 μl of sterile deionized water. The final yield of XylO DNA was about 100 μg. The DNA inserts were released with EcoRI digestion and subcloned into the EcoRI site of Bluescript vector (25).

Isolation and Northern Blot—Mouse brain was dissected into its various parts from decapitated mice and immediately frozen in liquid nitrogen. One g of the tissue was grinding in liquid nitrogen with a pestle and mortar. The tissue powder was added to 14 ml of guanidinium thiocyanate, 0.07% β-mercaptoethanol and homogenized with three 30-s bursts of a Polytron, according to the manufacturer's recommendation supplied with the RNA isolation kit (Stratagene). Following acid-phenol extraction and isopropanol precipitation, the samples were lyophilized, and the total cellular RNA was dissolved in RNase-free water and the concentration was determined by UV absorption at OD260 nm. The aliquots of RNA could be stored at −80 °C before running the gel. Equal amounts of RNA (40 μg) were mixed 1:1 with RNA denaturing solution (50% formamide, 2 X MOPS buffer and 6% formaldehyde), incubated at 65 °C for 5 min, then chilled on ice. After adding 0.1 volume of 10 × dye solution (50% glycerol, 0.25% bromphenol blue, 0.25% xylene cyanol, and 1 mM EDTA), the RNA samples were loaded onto 1% agarose-formaldehyde gels (1 X MOPS buffer (pH 7.0), 6.6% formaldehyde) and the electrophoresis was run at 100 V in 1 X MOPS buffer (pH 7.0). The RNA was sequenced with ethidium bromide in 10 mM sodium phosphate buffer, pH 7.2, for 30 min. The gel was destained in the same buffer and then soaked in 10 × SSC for 30 min. RNA was transferred to a Duron-UV membrane (Stratagene) and cross-linked to the membrane by UV. The labeled DNA probe for GAT3 was the full-length cDNA insert, and GAT4 was the insert of the partial cDNA clone of NTT13. Prehybridization for 2 h and hybridization overnight with specific probes were carried out in the same hybridization buffer (15% bovine serum albumin, 5% SDS, 0.5 M sodium phosphate buffer (pH 7.2), and 1 mM EDTA) at 65 °C. The filters were washed at 65 °C first with washing solution 1 (1% bovine serum albumin, 5% SDS, 40 mM sodium phosphate buffer (pH 7.2), and 1 mM EDTA) twice for 15 min each, then with washing solution 2 (0.6% sodium dodecyl sulfate, 1% SDS, 40 mM sodium phosphate buffer, and 1 mM EDTA) three times at 10 min/wash (27). The exposure of autoradiography for GAT1 and GAT4 was overnight, GAT3 for 1 week, and GAT2 for 2 weeks.
RESULTS

The tissue-specific localization of the two GABA-transporters (GAT1 and GAT2) prompted us to look for additional transporters active in sodium-dependent GABA uptake. Several positive clones isolated as described under "Experimental Procedures" were assayed for GABA uptake following expression in Xenopus oocytes. Two different cDNA clones exhibited GABA uptake activity and were named GAT3 and GAT4. Fig. 1 shows the nucleotide and predicted amino acid sequences of GAT3. The predicted open reading frame encodes a protein of 602 amino acids with a molecular mass of 68,293 Da. An unusual low isoelectric point was calculated to be pI = 6.1. Most neurotransmitter transporters have pI > 9. The hydropathy plot suggests 12 transmembrane segments, a similar structure to all the other neurotransmitter transporters sequenced so far (12-23).

Alignment of GAT3 with predicted amino acid sequences of the other transporters showed 69% identity with GAT2 (16), 64% identity with the taurine transporter (28), 51% identity with GAT1 (15), 42% identity with the glycine transporter (24), and 42-45% identity with the catecholamine transporters (17-21). Therefore, GAT3 is a novel neurotransmitter transporter related to GAT2.

The substrate specificity of GAT3 was determined by expression of its cRNA in Xenopus oocytes. The injected oocytes accumulated up to 350 times as much [H]GABA as uninjected oocytes or oocytes injected with mRNA of the glycine transporter. Cold GABA competed with the radioactive GABA uptake. The anion and cation specificity for GABA uptake by the expressed GAT3 was similar to that of GAT1 and GAT2. Fig. 2 shows the effect of GABA concentration on its uptake into GAT3 cRNA-injected oocytes. The Eadie-Hofstee plot revealed a Michaelis constant $K_m = 18 \mu M$ for GAT3. All of these substances were taken up at relatively high rates. This suggests that GAT3 is the transporter of GABA, β-alanine, and taurine.

Screening of the neonatal and adult mouse brain libraries

FIG. 2. Kinetics of GABA, β-alanine, and taurine uptake into Xenopus oocytes injected with synthetic mRNA of GAT3. The uptake of [H]labeled GABA, β-alanine, and taurine was assayed as described for GABA uptake under "Experimental Procedures." A, GABA uptake. B, β-alanine uptake. C, taurine uptake. The values obtained with uninjected oocytes were subtracted from the corresponding injected samples. The values obtained with the injected oocytes were 350- (GABA), 330- (β-alanine), and 72- (taurine) fold greater than with the uninjected oocytes. Eadie-Hofstee analysis is depicted in the insets.

Fig. 1. Nucleotide and deduced amino acid sequences of cDNA encoding the GABA-transporter GAT3. The cDNA was cloned and sequenced as described under "Experimental Procedures." Both DNA strands were sequenced by the dideoxy termination method (36).
yielded another GABA transporter (GAT4). Fig. 3 shows the
nucleotide and predicted amino acid sequences of GAT4. The
open reading frame encodes a protein of 627 amino acids with
a calculated molecular mass of 69,868 Da. Alignment of the
amino acids of GAT4 with the other GABA transporters
revealed that it is highly related to GAT2 and GAT3 and
more remotely related to GAT1 (Fig. 4). The alignment of
GAT4 with the known neurotransmitter transporters showed
76% identity with GAT3, 65% identity with GAT2 (16), 53%
identity with GAT1 (15), 66% identity with the taurine trans-
porter (28), 42% identity with the glyoxylate transporter (24),
and 42-44% identity with the catecholamine transporters (17-
21). The affinity of GAT1 to GABA was much higher than
that of GAT2 or GAT3; therefore, it was surprising that the
affinity of GAT4 with the other GABA transporters showed
more remotely related to GAT1 (Fig. 4). The alignment of
amino acids of GAT4 with the other GABA transporters
revealed that it is highly related to GAT2 and GAT3 and
amino acids of GAT4 with the other GABA transporters
showed 70% identity with GAT3, 65% identity with GAT2 (16),
53% identity with GAT1 (15), 66% identity with the taurine trans-
porter (28), 42% identity with the glyoxylate transporter (24),
and 42-44% identity with the catecholamine transporters (17-
21). The affinity of GAT1 to GABA was much higher than
that of GAT2 or GAT3; therefore, it was surprising that the
affinity of GAT4 to GABA was even greater than that of
GAT1 (Fig. 5). The Eadie-Hofstee plot revealed a Michaelis
constant ($K_m$) of 0.65 $\mu$M, and two other experiments gave $K_m$
values of 0.86 and 0.94 $\mu$M. The mean value of about 0.8 $\mu$M
suggests that GAT4 is a high affinity GABA transporter with
the highest affinity for GABA than all transporters cloned so
far. Like GAT3, GABA uptake by expressed GAT4 was also
inhibited by $\beta$-alanine and taurine. Moreover, $\beta$-alanine up-
take with $K_m = 99$ $\mu$M and taurine uptake with $K_m = 1.4$ $\mathrm{mM}$
were measured in the GAT4 cRNA injected oocytes (not shown).

The pharmacology of the four GABA transporters is de-
picted in Table I. $\beta$-Alanine inhibited the GABA uptake by
the expressed GAT3 and GAT4, and to much lesser extent by
GAT2. Betaine inhibited GABA transport only by GAT2.
GABA transport by GAT1 and GAT4 was more sensitive to
DABA, guvacine, and nipecotic acid than that of GAT2 and
GAT3. While GAT2 was the most sensitive to phloretin and
quinidine, GAT3 and GAT4 were the most sensitive to $\beta$-GPA,
DAPA, and hypotaurine. It is apparent that even though
GAT2, GAT3, and GAT4 exhibit high sequence homology
they have different substrate and inhibitor specificities. More-
over, GAT1 and GAT4 have the highest affinity for GABA
and have greater sensitivity to nipecotic acid.

The specificity of the various GABA transporters suggests
specialization for distinct functions. Northern hybridization
was performed with RNA isolated from different parts of
the mouse brain as well as from kidney and liver. The GAT4-
cDNA hybridized exclusively with the brain tissues and hy-
bridization with RNA from kidney and liver could not be
detected (Fig. 6). The 5-kilobase GAT4 mRNA was enriched
in the brain stem and the rest of the brain at least five times
more than the cerebellum and cerebral cortex. In contrast,
the GAT1 mRNA was distributed evenly in different parts of
the brain (13). GAT3 cDNA hybridized weakly with the RNA
isolated from adult mouse brain, however, it gave a strong
positive 2.5-kilobase hybridization band with liver and kidney
RNA. Since colonies containing the cDNA encoding GAT3
were abundant in the neonatal brain library, we looked for
the presence of mRNA in neonatal and young mice. As shown
in Fig. 7 neonatal brains contain substantial amounts of
GAT3 mRNA. Its amounts increase in the first few days after
birth and decreases in the adult brains. No significant pattern
of mRNA change for GAT1, GAT2, and GAT4 was observed
in the neonatal mouse brain in comparison with adult mouse
brain (not shown). Therefore, GAT3 may have a role in brain
development, and the amounts of its mRNA are develop-
mentally regulated.

**DISCUSSION**

Studies on GABA uptake into brain slices and isolated cells
revealed the presence of more than one sodium-dependent
GABA transport systems (1-7, 29). Recently, the presence of
two pharmacologically distinct GABA transporters was dem-
onstrated (10). It was shown that while one of the transport-
ers, presumably GAT1, is not inhibited by $\beta$-alanine, the
second GABA transporter is sensitive to $\beta$-alanine and may
also function in $\beta$-alanine transport. An apparent $K_m$ of about
44 $\mu$M was observed for the $\beta$-alanine uptake activity (10).
Out of the four GABA transporters analyzed in this work only
GAT3 and GAT4 transported $\beta$-alanine at appreciable rates
and with relatively high affinity of $K_m = 28$ and 99 $\mu$M,
respectively. These values are close enough to the reported
$K_m = 44$ $\mu$M for $\beta$-alanine uptake in the reconstituted prepa-
ration from rat synaptosomes (10). Recently, we cloned a
taurine transporter that also transports $\beta$-alanine with a $K_m$
= 56 $\mu$M (28). Therefore, the observed $\beta$-alanine transport in
preparations of brain synaptosomes may be the result of
uptake by more than a single transporter. The taurine trans-
porter, GAT3, and GAT4, can be distinguished by the sensi-
tivity of their $\beta$-alanine transport to inhibition by GABA.

**FIG. 3. Nucleotide and deduced amino acid sequences of cDNA encoding the GABA-transporter GAT4.** The experimen-
tal conditions were as described in Fig. 1.
The transport assay with the various GABA transporters was performed as described under "Experimental Procedures." The different chemicals were present at the indicated concentrations during the preincubation and assay periods.

The pharmacology of the four GABA transporters is influenced by their substrate specificity. Taurine and its analogs βGPA, DAPA, and hypotaurine specifically inhibited GAT3 and GAT4 that transport taurine. On the other hand GAT2 that transports betaine was the most sensitive to betaine and its analogs phloretin and quinidine. The two high affinity GABA-transporters GAT1 (15), GAT2, GAT3, and GAT4, were aligned using a DNAstar program. Identical amino acids are indicated by an asterisk (*). Putative transmembrane domains are underlined.

**Table I**

Inhibitor sensitivity of the four different GABA transporters expressed in Xenopus oocytes.

The transport assay with the various GABA transporters was performed as described under "Experimental Procedures." The different chemicals were present at the indicated concentrations during the preincubation and assay periods.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Conc. (μM)</th>
<th>GABA transport activity (% of control)</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Alanine</td>
<td>10</td>
<td>100 ± 2</td>
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<td></td>
<td>100</td>
<td>100 ± 11</td>
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<tr>
<td></td>
<td>500</td>
<td>500 ± 39</td>
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<tr>
<td></td>
<td>1000</td>
<td>100 ± 4</td>
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<tr>
<td>Betaine</td>
<td>100</td>
<td>100 ± 11</td>
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<tr>
<td></td>
<td>500</td>
<td>500 ± 39</td>
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<tr>
<td></td>
<td>1000</td>
<td>100 ± 4</td>
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<tr>
<td>DABA</td>
<td>100</td>
<td>100 ± 11</td>
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<tr>
<td></td>
<td>500</td>
<td>500 ± 39</td>
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<tr>
<td></td>
<td>1000</td>
<td>100 ± 4</td>
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<tr>
<td>Guvacine</td>
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Q.-R. Liu, unpublished results.

*Fig. 5. Effect of GABA concentration on GABA uptake into Xenopus oocytes injected with synthetic mRNA of GAT4. The experimental conditions were as described in Fig. 2.*

While the transport of β-alanine by GAT3 and GAT4 is strongly inhibited by GABA, the taurine transporter is insensitive to GABA. On the other hand taurine is a potent inhibitor of β-alanine transport by the taurine transporter, but relatively high concentrations of taurine are required to inhibit β-alanine transport by GAT3 and GAT4.

The pharmacology of the four GABA transporters is influenced by their substrate specificity. Taurine and its analogs βGPA, DAPA, and hypotaurine specifically inhibited GAT3 and GAT4 that transport taurine. On the other hand GAT2 that transports betaine was the most sensitive to betaine and its analogs phloretin and quinidine. The two high affinity GABA transporters GAT1 and GAT4 were the most sensitive to guvacine and nippecotic acid. Therefore, GABA transporters might not simply be divided into neuronal and glial subtypes according to their pharmacological properties, and sensitivity of GABA uptake to β-alanine is only first approximation for glial GABA transporters. GAT1 is considered to be neuronal because of its relatively high affinity for GABA and its sensitivity to nippecotic acid (12). Recent studies on the localization of its mRNA by in situ hybridization and immunocytochemistry with antibodies raised against this transporter confirmed its function in neurons (30, 31). However, the other GABA transporters may also contribute to the termination of neurotransmission by GABA. The affinity of GAT4 for GABA is about 8-fold greater than that of GAT1, and therefore it may remove GABA into much lower concentrations. On the other hand the rate of reuptake in the synaptic clefts is
Northern hybridization was performed as described under “Experimental Procedures.” About 40 g of total RNA were applied to each well. The equal amount of RNA in each well was verified by staining with ethidium bromide. The size of the transcripts was determined by RNA standards. The source of RNA was as follows: 1) liver, 2) kidney, 3) cerebellum, 4) cerebral cortex, 5) brain stem, and 6) the rest of the brain.

The expression of the various GABA transporters was studied by Northern hybridization of the transporters cDNA with RNA isolated from different parts of the brain. As was shown before, GAT1 is specific to the central nervous system, and it could not be detected in other tissues such as kidney and liver (13, 15). Transcripts of GAT1 could be found in all brain parts in recent studies with in situ hybridization and immunocytochemistry revealed that it is primarily a neuronal transporter (30, 31). Transcripts of GAT2 are also present in all brain parts, kidney and liver (16). Similar but not identical transporter of canine kidney was found to be regulated by hypertonicity and suggested to be functional in betaine transport in the kidney (34). The pharmacology of GAT2 is consistent with its being a glial GABA transporter (16). Like GAT1 the transcript of GAT4 is specific for the central nervous system, and it is not present in kidney or liver. However, GAT4 mRNA was more concentrated in brain stem in comparison with cerebellum and cerebral cortex. Its high affinity to GABA suggests an important role for GAT4 in termination of GABA transmission in the central nervous system. Initially, we detected transcripts of GAT3 only in kidney and liver. This was surprising because the cDNA encoding GAT3 was very abundant in the neonatal mouse library. Indeed northern hybridization (Fig. 7) revealed that its expression is developmentally regulated, and its transcript is much more abundant in the brains of newly born mice than in the adult brain. The interchange of GABA transporters expression may play an important role in the development of the GABAergic neuronal functions in the brains of young mice.

The emerging family of neurotransmitter transporters can now be divided into subfamilies that share not only similar substrates but also extensive amino acid sequence identity. The subfamily of GABA transporters contains the transporters GAT1 (12, 13, 15), GAT2 (16), GAT3, GAT4, choline transporter (17), and taurine (28). Sequence identity among all of these transporters suggests that they evolved from a common ancestor highly related to the taurine transporter (28). Another subfamily of catecholamine transporters contains the norepinephrine, serotonin, and dopamine transporters (17–21). The glycine (23, 24) and proline (22) transporters may belong to a subfamily of amino acid transporters. The evolutionary distance revealed by the percentage of sequence identity among the subfamilies is about equal. Moreover, a common exon-intron junction was identified at the most conserved region between the first and second transmembrane domains in all the genes encoding neurotransmitter transporters (15). Therefore, it seems likely that the subfamilies evolved from a single ancestral gene, and they diverged at about the same time. The challenge now is to learn about the mechanism of action and regulation of the various neurotransmitter transporters as well as their specific function in the central nervous system and in other tissues.

**Acknowledgments**—We thank Dr. John Reeves and Dr. Nat Brot for critical reading of the manuscript.

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Four Different GABA Transporters