Branched-Chain Amino Acids and Neurotransmitter Metabolism: Expression of Cytosolic Branched-Chain Aminotransferase (BCATc) in the Cerebellum and Hippocampus

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ABSTRACT

In the brain, catabolism of the branched-chain amino acids (BCAAs) provides nitrogen for the synthesis of glutamate and glutamine. Glutamate is formed through transfer of an amino group from BCAA to α-ketoglutarate in reaction catalyzed by branched-chain aminotransferases (BCAT). There are two isozymes of BCAT: cytosolic BCATc, which is found in the nervous system, ovary, and placenta, and mitochondrial BCATm, which is found in all organs except rat liver. In cell culture systems, BCATc is found only in neurons and developing oligodendrocytes, whereas BCATm is the isofrom in astroglia. In this study, we used immunohistochemistry to examine the distribution of BCATc in the rat brain, focusing on the well-known neural architecture of the cerebellum and hippocampus. We show that BCATc is expressed only in neurons in the adult rat brain. In glutamatergic neurons such as granule cells of the cerebellar cortex and of the dentate gyrus, BCATc is localized to axons and nerve terminals. In contrast, in GABAergic neurons such as cerebellar Purkinje cells and hippocampal pyramidal basket cells, BCATc is concentrated in cell bodies. A common function for BCATc in these neurotransmitter systems may be to modulate amounts of glutamate available either for release as neurotransmitter or for use as precursor for synthesis of GABA. Particularly striking in our findings is the strong expression of BCATc in the mossy fiber pathway of the hippocampal formation. This result is discussed in light of the effectiveness of the anticonvulsant drug gabapentin, which is a specific inhibitor of BCATc. J. Comp. Neurol. 477:360–370, 2004. © 2004 Wiley-Liss, Inc.

Indexing terms: glutamate; gamma-amino butyric acid; gabapentin; epilepsy; dentate gyrus; Purkinje cell

Leucine, isoleucine, and valine, the branched-chain amino acids (BCAAs), readily cross the blood–brain barrier (Oldendorf, 1971), with influx of leucine exceeding that of isoleucine and valine (Smith et al., 1987). In brain slices, metabolism of BCAAs exceeds their rate of incorporation into brain tissue protein (Chaplin et al., 1976), and it has been shown that the BCAAs are nitrogen donors for synthesis of glutamate and glutamine in brain explants and in primary brain cell cultures (Yudkoff et al., 1983; Cooper and Plum, 1987). In recent studies in ex vivo retinas and in in vivo rat brain (Lieth et al., 2001), as well as astroglial and neuronal cells in primary culture (Gam-berino et al., 1997; Waagepetersen et al., 2001a), maintenance of glutamate levels was shown to depend on de novo
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(anaerobic) synthesis of glutamate. De novo glutamate synthesis involves the combined actions of astroglial pyruvate carboxylase and citric acid cycle enzymes to provide α-ketoglutarate (Gambarino et al., 1997; Lieth et al., 2001). Subsequently, transfer of an α-amino group from an amino acid donor to α-ketoglutarate produces glutamate and the α-keto-acid of the donor amino acid.

The BCAAs are important nitrogen donors for glutamate synthesis through transamination. Transamination of BCAAs with α-ketoglutarate is catalyzed by branch-chain aminotransferase isozymes (BCATs), mitochondrial BCATm and cytosolic BCATc (Ichihara, 1985; Hutson, 1988; Hutson et al., 1992). BCATm is present in all organs except liver, whereas BCATc is found only in the nervous system, ovary, and placenta (Ichihara, 1985; Hall et al., 1993; Hutson et al., 1995,1998; Sweatt et al., 2004). In rat brain, BCATc accounts for about 70% of all BCAT activity (Hall et al., 1993). In addition, it has been shown that BCAAs provide up to 30% of the nitrogen for glutamate synthesis in the in vivo rat brain (Kanamori et al., 1998) and that BCATc and BCAA are important for de novo glutamate synthesis in ex vivo rat retina (LaNoe et al., 2001; Lieth et al., 2001). In rat brain primary cell cultures the BCAT isozymes are expressed in different cell types. BCATc is found in neurons and oligodendroglia, whereas BCATm is found in astroglia and microglia cells (Bixel et al., 1997,2001). It has been proposed that the BCATs participate in an astroglial-neuronal nitrogen shuttle that replenishes amino-nitrogen lost from astrocytes in the form of glutamine (Yudkoff et al., 1996; Bixel et al., 1997,2001; Hutson et al., 1998; Daikhin and Yudkoff, 2000).

Regulation or buffering of the supply of glutamate metabolism, nitrogen shuttling, and in regulation of seizure activity in epilepsy and mechanism of action of drugs that may interfere with BCAA metabolism.

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MATERIALS AND METHODS

Tissue

Adult male Sprague Dawley rats (250–300 g) were anesthetized with pentobarbital (20 mg / 100 g) and perfused through the left ventricle with 150 ml PBS, followed by 300 ml 4% paraformaldehyde / 0.1 M sodium phosphate buffer, pH 7.4. Brains were removed and placed in the fixative for 24 hours. To cryoprotect the tissue, the brains were transferred sequentially into 10%, 20%, and 30% sucrose solutions for 24 hours each. The brains were embedded in OCT Compound (Sakura Finetek, Torrance, CA) by freezing on dry ice. Serial coronal and sagittal sections, 34 μm in thickness, were collected and stored as floating sections at −20°C in antifreeze media (25% glycerol, 25% ethylene glycol in 50 mM phosphate buffer, pH 7.4). For anatomical reference, sets of sections chosen at various intervals across the brain were mounted on slides and stained with cresyl violet (Nissl stain). Experimental procedures involving animals conformed to NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the Wake Forest University School of Medicine.

Antibodies

Polyclonal antibodies were raised in rabbits against purified recombinant human BCATc. Characterization and affinity purification of the antibodies has been described previously (Sweatt et al., 2004). On immunoblots of rat brain extract, the affinity-purified antirecombinant human BCATc antibody recognizes a single band of 47 kDa, the expected molecular mass of BCATc (Sweatt et al., 2004). The BCATc antibody has been used to identify BCATc in rat tissues by immunoblotting and immunohistochemistry (Sweatt et al., 2004). The antibody was used for immunohistochemistry at concentrations of 2–10 μg protein/ml. A monoclonal antibody recognizing glutamic acid decarboxylase (GAD67) was used to identify GABAergic neurons (Chemicon International, Temecula, CA; MAb5406). The isozymes GAD67 and GAD65 catalyze synthesis of GABA from glutamate. GAD67 is found throughout GABA-synthesizing neurons, whereas GAD65 is found mostly in GABAergic nerve terminals (Sogomonian and Martin, 1998). The antibody was used at a concentration of 1 μg protein/ml.

RESULTS

BCATc in cerebellar cortex and deep nuclei

For immunolocalization studies, floating sections were rinsed first in phosphate-buffered saline (PBS) for 25 minutes. Nonspecific binding sites were blocked by incubation of the sections in PBS containing 1% bovine serum albumin and 0.25% Triton X-100 for 1–1.5 hours. Sections were then incubated with the primary antibodies diluted in the blocking buffer at the stated concentrations. Incubations with primary antibodies were carried out at 4°C overnight, followed by 2 hours at 25°C. Sections were rinsed three times (10 minutes each) with PBS before incubation with affinity purified-goat antirabbit IgG, conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, West Grove PA; 8 μg/ml). After three rinses with PBS, color was developed using diaminobenzidine HCl/H2O2. Controls consisted of incubations of sections with secondary antibodies only or with primary antibodies that had been preincubated overnight with a 10-fold excess of competing antigen, followed by secondary antibodies. Sections were viewed with a Zeiss AxioPlan 2 microscope, and images were obtained using an AxioCam digital camera and AxioVision imaging software (Carl Zeiss USA, Thornwood, NY). Images were adjusted and assembled using Adobe PhotoShop 6.0 (Adobe Systems, San Jose, CA).

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reactive for BCATc. Figure 1 shows Nissl-stained and immunolabeled sections of cerebellar cortex and white matter. Over most of the cerebellar cortex (lobules I–VIII; Paxinos and Watson, 1998), BCATc immunoreactivity was seen in granule cell axons and in the parallel fibers derived from those axons (Fig. 1B,C). The radially oriented processes were not derived from Bergmann glial cells because, in sections of cerebellum immunolabeled for GFAP, radial processes of Bergmann glial cells extended far past the BCATc-immunoreactive zone, reaching the pial surface (not shown). In the caudal cerebellum (lobules IX and X), immunoreactivity for BCATc was present in the cell bodies of Purkinje cells and in their proximal dendrites (Fig. 1D). In Nissl-stained sections of cerebellum, Purkinje cells were evenly distributed across all the cerebellar lobules, and always exhibited a characteristic large cell body (Fig. 1A). Thus, the difference in the BCATc labeling pattern between the cerebellar lobules was not due to variation in Purkinje cell distribution or orientation. In addition, this staining pattern for BCATc was
consistent between animals, i.e., the same pattern was observed in all rat brains. Throughout the cerebellar cortex, cell bodies of the GABAergic Purkinje cells were heavily labeled with this antibody (not shown). However, the large BCATc-immunoreactive neurons of the deep cerebellar nuclei were not labeled for GAD67 (Fig. 2B). Instead, anti-GAD67 labeled many neuronal processes in the deep cerebellar nuclei and the unlabeled cell bodies were clearly outlined by synaptic terminals labeled for GAD67.

BCATc in hippocampal formation: dentate gyrus and mossy fiber projection

In the hippocampal formation, there was intense immunoreactivity for BCATc in the mossy fiber projection from the granule cells of the dentate gyrus. Figure 3A shows a Nissl-stained section of the dentate gyrus and field CA3, along with a nearby section labeled for BCATc (Fig. 3B). The most striking feature of the labeling pattern was the almost total lack of BCATc immunoreactivity in granule cell bodies. Instead, the BCATc labeling was very intense over the processes that make up the mossy fiber projection from the dentate gyrus to field CA3. Figure 4A shows a higher-resolution image of labeling for BCATc in the hilus (area in Box 1 in Fig. 3B). Of the small proportion of cell bodies in this area that were labeled for BCATc, most were located in the polymorphic layer, and included large mossy cells as well as smaller multipolar and fusiform cells (Fig. 4A). At the inner border of the granule cell layer there were also some heavily labeled pyramidal cells (PBC in Fig. 4A). Apical dendrites of some of these labeled cells extended through the granule cell layer into the overlying molecular layer of the dentate gyrus (Fig. 4A). Based on the location and shape of these labeled neurons, they are identified as pyramidal basket cells, many of which are GABAergic (Amaral and Witter, 1995). In a section through the hilus labeled with anti-GAD67, the labeling pattern for pyramidal cell bodies in the dentate gyrus corresponded to that seen for BCATc (Fig. 4B, PBC). Occasional cells in the molecular and polymorphic layers were labeled by anti-BCATc (Fig. 3B), and cells with the same respective positions and morphology were also labeled for GAD67 (not shown).

Figure 4C shows the region outlined by Box 2 in Figure 3B at higher resolution. Immunolabeled mossy fibers originating at the inner border of the granular layer traverse the polymorphic layer, running toward field CA3. These processes constitute the mossy fiber projection to field CA3. Some of the immunolabeled processes in the polymorphic layer may be granule cell axon collaterals that make local synaptic contacts with neurons of the polymorphic layer and with the basal dendrites of pyramidal basket cells (Amaral and Witter, 1995). The extensive basal dendritic trees of the pyramidal basket cells are also in a position to contribute to the network of immunoreactive processes. Where the mossy fibers reach hippocampal field CA3, immunolabeling for BCATc in processes gave way to dense labeling of larger structures. These labeled structures are the terminal expansions of the mossy fibers that form synaptic complexes, or varicosities, with target CA3 pyramidal cells. However, immunolabeling for BCATc in dendritic components of these contacts cannot be ruled out. In Figure 4C, the labeled varicosities are located at the upper and lower borders of the CA3 pyramidal cell layer, as well as within the pyramidal
cell layer in this region. These groupings of varicosities reflect the several routes by which the mossy fibers reach the most proximal region of field CA3, including the supra-, infra-, and intrapyramidal bundles (Amaral and Witter, 1995).

**BCATc in hippocampal fields CA3, CA1, and the subiculum**

In regions of field CA3 distant from the dentate gyrus (i.e., to the extreme right in Fig. 3), the principal BCAT-

![Image of hippocampal fields CA3, CA1, and the subiculum](image_url)
immunoreactive structures were again the varicosities located in the stratum lucidum, representing the synaptic complexes between mossy fibers and CA3 pyramidal cells (Fig. 5A). In the stratum radiatum and stratum oriens, cell bodies of large multipolar interneurons were labeled by anti-BCATc, as were many processes. The labeled processes in the stratum radiatum could be derived from neurons with cell bodies either in the stratum radiatum, in the pyramidal cell layer, or in the stratum oriens (Amaral and Witter, 1995). In the stratum oriens, some of the labeled cells correspond in position and morphology to CA3 basket cells, which form inhibitory (GABAergic) contacts on the basal dendrites of CA3 pyramidal cells (Ribak et al., 1978; Amaral and Witter, 1995). Immunolabeling for BCATc was also observed in the alveus (Fig. 5A), which consists of axons of CA3 pyramidal cells that project to various regions within and outside of the hippocampal formation. The alveus also includes axons of local circuit neurons and incoming axons from brain areas that project to the hippocampal formation.

In companion sections labeled for GAD67, there was heavy labeling in field CA3 of the stratum lucidum, as there was for BCATc (Fig. 5B). Again, the labeling is associated with the synaptic varicosities formed between mossy fibers and their target pyramidal cells. Additional labeling was present in a punctuate pattern over the lower portion of the pyramidal cell layer and in the stratum oriens. This pattern indicates labeling of synaptic terminals on the basal dendritic trees of the pyramidal cells. Above and below the pyramidal cell layer, anti-GAD67 labeled large GABAergic neurons (Fig. 5B) that resembled those that were labeled by anti-BCATc (Fig. 5A). The alveus was not immunolabeled for GAD67.

In hippocampal field CA1, the pattern of immunoreactivity for BCATc changed markedly, as immunolabeled varicosities became less frequent in field CA2 (not shown) and disappeared altogether in field CA1. Labeling of cell bodies was restricted to a small proportion of neurons in the CA1 pyramidal cell layer and to neurons in the stratum oriens (Fig. 6A). Neuronal processes in all strata were labeled for BCATc, although not as heavily as in field CA3. An exception was the alveus, which was heavily labeled for BCATc. In field CA1, the alveus projects largely to the adjacent subiculum.

In adjacent sections of field CA1 labeled with anti-GAD67 (Fig. 6B), the pattern of labeling of cell bodies was similar to that observed for BCATc. However, anti-GAD67 also labeled many fine processes and synaptic varicosities in the lower portion of the pyramidal cell layer and nearby in the stratum oriens. Based on these findings, it appears

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that some of the anti-BCATc-labeled neurons at the border of the pyramidal cell and stratum oriens are GABAergic pyramidal basket cells, with processes that are known to terminate among the basal dendrites of the pyramidal cells. The alveus in field CA1 was not immunoreactive for GAD67 (Fig. 6B).

In the subiculum, immunoreactivity for BCATc was seen over the cell bodies and large-caliber proximal processes of a significant proportion of neurons, as well as over many fine neuronal processes (Fig. 7A). The labeled neurons were mostly medium to large multipolar cells, although some resemble pyramidal cells. As the postsubicular was approached, immunolabeled cells became more numerous (not shown). Similar to findings for fields CA3 and CA1, the alveus was labeled for BCATc (not shown). A major output pathway for the subiculum is the fornix, in which nearly all the fine myelinated axons were immunoreactive for BCATc, similar to the findings for the cerebellar white matter (see Fig. 1E). The distribution of GAD67 in the subiculum was similar to that of BCATc, with many neuronal cell bodies and processes showing immunoreactivity (Fig. 7B). In addition, fine punctate immunoreactivity, associated with synaptic varicosities, was observed throughout the subiculum.

DISCUSSION

In rat brain cell cultures, the BCAA catabolic enzyme and gabapentin target BCATc was the sole BCAT isozyme expressed in rat cortical neurons prepared from brains of 16-day-old rat embryos (Bixel et al., 2001). BCATc was also found in oligodendroglial and O2A progenitor cells in astroglia-rich primary cultures derived from brains of newborn rats (Bixel et al., 1997). The pattern of immunolabeling for BCATc in intact adult rat cerebellum and hippocampus shows that the enzyme is located in glutamatergic and GABAergic neurons. BCATc is also found in some cerebral cortical pyramidal cells (Hutson et al., 2001) and in other brain regions (data not shown). No immunostaining for BCATc was observed in glial cells.

Distinct differences in the localization of BCATc in glutamatergic and GABAergic neurons were observed. In most glutamatergic neurons, little immunoreactive BCATc was found in the cell body (e.g., granule cells of the cerebellum and of the dentate gyrus). This suggests that BCATc is synthesized in neurons in the cerebellum and hippocampus, and is transported into the processes, especially into axons. This is most clearly the case for the terminals of the mossy fiber projection in the hippocampal field CA3. Axonal transport of BCATc also accounts for the presence of immunoreactive BCATc in granule cell axons / parallel fibers and in myelinated projection axons such as those in the cerebellum and in the fornix. The localization of BCATc in glutamatergic neurons is consistent with the hypothesis that this enzyme contributes to the mainte-
The nature of a pool of neurotransmitter glutamate. Thus, BCATc is concentrated at regions of high turnover of the neurotransmitters, namely, at synaptic terminals. For GABAergic neurons, significant BCATc is found in the cell bodies scattered throughout the pyramidal cell layer characteristic of this region of the hippocampal formation. Many finer processes are also immunoreactive for BCATc, and may be derived from axons arriving from field CA1. B: Labeling of cells and processes for GAD67 is very similar to that for BCATc, but many more fine processes are immunoreactive for GAD67. In addition, much of the labeling for GAD67 has a punctate/granular appearance. Scale bars = 100 μm.

Fig. 7. Immunolocalization of BCATc and GAD67 in the subiculum. A: BCATc is expressed in the cell bodies and proximal processes of medium and large multipolar neurons. The labeled cells are scattered in the wide pyramidal cell layer characteristic of this region of the hippocampal formation. Many finer processes are also immunoreactive for BCATc, and may be derived from axons arriving from field CA1. B: Labeling of cells and processes for GAD67 is very similar to that for BCATc, but many more fine processes are immunoreactive for GAD67. In addition, much of the labeling for GAD67 has a punctate/granular appearance. Scale bars = 100 μm.

Fig. 6. Immunolocalization of BCATc and GAD67 in hippocampal field CA1. A: Labeling for BCATc is present in cell bodies scattered throughout the pyramidal cell layer (Pyr) and in the stratum oriens (SO). Heavy labeling is seen in the alveus (AL). B: Immunoreactivity for GAD67 in field CA1 is present in cell bodies in the stratum radiatum (SR), pyramidal cell layer (Pyr), and stratum oriens (SO), as well as in fine processes among the inferior ranks of pyramidal cells. The alveus is not labeled for GAD67. Scale bars = 100 μm.
body (e.g., cerebellar Purkinje cells and pyramidal basket cells in the dentate gyrus). The presence of BCATc in cell bodies in GABAergic neurons may result from differences in targeting properties for the BCATc protein in these neurons. BCATc in GABAergic neurons may be involved in the regulation of a cytosolic, non-neurotransmitter pool of GABA (see below). The results do suggest that BCATc may have different roles in glutamatergic and GABAergic neurons.

We have not yet performed a detailed analysis in adult rats of expression of BCATc in brain regions with neurons that are primarily dopaminergic, such as the substantia nigra (SN). However, a preliminary result in neonatal rats indicates that dopaminergic neurons do not express BCATc at this stage of development. In a study using 12-day-old rats, 30% of the neurons in the SN pars reticulata underwent apoptosis following a lesion to their target striatum (Kholodilov et al., 2000). Concurrently, BCATc mRNA was upregulated in SN. Expression of immunoreactive active BCATc protein was increased mainly in the SN pars reticulata, where neurons are predominately GABAergic (Oertel et al., 1982). BCATc was not expressed in many neurons in the nearby SN pars compacta, where dopaminergic neurons are numerous (Kholodilov et al., 2000).

There are striking regional differences in the pattern of BCATc immunoreactivity in the cerebellar cortex (Fig. 1). Differences in connectivity and function along the paravermal region of the cerebellar cortex appear to be correlated with variation in the pattern of expression of BCATc, with heavy expression in glutamatergic inputs to most of the cortex, but with expression in the vestibulocerebellum being limited to the GABAergic output cells. Thus, in the paravermal zone of the cerebellar cortex, BCATc is localized in granule cell axons and parallel fibers in cerebellar lobules I–VIII, but is found in Purkinje cell bodies and their proximal dendrites in lobules IX and X. The paravermal regions of lobules I–VIII primarily receive spinal and trigeminal somatosensory inputs and are involved in proprioception and coordination of limb movements. In contrast, lobules IX and X have reciprocal connections with the brainstem vestibular nuclei and are involved in coordination of eye and head movements.

In the DCN, BCATc was present in the cell bodies of many large neurons, but they do not appear to be GABAergic neurons. Based on their size and location, these cells belong to a class of neurons identified as glutamatergic projection neurons (Sultan et al., 2003). Consistent with this classification, these DCN neurons were not immunopositive for the GABA-synthetic enzyme GAD67, although they received heavy GABAergic input from the cerebellar cortex (Fig. 2). So in this instance, immunoreactive BCATc is present in the cell bodies of a subclass of glutamatergic neurons in the DCN. Therefore, although most glutamatergic neurons in this region have axonal/synaptic BCATc, there are exceptions, indicating that BCATc expression can be differentially regulated in a given class of neurons. Evidence that BCATc expression can be regulated comes from the SN target-deprivation model in neonatal rats, mentioned above, in which BCATc protein expression was markedly upregulated in surviving neurons (Kholodilov et al., 2000). In the brain of normal adult rats, DCN neurons are not target-deprived, but their expression and/or trafficking of BCATc may reflect circumstances peculiar to their patterns of connection and activity.

The pattern of expression of BCATc in the hippocampal formation was consistent along the septotemporal axis. Most generally, BCATc was expressed in the mossy fiber inputs to the CA3 field and in output pathways (alveus) from the CA3 and CA1 fields. In addition to being found in these largely glutamatergic pathways, BCATc was also expressed in the cell bodies of many GABAergic interneurons. The widespread expression of BCATc in hippocampal pathways raises the possibility that manipulation of BCATc activity may affect hippocampal functions. One of the drugs that is used to control seizures in epileptics is gabapentin, which was synthesized as a GABA analog, but which resembles leucine (Su et al., 1995). Although its mode of action is not well understood, gabapentin is believed to enhance GABAergic, or inhibitory, neuronal activity, with resulting anticonvulsive, as well as antinociceptive, antidepressant, and anxiolytic effects (Ashton and Young, 2003; Finnerup et al., 2002; Ernst and Goldberg, 2003).

An early study on the mechanism of action of gabapentin concluded that it was an inhibitor of the BCAT isozymes (Goldlust et al., 1995), and a metabolic hypothesis of gabapentin action was proposed (Goldlust et al., 1995; Welty et al., 1995). Consistent with an intracellular site of action, uptake of gabapentin appears to be mediated by the L-amino acid transport system, which is present at the blood–brain barrier and in astrocytes and neurons (Su et al., 1995; Reichel et al., 2000). We have shown that gabapentin is a specific inhibitor of the cytosolic isozyme BCATc, but not of the mitochondrial isozyme BCATm (Hutson et al., 1998). Gabapentin also binds with high affinity to the α2δ subunit of the voltage-sensitive L-type calcium channel (Gee et al., 1996), a property it shares with leucine (Brown et al., 1998). L- and N-type calcium channels have been implicated in its mechanism of action (Luo et al., 2002; Bertrand et al., 2003). In addition to its role as an amino acid analog, it has been argued that gabapentin is a GABA<sub>A</sub> receptor agonist, but this hypothesis is controversial (Ng et al., 2001; Bertrand et al., 2001,2003; Jensen et al., 2002). Although no single molecular mechanism is sufficient to explain all the different therapeutic effects of gabapentin (Luo et al., 2002), the pattern of BCATc immunostaining supports a role for BCATc in the action of this drug.

Regarding epilepsy, inhibition of neural pathways in which hyperactivity leads to seizures is assumed to account for the anticonvulsant effects of the drug. Since BCATc is heavily expressed in the hippocampal formation, gabapentin’s efficacy as an anticonvulsant may derive in part from inhibition of BCATc activity that may influence the neurotransmitter glutamate pool or levels of the GABA precursor glutamate in GABAergic neurons. In the hippocampal formation, several BCATcexpressing cell types provide targets for gabapentin’s modulation of neurotransmitter metabolism. These include the glutamatergic granule cells that contribute axons to the mossy fiber projection, as well as the GABAergic pyramidal basket cells that exert an inhibitory influence locally on granule cells. There is increasing evidence that granule cells may also synthesize and release GABA, particularly after seizures (Ramirez and Gutierrez, 2001; Gomez-Lira et al., 2002; Walker et al., 2002). Because GAD67 is in the region where mossy fibers terminate on CA3 pyramidal cells, our results support a role for GABA in the mossy fiber projection, an area that is also rich in BCATc.
Translation of the effects of gabapentin on enzyme activity into changes in neuronal activity would depend on aspects of the intra- and intercellular environment. For example, effects on BCATc activity may be reflected in changes in specific intracellular pools of neurotransmitters. There is a growing body of evidence from cell culture studies that GABA, glutamate, and glutamine may exist in metabolic (cytosolic/mitochondrial) and neurotransmitter (synaptic) pools in neurons and astroglia (Waagepetersen et al., 2001b, 2003). In glutamatergic neurons, where BCATc is localized to processes and terminals, the enzyme would be more likely to interact with the neurotransmitter pool of glutamate. In GABAergic neurons, where most BCATc is concentrated in the cell bodies and proximal processes, the enzyme is well situated to have access to the cytosolic pool of glutamate. This pool of glutamate is derived from uptake of glutamate released from excitatory nerve terminals that synapase on GABAergic cell bodies and proximal dendrites and of astrocytic glutamine (Conti et al., 1998). The pattern of connections between the neurons should also contribute to the effect of changes in BCATc activity. In the GABAergic pyramidal basket cells, with local inputs to many dentate granule cells, changes in BCATc activity may have a more widespread effect on overall hippocampal output than would changes in BCATc activity in GABAergic neurons located downstream in the CA3 or CA1 fields and subiculum.

In summary, the expression of BCATc in select groups of glutamatergic and GABAergic neurons suggests that this enzyme plays a role in BCAA metabolism in neurons, and opens an avenue for improving our understanding of the metabolism that maintains neurotransmitter systems. The striking difference in intracellular distribution of BCATc between glutamatergic and GABAergic neurons suggests a different role for BCAA metabolism in these two neurotransmitter systems. Expression of BCATc in the hippocampal formation could explain some of the anticonvulsant properties of gabapentin. In addition, gaba- papent may modulate effects on aspects of neuronal activity in the cerebellum, where BCATc is heavily expressed in the cerebellar cortex.

LITERATURE CITED


