■ REVIEW

# GABA and Glutamate in the Human Brain

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Cortical excitability reflects a balance between excitation and inhibition. Glutamate is the main excitatory and GABA the main inhibitory neurotransmitter in the mammalian cortex. Changes in glutamate and GABA metabolism may play important roles in the control of cortical excitability. Glutamate is the metabolic precursor of GABA, which can be recycled through the tricarboxylic acid cycle to synthesize glutamate. GABA synthesis is unique among neurotransmitters, having two separate isoforms of the rate-controlling enzyme, glutamic acid decarboxylase. The need for two separate genes on two chromosomes to control GABA synthesis is unexplained. Two metabolites of GABA are present in uniquely high concentrations in the human brain. Homocarnosine and pyrrolidinone have a major impact on GABA metabolism in the human brain. Both of these GABA metabolites have anticonvulsant properties and can have a major impact on cortical excitability. NEUROSCIENTIST 8(6):562–573, 2002. DOI: 10.1177/1073858402238515

***KEY WORDS*** *Gamma-aminobutyric acid, Homocarnosine, Pyrrolidinone, Human, Brain*

Gamma-aminobutyric acid (GABA) has had a central role in neural control theory since it was first discovered in 1950 (Roberts 1986; Martin and Tobin 2000). It is the major inhibitory neurotransmitter in the human cortex. GABA serves as the primary inhibitory neurotransmitter at 20% to 44% of cortical neurons (DeFelipe 1993; Ribak and Yan 2000). Changes in GABA metabolism may play an important role in the origin and spread of seizure activity (Meldrum 1975; Hamberger and van Gelder 1993; Sherwin 1999; Ribak and Yan 2000). Several reports suggest that GABAergic neurons are decreased in the epileptic neocortex (Haglund and others 1992; Marco and others 1996; Spreafico and others 1998; Ribak and Yan 2000). Significant reductions in cerebrospinal fluid (CSF) GABA concentration are seen in patients with various epileptic syndromes (Wood and others 1979). Microdialysis-based GABA measurements show a decrease in the seizure-induced release of GABA in the epileptogenic hippocampus, which may reflect a functional defect in the GABA transporter (During and Spencer 1993; During and others 1995). Occipital lobe GABA concentrations, measured using magnetic resonance spectroscopy (MRS), are often below normal in epileptic patients who have frequent complex partial seizures (Petroff, Rothman, Behar, Mattson, and others 1996; Petroff and Rothman 1998; Petroff, Behar, and others 1999). Whether low GABA levels are the cause or the result of frequent seizures is unknown.

There are several ways of increasing GABAergic activity in the human brain (Kocsis and Mattson 1996). GABA agonists, for example, progabide, diazepam, phenobarbital, and propofol, directly increase inhibitory chloride conductances or up-regulate the effect of synaptically released GABA on the GABA-A receptor. GABA transporter blockers, for example, tiagabine, prolong the action of GABA in the synaptic cleft by inhibiting uptake. Stimulating GABA synthesis and release by valproate or gabapentin would increase synaptic GABA during neuronal activation. Slowing degradation of GABA by vigabatrin or valproate increases intracellular and extracellular GABA concentrations. Recent studies in animal models and human patients show that multiple feedback mechanisms control both GABA concentration and inhibitory activity.

GABA is formed from the alpha-decarboxylation of glutamate by glutamic acid decarboxylase (GAD) and is metabolized to succinate by the sequential actions of GABA-transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH). The activity of GAD is believed to be primarily responsible for regulating the steady-state concentration of GABA in vivo through the pyridoxal-5'-phosphate-dependent interconversion of active (holo-GAD) and inactive forms (apo-GAD) (Martin 1993; Martin and Rimvall 1993; Martin and Tobin 2000). A schematic diagram is shown in Figure 1. Apo-GAD accounts for at least 50% of total GAD present in the brain. The activation of GAD is stimulated by inorganic phosphate and inhibited by ATP, GABA, and aspartate. ATP promotes the formation of apo-GAD and stabilizes it. Without ATP, apo-GAD has a half-life of a few minutes at 37 °C. The activation of apo-GAD to holo-GAD by pyridoxal-phosphate is a two-step process.

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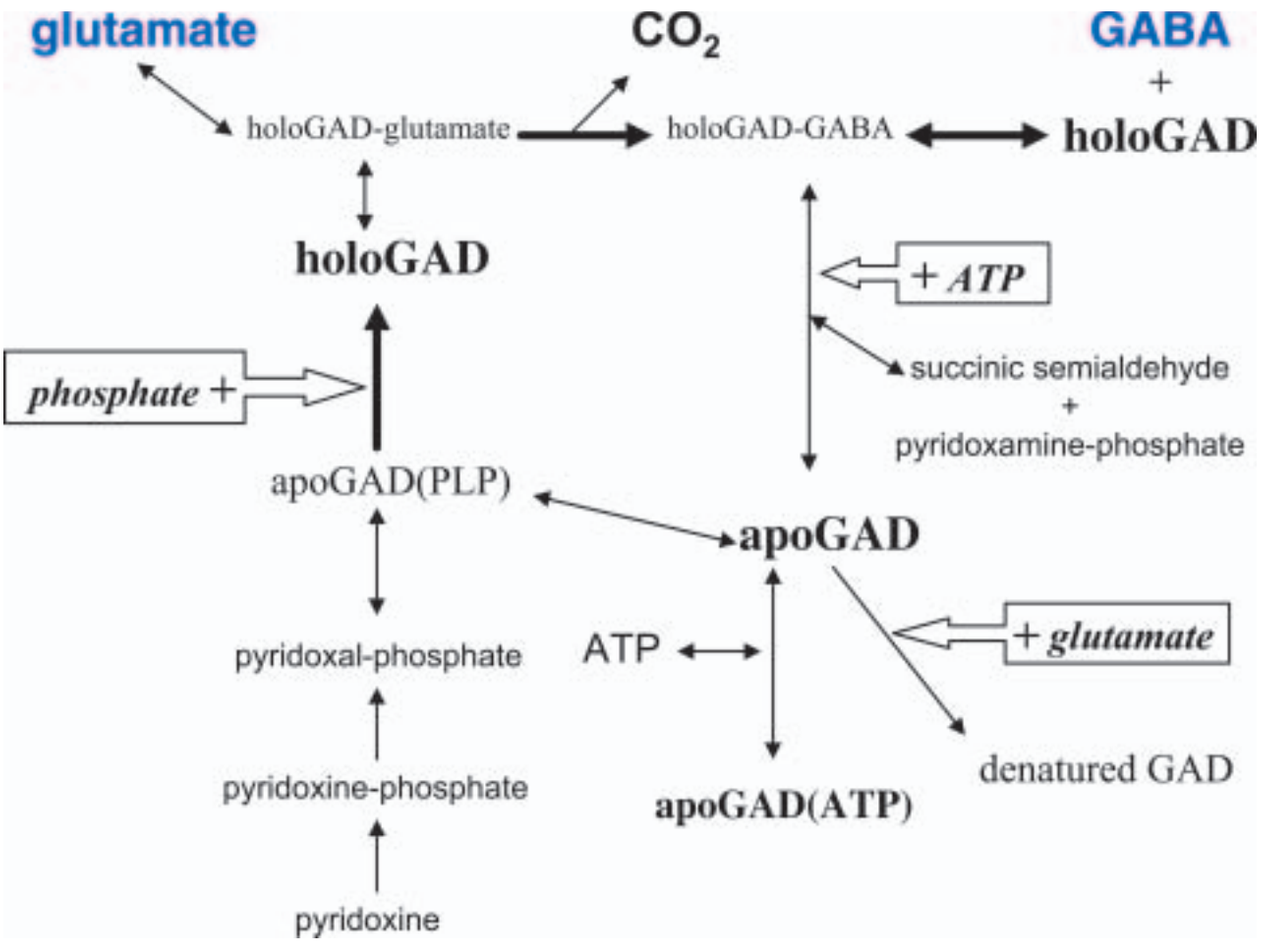


**Fig. 1.** Schematic of the regulation of GABA synthesis. GABA = gamma-aminobutyric acid; ATP = adenosine triphosphate; PLP = pyridoxal phosphate; $CO_2$ = carbon dioxide; holoGAD = holo-enzyme (active) isoform of glutamic acid decarboxylase; apoGAD = apo-enzyme (inactive) isoform of glutamic acid decarboxylase.

The reversible association of apo-GAD with activated pyridoxine is rapid (ATP inhibits binding of apo-GAD with pyridoxal-phosphate). Inorganic phosphate antagonizes the inhibitory effects of ATP and, through allosteric mechanisms, accelerates the formation of holo-GAD from the apo-GAD/pyridoxyl-phosphate intermediate. GAD is activated by changes in energy state—depolarization, acidosis, increased carbon dioxide, low bicarbonate, low phosphocreatine, increased magnesium, increased ADP, and decreased ATP.

GAD consists of two major isoforms with differing molecular weights (65 and 67 kD) that are the products of two different genes located in humans on chromosomes 2 and 10 (Erlander and Tobin 1991; Bu and others 1992). GAD65 appears to comprise the bulk of GAD protein in the brain, but most of it is in the inactive, apoenzyme form (Soghomonian and Martin 1998; Martin and Tobin 2000). Thus, GAD65 operates at a small fraction of its maximal catalytic capacity. Because the equilibria under physiological conditions strongly favor the apoenzyme form, GAD65 activity is very sensitive to changes in energy state (inorganic phosphate, phosphocreatine, pH, magnesium, ADP, ATP) and the availability of pyridoxal-phosphate (activated vitamin B6) (Martin 1993; Martin and Rimvall 1993). This isoform is concentrated in synaptosomes and readily binds to membranes. It appears to be used to control turnover in the vesicular, "neurotransmitter," pool of GABA. It has been suggested that GAD65 may be involved in short-term changes in GABA synthesis flux and GABAergic function in phasic inhibitory neurons (Rimvall and Martin 1994). Holo-GAD (active enzyme) levels remain unchanged in mice deficient in GAD65, but apo-GAD (inactive) levels are greatly reduced (Asada and others 1996, 1997; Kash and others 1997). Not unexpectedly, GABA levels are about the same in wild-type and homozygous GAD65-deficient mice. Mild stress induces spontaneous seizures affecting limbic structures in these mice, and they are very sensitive to convulsants.

The GABA concentration of the neocortex primarily reflects the fractional volume of GABAergic neurons weighted by the GABA content of those neurons. Under normal conditions, the GABA content of GABAergic neurons (50 to 100 mM in nerve terminals) is far greater than that of non-GABAergic neurons or glia (Fonnum

and Fyske 2000). The vesicular pool of GABA, thought to be synthesized under normal conditions primarily by GAD65, comprises ~30% of the GABA content formed, whereas GAD67 appears to synthesize ~70% of brain GABA (Soghomonian and Martin 1998; Martin and Tobin 2000). GAD67 has a wider distribution in the neuronal cytosol, being expressed in cell bodies, axons, dendrites, and synapses (Erlander and Tobin 1991; Martin and Tobin 2000). In individual neurons, the ratio of GAD65 to GAD67 mRNA varies with cell type. Higher levels of GAD67 mRNA are seen usually in neurons known to be tonically firing. GAD67 mRNA is more abundant than GAD65 mRNA in most regions of the brain. In the adult primate visual cortex, 18% of neurons express GAD67 mRNA whereas 13% express GAD65 mRNA (Hendrickson and others 1994). Most of GAD67 is in the holo-GAD form, actively synthesizing GABA. These observations suggest that changes in GAD67 activity have a greater impact on brain GABA content than changes in GAD65.

Experiments with GAD67 "knockout" mice lend further support to the hypothesis that changes in brain GABA content are more affected by changes in GAD67 expression and activity than by changes in GAD65. Mice completely deficient in GAD67 (–/–) die shortly after birth because of severe cleft palate malformations (Asada and others 1997; Ji and others 1999). These mice have less than 20% of the GAD activity and 7% of the brain GABA concentration compared with the wild type. The mice heterozygous for GAD67 (+/–) deficiency have 65% of the brain GABA concentration of the wild type as newborns and 79% as adults. Mice (embryonic day 14) deficient in both GAD isoforms had virtually no GABA present in the forebrain (Ji and others 1999). Those completely deficient in GAD67 (–/–) yet having one functioning GAD65 (+/–) gene have 9% of normal GABA concentrations.

Feedback inhibition of GAD by GABA is not considered important owing to the high concentration of GABA required for inactivation of GAD (Ki ~17 mM) relative to the concentration of GABA (1 to 2 mM). However, increased GABA concentrations reduce GABA synthesis and GAD activity (Rimvall and Martin 1994; Manor and others 1996). Elevated GABA decreases the activity of GAD and thus the rate of GABA synthesis by ~70% within 24 h. The genetic expression of GAD67 decreases as GABA levels rise (Rimvall and Martin 1994; Sheikh and Martin 1998). The first dose of vigabatrin irreversibly inhibits GABA-T thus increasing GABA levels (Jung and Palfreyman 1995; Petroff and Rothman 1998). GAD67 expression decreases by ~80% within 24 h of the first dose of vigabatrin and accounts for most of the decrease in GABA synthesis (Behar and Martin, personal communication). In epilepsy patients, the median rate of rise in GABA in response to the second dose of vigabatrin, administered 48 h after the first, is slowed by over 50% (Petroff, Hyder, Collins, and others 1999). The slower rate could be attributed to decreased GABA synthesis and decreased GAD expression, probably of the GAD67 isoform.

The synaptic actions of GABA are terminated by the high-affinity uptake systems (GABA transporters) of neurons and glia (Schousboe and Westergaard 1995; Roettger and Amara 1999). GABA taken up by the GABAergic neuron can be repackaged in vesicles for neurotransmission (Fonnum and Fyske 2000). When catabolized by GABA-T and SSADH, it can replenish intermediates in the mitochondrial TCA cycle (GABA shunt). It has been suggested that the flux through the GABA shunt regulates intracellular glutamate levels in cells that express GAD and GABA-T/SSADH (Martin and Tobin 2000). The flux through the GABA shunt would be expected to increase when cytosolic glutamate levels rise (effect primarily on GAD67) or when the redox potential shifts to the oxidizing state (effect on GABA-T/SSADH complex).

Glutamate-glutamine cycling reflects one aspect of metabolic interaction between neurons and glia. Changes in glutamatergic neuronal activity are reflected by proportionate changes in glutamate-glutamine cycling (Rothman and others 1999; Shen and others 1999). Glutamate released by neurons is taken up primarily by glia (Schousboe and others 1992; Schousboe and Westergaard 1995; Roettger and Amara 1999). The loss of glutamate from the neuron reflects an obligate loss of tricarboxylic acid (TCA) cycle intermediates that are critical for mitochondrial functioning. Only glia contain enzymes (primarily pyruvate decarboxylase) required for synthesis of new TCA cycle intermediates from glucose (Schousboe and others 1992; Wiesinger 1995). Glutamate taken up by glia is transformed to glutamine for recycling to neurons. Glutamine synthetase is localized in glia not neurons (Wiesinger 1995). Glia detoxify ammonia by making glutamine that is released into the blood stream for utilization by the liver and kidneys. Glutamine that is taken up by neurons can replenish glutamate stores lost by neurotransmission.

Recent developments in MRS have allowed the study of neuronal glutamate and GABA metabolism and the relationship of amino acid metabolism to functional neuroenergetics (Rothman and others 1999; Shen and others 1999). Under nonfasting conditions, glucose is the almost exclusive source of energy for the brain. By following the flow of glucose, labeled with the nonradioactive, stable isotope of carbon (13C) into these metabolites (Fig. 2). MRS has been used to determine the separate rates of glucose oxidation in glutamatergic and GABAergic neurons and glia. The metabolism of these cell types is coupled by several neurotransmitter cycles. In the glutamate-glutamine cycle, glutamate is released from cells (by either vesicular release or transporter reversal), taken up primarily by surrounding glia, converted to glutamine, released by glia, taken up by neurons, and is converted back to glutamate, thereby completing the cycle. By following the flow of 13C label from glutamate into glutamine, the rate of the glutamate-glutamine cycle may be determined using MRS. Through a similar strategy, the GABA-glutamine cycle may be measured.

If GABA is taken up by non-GABAergic neurons or glia or lost to the circulation, the GABAergic neuron is

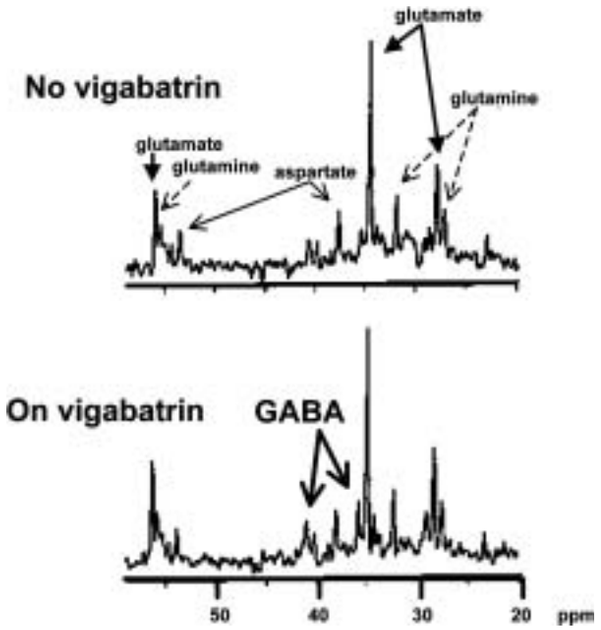


**Fig. 2.** Carbon magnetic resonance spectra following the infusion of 1-13C-glucose. The spectra were obtained at 2.1 Tesla of an 81-mL volume of the human occipital-parietal lobe. The GABA resonances (C4–40.4, C3–24.5, C2–35.3 ppm) are increased (bottom spectrum) in the patient taking vigabatrin. The resonances of glutamate (C4–34.3, C3–27.9, C2–55.5 ppm), glutamine (C4–31.7, C3–27.3, C2–55.0 ppm), and aspartate (C3–37.5, C2–53.0 ppm) are seen. The rate of synthesis of glutamate, glutamine, GABA, and other metabolites may be determined by measuring the rate of entry of carbon label into the various resonances. (Adapted from DeGraaf and others, Proc Intl Soc Mag Reson Med 2000;8:13.)

obligated to replace the four carbon skeleton of GABA made from glutamine or TCA cycle intermediates (Schousboe and others 1992). Only glia contain the enzymes needed to synthesize glutamine and new TCA cycle intermediates (Wiesinger 1995). Glutamine, synthesized by glia, appears to be the main route for replenishment of glutamate, and thus GABA, in GABAergic neurons. Glutamine produced from exogenous glutamate is readily released in cultured astrocytes. GABA synthesis is stimulated by glutamine in synaptosomes, cell cultures, and brain slices, where it serves as a major precursor of the releasable pool of glutamate and GABA (Schousboe and others 1992; Kapetanovic and others 1993; Sonnewald and others 1993; Martin and Tobin 2000). This suggests that GAD activity, and thus GABA synthesis, is limited by the availability of glutamate.

Another cycle, the glutamate-GABA cycle, potentially exists. Glutamatergic neurons appear to express GABA transporters (GAT1) and GABA-T/SSADH (Roettger and Amara 1999; Martin and Tobin 2000). GABA released by GABA-containing cells can be taken up by glutamatergic cells and recycled to form glutamate. Similarly, GABAergic neurons can take up extracellular glutamate (Roettger and Amara 1999). Glutamate taken up by GABAergic neurons stimulates GAD and thus GABA synthesis, thereby increasing GABA levels. GAD67 transcription and expression is up-regulated in response to injury (Martin and Tobin 2000). Enhanced activity of the glutamate-GABA cycle could be an adaptive metabolic response to excess glutamate release, helping to mitigate excitotoxicity.

The application of MRS to study brain glutamate and GABA metabolism, and the coupling of neurotransmitter cycling to neuroenergetics, has provided several new, and controversial, insights into the relationship of brain metabolism and function (Rothman and others 1999). Contrary to the previous view of a separate metabolic and neurotransmitter pool of glutamate, glutamate release and recycling were shown to be major metabolic pathways. MRS studies of GABA metabolism in the rodent and human brain have suggested that there is also an important role of the metabolic pool of GABA in inhibitory function. Another key finding is that the glutamate/glutamine cycle in the cerebral cortex is coupled in a close to 1:1 ratio to neuronal (primarily glutamatergic) glucose oxidation above that associated with an isoelectric electroencephalogram. This finding in combination with cellular studies has led to a model for the coupling between functional neuroenergetics and glutamate neurotransmission. The coupling between neurotransmission and neuroenergetics provides a linkage between the functional imaging signal and specific neuronal processes.

Carbamazepine, phenytoin, phenobarbital, and primidone do not increase CSF GABA significantly in adult patients with epilepsy (Wood and others 1979; Crawford and Chadwick 1987; Pitkänen and others 1989). Occipital lobe GABA measurements made using proton MRS (Fig. 3) show no distinct increase with the use of carbamazepine, phenytoin, phenobarbital, or primidone (Petroff, Rothman, Behar, Mattson, and others 1996; Petroff, Behar, and others 1999; Petroff and others 2000b). Brain GABA concentrations in the occipital lobe remote from the presumed epileptogenic zone are below normal in 67% of patients with refractory complex partial seizures treated with carbamazepine, 36% on phenytoin, and 33% on barbiturates (Petroff and others 2000b) (Fig. 4). Patients with poor seizure control have the lower GABA levels (Petroff, Rothman, Behar, Mattson, and others 1996; Petroff and Rothman 1998; Petroff, Behar, and others 1999). Both GABA synthesis and brain GABA concentrations increase in most seizure models (Wasterlain and others 1993; Kaura and others 1995; Loscher and others 1999). Enhanced GAD67 mRNA expression, enzyme activity, and GABA levels seen in animal models of stress, seizures, and other injuries could reflect a protective response (Martin and Tobin 2000). Therefore, it is surprising that GABA levels are below normal in many patients with poor seizure control, particularly in regions presumably remote from the epileptogenic areas. The studies with GAD knockout mice suggest that our patients with low GABA should have down-regulation of GAD67 activity or low levels of GAD67 protein. Studies in human temporal lobe epilepsy show decreased GAD expression and activity (Lloyd and others 1986; During and Spencer 1993; Mathern and others 1999; Sherwin 1999; Ribak and Yan 2000).

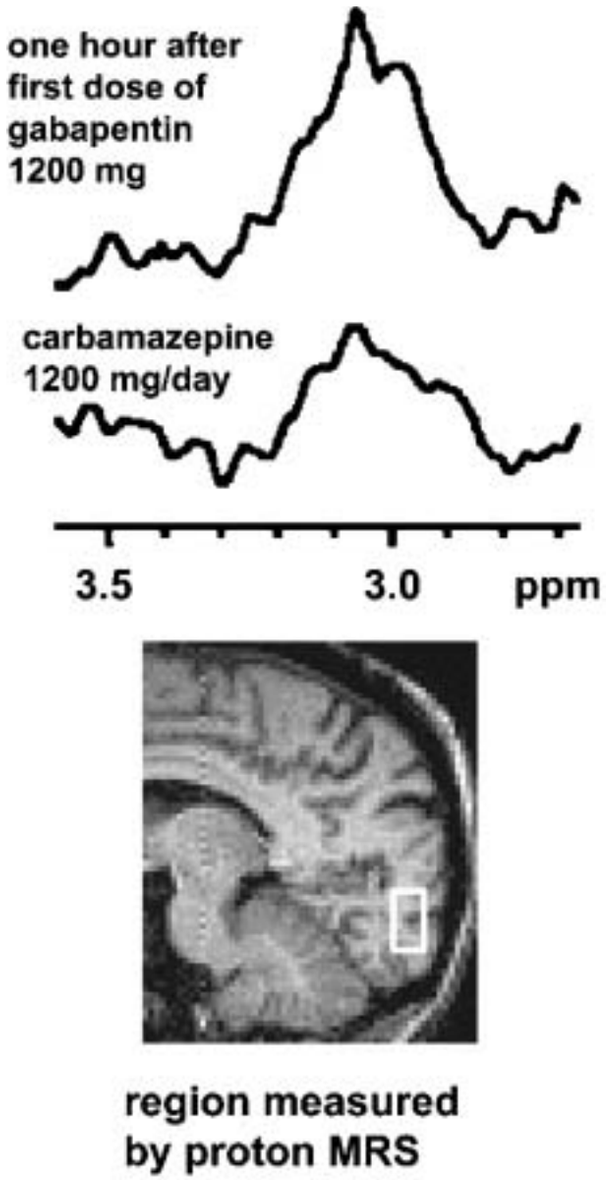


**Fig. 3.** Serial proton magnetic resonance spectra of GABA of a single patient before and after the first dose of gabapentin showing the rise in GABA. Spectra were obtained of a 14-cm3 volume in the visual cortex by proton spectroscopy using a 2.1 Tesla magnetic resonance spectrometer and an 8-cm in diameter surface coil. The localization techniques were 3D-ISIS sequence, outer volume suppression, plane selective excitation, and a surface spoiler coil. Homonuclear editing of the 3.0 ppm C4-GABA, homocarnosine, and pyrrolidinone resonances was performed using spin-spin (J) editing pulse sequence. (Adapted from Petroff and others, Epilepsia 2000a;41:675–80.)

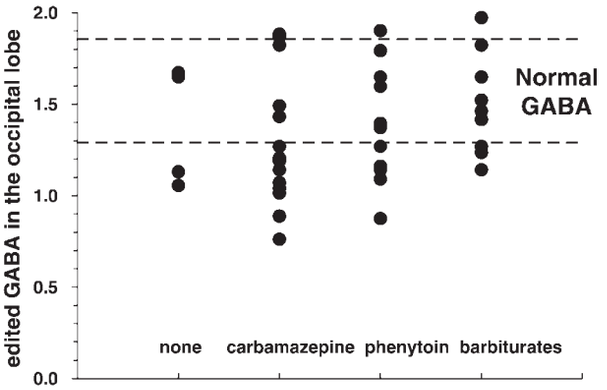


**Fig. 4.** The edited GABA concentrations measured using proton magnetic resonance spectroscopy in the occipital lobe of patients with complex partial seizures taking no medications (4 patients), carbamazepine alone (18 patients), phenytoin alone (9 patients), or carbamazepine (3 patients) and barbiturates alone (3 patients), or in combination with carbamazepine (4 patients) or phenytoin (2 patients). The dashed lines reflect two standard deviations about the mean edited GABA (GABA plus homocarnosine) of 25 drug-free, seizure-free volunteers.

Preclinical studies in rodents of the neurochemical effects of valproate show that valproate increased brain GABA content (Löscher 1999). The increase in GABA is attributed to inhibition of SSADH. This mitochondrial enzyme can complex with GABA-T; therefore, inhibition of one would inhibit both (Martin and Tobin 2000). Some studies of pediatric patients treated with valproate report increased CSF GABA concentrations, but others of adults do not (Löscher and Siemes 1985; Araki and others 1988; Pitkänen and others 1989). MRI-MRS measurements of the occipital lobe suggest that valproate does not increase occipital lobe GABA of patients with complex partial seizures (Petroff, Rothman, and others 1999). Although serial measurements have not been reported, it is unlikely that the large increase in GABA seen in the rodent forebrain will be seen in the human cerebrum.

Gabapentin, topiramate, and vigabatrin are three new antiepileptic drugs (Cramer and others 1999; Petroff and others 2000b). As shown in Figure 5 and Table 1, daily use of vigabatrin, topiramate, and gabapentin increases GABA in the human neocortex. Vigabatrin increases GABA in rodents and humans by irreversibly inhibiting GABA-T (Jung and Palfreyman 1995; Petroff and Rothman 1998). Vigabatrin is taken up by the GABA transporter and therefore preferentially inhibits GABA-T expressing more of the GABA transporter. Intracellular GABA concentrations rise not only in GABAergic cells but also in glia. In epilepsy patients, vigabatrin triples occipital lobe GABA concentrations within 90 minutes of the first oral dose (Petroff, Hyder, Collins, and others 1999) (Fig. 6). GABA levels remain nearly constant for 48 h after the first dose, slowly coming down over the course of a week (Petroff, Rothman, Behar, Collins, and others 1996; Petroff, Hyder, Collins, and others 1999). The increase in tissue GABA concentration reflects not only the rise in GABAergic cells but also the larger volume of cells (mainly glia) that now contain significant amounts of GABA. The rising GABA levels in glia would explain some of the quantitative differences between the effects of vigabatrin and gabapentin (Fig. 6). Vigabatrin increases tissue GABA levels more than gabapentin. After 1 h, the GABA levels achieved appear to be similar. With vigabatrin, GABA levels continue to rise over the first 90 min. The differ-

**Table 1.** Daily Treatment with Vigabatrin, Topiramate, or Gabapentin Mean in mM (95% confidence interval)

| Drug | Increased GABA | Increased Homocarnosine | Increased Pyrrolidinone |
|---|---|---|---|
| Vigabatrin | 0.7 (0.5–0.9) | 0.5 (0.4–0.6) | 0.15 (0.11–0.19) |
| Topiramate | 0.6 (0.3–0.8) | 0.5 (0.3–0.6) | 0.18 (0.13–0.22) |
| Gabapentin | 0.3 (0.1–0.6) | 0.3 (0.2–0.4) | 0.09 (0.06–0.13) |

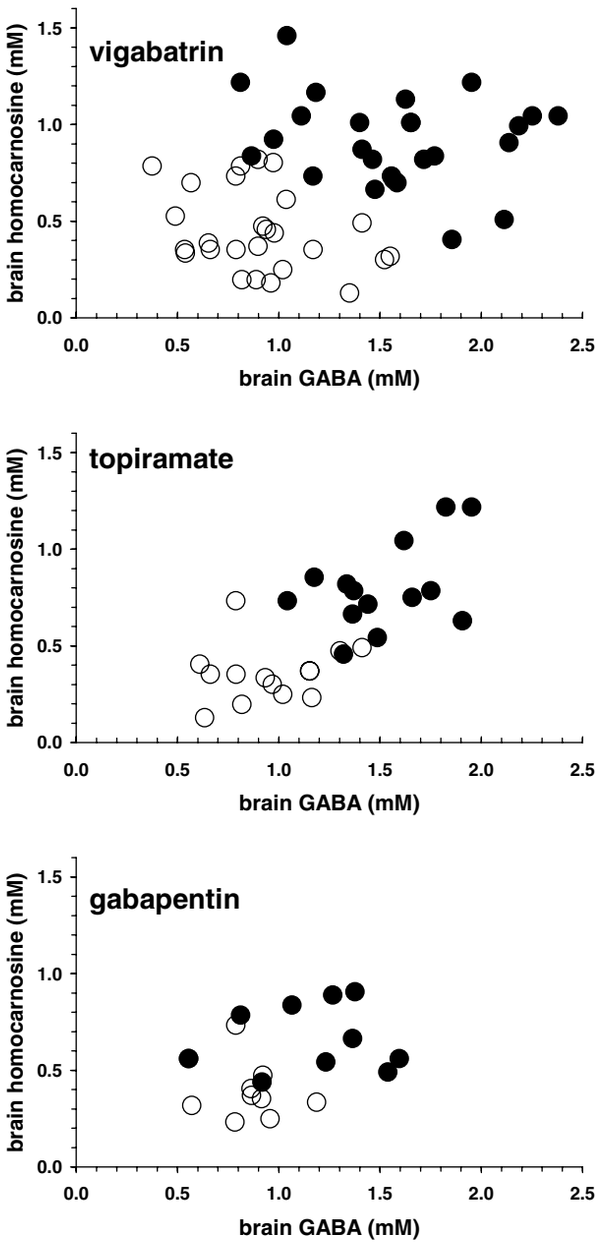


**Fig. 5.** GABA and homocarnosine concentrations serially measured using proton magnetic resonance spectroscopy in the occipital lobe of patients with complex partial seizures before (open circles) and with daily use (filled circles) of vigabatrin (*top* scattergram), topiramate (*middle* graph), and gabapentin (*bottom*).

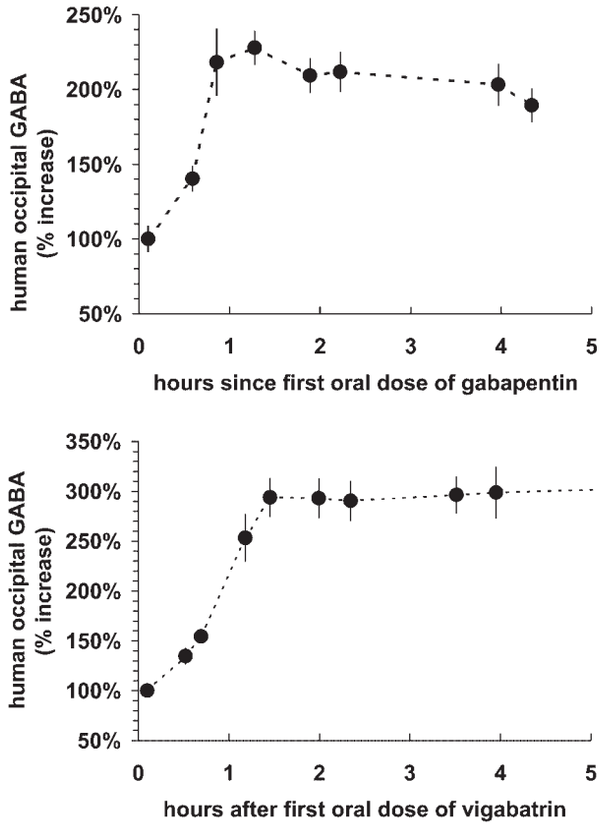


**Fig. 6.** The increase in GABA following the first dose of gabapentin (*top* graph) and vigabatrin (*bottom*). Serial GABA measurements were made of six patients with complex partial seizures following the first oral dose of gabapentin. Similar measurements were made in 11 patients after the first oral dose of vigabatrin. GABA levels increased by ~125% in 60 min with gabapentin and by ~200% in 90 min with vigabatrin.

ence between the effects of gabapentin and vigabatrin could be explained by proposing that gabapentin increases GABA levels in GABAergic neurons alone with no appreciable increase in GABA in glia or non-GABAergic neurons.

In epilepsy patients, gabapentin rapidly doubles occipital GABA concentration within 1 h after the first oral dose (Petroff and others 2000a) (Figs. 3 and 6). Brain GABA levels run to basal levels by the next day. Effects of gabapentin on brain amino acid neurotransmitters are not completely understood. In vitro studies with gabapentin at clinically relevant concentrations do not show direct effects on GABA pathways (Goldlust

and others 1995; Taylor and others 1998). Gabapentin does not directly affect GABA-specific enzymes, GABA receptors, GABA uptake, or the electrophysiological responses to GABA application. At higher concentration, gabapentin inhibits GABA degradation by GABA-transaminase and stimulates GAD, actions that promote high levels of GABA. Gabapentin fails to increase forebrain GABA concentrations in mice (Leach and others 1997). After pretreating with GABA-T inhibitors, animal models suggest that gabapentin stimulates very modest regional increases in GABA synthesis (Löscher and others 1991). At clinically relevant concentrations, gabapentin modulates the in vitro activities of some enzymes and transporters that affect glutamate metabolism. Gabapentin stimulates some isoforms of glutamate dehydrogenase (Goldlust and others 1995; Taylor and others 1998). Gabapentin is a competitive inhibitor of the neuronal-cytosolic-form of branched-chained-amino-acid-transaminase that synthesizes glutamate from branch-chained-amino-acids (BCAAs) and alpha-ketoglutarate and competes with BCAAs for uptake and release via the BCAA transporter (Taylor and others 1998). Potentially, these effects could increase glutamate concentrations in GAD-containing neurons, thus stimulating GABA synthesis. Further studies should clarify which mechanisms account for the large increase in GABA seen in human subjects.

Topiramate rapidly doubles brain GABA concentrations of subjects with and without epilepsy (Kuzniecky and others 1998; Petroff, Hyder, Mattson, and others 1999; Petroff and others, 2001b). Brain GABA remains elevated for more than 24 h after the first dose. The mechanisms by which topiramate increased human brain GABA are not understood. Preclinical data obtained in rodent models and by standard enzymology failed to predict significant increases in GABA (Shank and others 2000; Sills and others 2000). Topiramate is a weak carbonic anhydrase inhibitor and thus increases intracellular carbon dioxide content and lowers bicarbonate. GAD activity is stimulated by intracellular acidification that increases inorganic phosphate, therefore increasing GABA concentrations. This hypothesis can be tested in human subjects using acetazolamide, a more potent carbonic anhydrase inhibitor that is used to treat some types of human epilepsy. Alternative hypotheses would posit that topiramate increases inorganic phosphate directly, has an allosteric interaction with GAD similar to phosphate, or increases the availability of glutamate (or glutamine) in the GABAergic neuron.

Human CSF GABA consists of micromolar concentrations of homocarnosine and pyrrolidinone (the internal lactam of GABA), small amounts of other GABA-containing peptides, and nanomolar quantities of free GABA (Grove and others 1982; Haegele and others 1987). Forty years ago, a group at the NIH discovered homocarnosine, a dipeptide of GABA and histidine unique to the brain (Pisano and others 1961; Abrahams and others 1962; Kish and others 1979; Jackson and others 1994). Homocarnosine comprises ~40% of the GABA measured in human CSF after acid extraction of human CSF. A unique aspect of primate GABA metabolism, homocarnosine, is present in the human brain in greater amounts (0.3 to 1.6 mM, higher levels in subcortical gray matter) than in other mammals, for example, rats and mice < 0.07 mM (72, 73). In the occipital lobe of healthy subjects, values for homocarnosine levels are about 50% of the ones for GABA (Petroff, Hyder, Collins, and others 1999; Petroff, Hyder, Mattson, and others 1999; Petroff and others 2000a, 2001a, 2001b). Homocarnosine is an inhibitory neuromodulator synthesized in the neuron from GABA and histidine (Kish and others 1979; Jackson and others 1994). Immunohistology studies for homocarnosine suggest a cytosolic localization in the human brain, most likely in subclasses of GABAergic neurons. In the human neocortex and white matter tracts, homocarnosine immunoreactivity is seen along projecting fibers, rather than in local GABAergic interneurons. Autopsy-based studies show that regional homocarnosine and GABA levels vary independently (Perry and others 1971; Perry 1982). Homocarnosine concentrations are three- to sixfold higher in adults than in infants.

Like its beta-alanine analog, carnosine, the histidine part of homocarnosine, is sensitive to pH with a pK in the physiologic range (6.86) (Rothman and others 1997). The chemical shift of the homocarnosine resonances can be used to measure the pH of the human brain (7.06) in the homocarnosine-specific (neuronal cytosol) compartment. It has been proposed that homocarnosine contributes to intracellular buffering.

Although homocarnosine may serve as an important inhibitory neuromodulator in the human brain, little is known about the regulation of its synthesis (Gjessing and others 1990). Homocarnosine is synthesized in a subclass of GABAergic neurons by the enzyme homocarnosine synthetase (Kish and others 1979). The substrates for the enzyme are histidine and GABA and ATP (Fig. 7). Human homocarnosine synthetase activity is highest in the occipital cortex, basal ganglia, and cervical cord and lowest in the cerebellar cortex. Immunoreactivity is seen in neuronal cell bodies, axons, and synapses, correlating well with the cytosolic distribution of homocarnosine itself (Jackson and others 1994). The distribution of homocarnosine synthetase appears to correlate better with the reported distribution of GAD67 than for GAD65. Not unexpectedly, regional homocarnosine and GABA levels do not correlate (Perry and others 1971; Kish and others 1979).

In vitro human homocarnosine synthetase has a Vmax 18 nmole/g/h and a Km for GABA of 8.8 mM (Kish and others 1979). The Km for histidine is approximately 1 mM based on studies of the rat enzyme (carnosine-homocarnosine synthetase) (Skaper and others 1973). Substrate availability based on estimated cytosolic concentrations of GABA (5 to 10 mM) and histidine (0.1 to 0.2 mM) may limit homocarnosine synthetase activity. Consistent with substrate limitation, our previous studies have shown that homocarnosine concentrations increase following administration of drugs that elevate GABA, but only after a delay of at least 24 h (Petroff, Hyder, Collins, and others, 1999; Petroff and others 2000a,

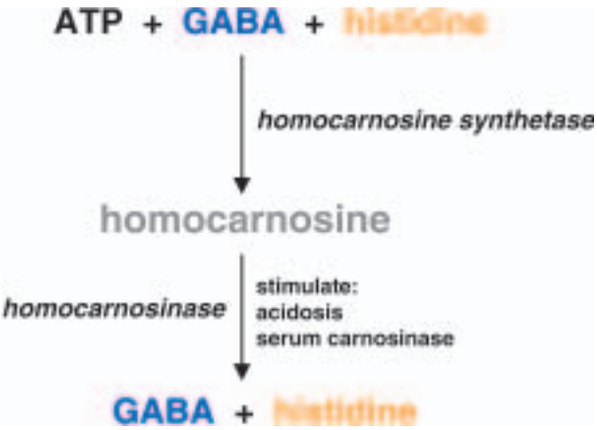


**Fig. 7.** Schematic of the synthesis and degradation of homocarnosine.

2001b). Low homocarnosine could reflect decreased fractional volumes of homocarnosine-containing neurons, expression of homocarnosine synthetase, or down-regulation of homocarnosine synthetase activity.

A specific enzyme, homocarnosinase (human serum carnosinase), rapidly hydrolyzes homocarnosine. It has an extracellular location associated with a subtype of GABAergic synapses. Homocarnosinase immunoreactivity is associated with synapses of projecting fibers, localization suggesting that homocarnosine may act as an important modulator of excitability in the human neocortex. Homocarnosinosis secondary to the inherited absence of homocarnosinase has been reported (Perry and others 1979; Gjessing and others 1990; Jakobs and others 1993). The phenotype is unclear and diagnosis required CSF analysis for homocarnosine. Homocarnosine that enters the blood is rapidly hydrolyzed by carnosinase, a serum enzyme that is present in normal amounts in homocarnosinosis. Dietary restriction of histidine lowers CSF homocarnosine levels (Lunde and others 1986).

As shown in Figure 5 and Table 1, daily use of vigabatrin, topiramate, and gabapentin increases homocarnosine in the human neocortex (Petroff and others 1998; Petroff, Hyder, Collins, and others 1999; Petroff, Hyder, Mattson, and others 1999; Petroff and others 2000a). Daily therapy with topiramate or vigabatrin increases homocarnosine and GABA more than gabapentin. The increase in homocarnosine levels reflected 70% to 80% of the rise in GABA. It seems reasonable to assume that homocarnosine synthesis is stimulated by the greater availability of GABA.

The regulation of homocarnosine concentrations is poorly understood. Restriction of histidine lowers the abnormally elevated homocarnosine levels associated with a dysfunctional degradative enzyme, homocarnosinase (Lunde and others 1986). CSF homocarnosine levels are elevated 6 to 24 h after the first dose of vigabatrin (Ben-Menachem and others 1989). However, brain concentrations increase after about a week (Petroff, Hyder, Collins, and others 1999). The first dose of topiramate increases brain homocarnosine within 24 h, much faster than the response to vigabatrin (Petroff and others 2001b). Although there is a general relationship between higher GABA and homocarnosine levels, the association is not a tight one (Fig. 5). The strongest association between GABA and homocarnosine increases appears to be for those patients taking topiramate. Based on the distribution of GAD67 and homocarnosine synthetase, homocarnosine is synthesized probably from GABA produced by GAD67 rather than GAD65. It is unlikely that homocarnosinase is inhibited by vigabatrin, topiramate, or gabapentin because this degradative enzyme has an extracellular localization.

Hydrolysis of homocarnosine has been proposed as an alternate metabolic pathway to rapidly increase GABAergic activity and thus serve as an important inhibitory neuromodulator in the human neocortex. Some studies in patients suggest that above-normal levels of homocarnosine may contribute to improved seizure control (Reikkinen and others 1989; Petroff and others 1998; Petroff and others 2001a). Intraventricular injections show that homocarnosine has anticonvulsant properties. It may interact directly with GABA receptors or after hydrolysis to GABA and histidine (Jackson and others 1994). The mechanisms of homocarnosine release, possible synaptic co-release with GABA, or more likely, nonsynaptic routes are obscure. Paracrine models appear to be favored with homocarnosine serving as an inhibitory modulator rather than a point-to-point synaptic signaling (Jackson and others 1994). Release of homocarnosine could contribute to glutamate-GABA cycling and reflect an adaptive response to excess extracellular glutamate.

Like its analog, carnosine (histidine and beta-alanine), homocarnosine may modulate synaptic transmission directly by altering the availability of zinc (Trombley and others 1998). Because homocarnosine is an excellent chelator of zinc and copper with relatively little affinity for calcium or magnesium, it can buffer zinc and copper levels with little impact on calcium. Alternatively, histidine, another excellent chelator of zinc, may be the primary effector. Having a strong zinc chelator at the GABA receptor should enhance the inhibitory action of GABA, particularly at those GABA receptors expressing the gamma-subunit (Smart and others 1994; Buhl and others 1996). Zinc also synchronizes release of GABA and modulates glutamatergic receptors (Williamson and Patrylo 1999). Thus, homocarnosine can modulate neuronal excitability through a number of mechanisms.

Even less is known about the internal lactam of GABA, pyrrolidinone. Under perchloric brain extract conditions, it rapidly hydrolyzes to GABA. The route of pyrrolidinone synthesis is unclear; it can be synthesized directly from GABA or indirectly from ornithine and polyamines (Lundgreen and Hankins 1978; Lundgreen and others 1980; Seiler 1980; Haegele and others 1987). The alternative synthetic pathways involve the conversion of putrescine to GABA and pyrrolidinone (Fig. 8). It is unknown whether a specific enzyme catalyzes the interconversion of GABA and pyrrolidinone or the equi-

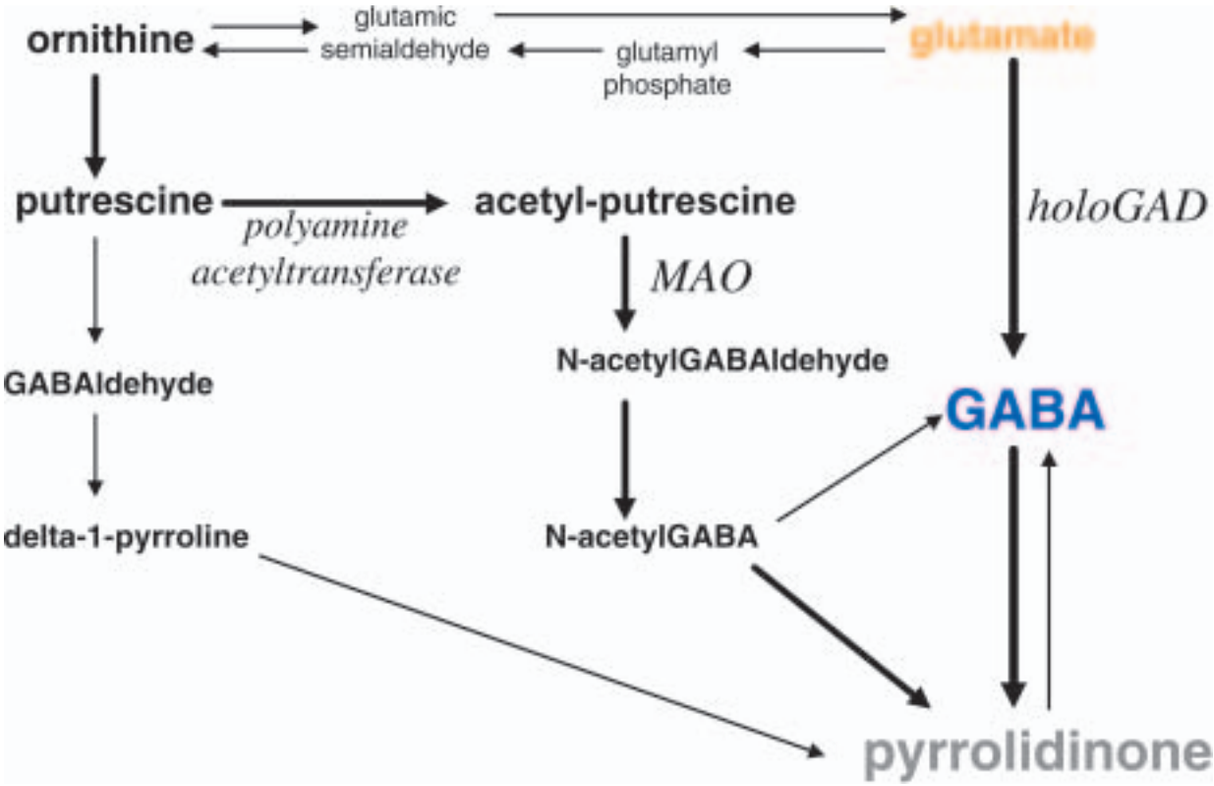


**Fig. 8.** Schematic of some metabolic pathways involved in the synthesis and catabolism of pyrrolidinone. GABA = gamma-aminobutyric acid; GABAldehyde = gamma-aminobutyraldehyde; MAO = monoamine oxidase; holoGAD = holo-enzyme (active) isoform of glutamic acid decarboxylase.

librium is mediated purely by acid-base considerations. Similar statements apply to the conversion of N-acetylGABA, delta-1-pyrroline, GABAldehyde, and other potential synthetic routes to pyrrolidinone (Haegele and others 1987).

Pyrrolidinone represents ~50% of GABA measured in human CSF after acid hydrolysis (Haegele and others 1987). In the rat and mouse brain, pyrrolidinone concentrations are very low, comprising less than 0.1% of GABA (Callery and others 1978, 1979; Bandle and others 1984; Fasolato and others 1988). Human brain concentrations appear to be at least an order of magnitude higher with levels about 20% to 25% of GABA (Hyder and others 1999; Petroff, Hyder, Mattson, and others 1999; Petroff and others 2000a, 2001a, 2001b). Because pyrrolidinone is an uncharged molecule that dissolves easily in both lipids and water, it crosses cell membranes and the blood-brain barrier far better than GABA. It has been proposed that pyrrolidinone is an intermediate coupling GABA and glutamate metabolism between the brain and liver (Fasolato and others 1988). Alternatively, it may couple brain and liver polyamine metabolism.

Exogenous administered pyrrolidinone has a half-life of 7 h in the blood and 14 h in the brain. Chronic oral administration in rats increases brain GABA levels by ~10% and decreases GAD activity by ~13% without affecting GABA-T activity (Fasolato and others 1988). Pyrrolidinone and some of its derivatives have anticonvulsant properties (Hawkins and Sarett 1957; Lightowler and MacLean 1963; Fasolato and others 1988; Sasaki and others 1991). The pyrrolidinone ring structure forms the core of a new class of antiepileptic drugs including levetiracetam (Cereghino and others 2000). Pyrrolidinone does not appear to have a direct anticonvulsant effect; conversion to GABA is assumed to be the primary mechanism (Callery and others 1979; Fasolato and others 1988). However, an indirect effect is possible, mediated perhaps by the linkage between GABA and polyamine metabolism (Seiler and Bolkenius 1985; Halonen and others 1993; Morrison and others 1995).

Gabapentin, topiramate, and vigabatrin increase cortical levels of pyrrolidinone in epileptic patients (Hyder and others 1999; Petroff, Hyder, Mattson, and others 1999; Petroff and others 2000a, 2001a, 2001b). The large increases in pyrrolidinone in response to these drugs are unexpected from the published results in animal models. The increase in pyrrolidinone reflects 22% of the increase in GABA with vigabatrin. Larger relative increases in pyrrolidinone are seen with gabapentin (27%) and topiramate (31%). The relative increase in pyrrolidinone with respect to the increase in homocarnosine ranges from 32% (vigabatrin) to 39% (topiramate). Topiramate appears to have a greater impact on pyrrolidinone levels than vigabatrin or gabapentin. Pyrrolidinone concentrations, measured before and after starting vigabatrin, topiramate, or gabapentin, correlate

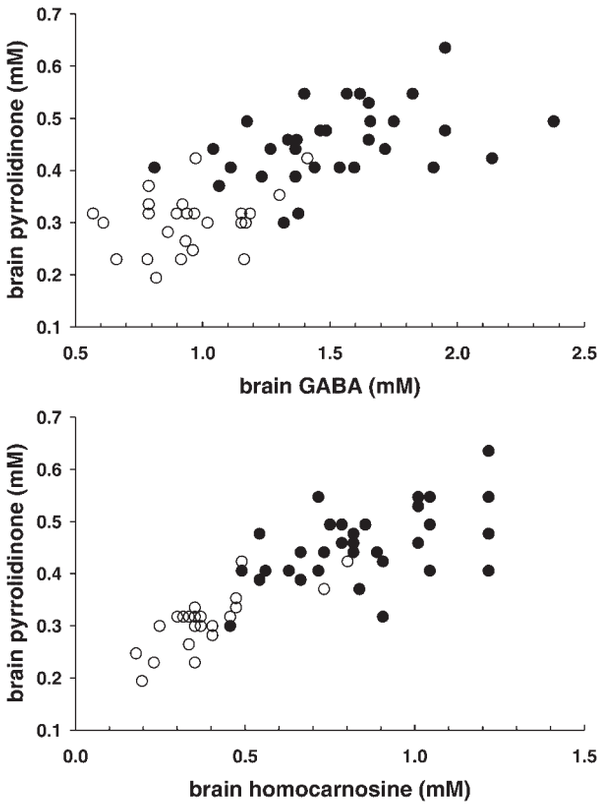


**Fig. 9.** Graphs showing the relationship between pyrrolidinone and GABA (*top* scattergram) and homocarnosine (*bottom*). Concentrations were serially measured using proton magnetic resonance spectroscopy in the occipital lobe of patients with complex partial seizures before (open circles) and with daily use (filled circles) of vigabatrin, topiramate, and gabapentin.

better with homocarnosine than with GABA concentrations (Fig. 9). This suggests that pyrrolidinone may be synthesized in the same neuronal compartment as homocarnosine. Potentially, homocarnosine synthetase could cyclize GABA to pyrrolidinone in the absence of sufficient histidine.

Human GABA and glutamate metabolism appears to be unique in a number of respects. Neocortical GABA concentrations appear to be lower than in rodent models. Homocarnosine and pyrrolidinone concentrations appear to be more than 10- to 100-fold higher in the human cerebrum than in the rodent forebrain. The large increases in brain GABA, homocarnosine, and pyrrolidinone measured in epilepsy patients taking gabapentin or topiramate are unexpected. The increases in homocarnosine and pyrrolidinone could contribute to the effectiveness of these drugs in controlling seizures by modulating cortical excitability. The mechanisms that regulate pyrrolidinone and homocarnosine concentrations remain unknown. The metabolic effects of increased concentrations of homocarnosine and pyrrolidinone await further study.

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