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Aspartate Aminotransferase and Glutaminase Activities in Rat Olfactory Bulb and Cochlear Nucleus; Comparisons with Retina and with Concentrations of Substrate and Product Amino Acids

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The quantitative distributions of aspartate aminotransferase and glutaminase were mapped in subregions of olfactory bulb and cochlear nucleus of rat, and were compared with similar data for retina and with the distributions of their substrate and product amino acids aspartate, glutamate, and glutamine. The distributions of both enzymes paralleled that of aspartate in the olfactory bulb and that of glutamate in the cochlear nucleus. In retina (excluding inner segments), there were similarities between aspartate aminotransferase and both glutamate and aspartate distributions. The distribution of γ-aminobutyrate (GABA) was similar to those of both enzymes in olfactory bulb, to aspartate aminotransferase in cochlear nucleus, and to glutaminase in retina (excluding inner segments). The results are consistent with significant involvement of aspartate aminotransferase, especially the cytosolic isoenzyme, and glutaminase in accumulation of the neurotransmitter amino acids glutamate, aspartate, and GABA, although with preferential accumulation of different amino acids in different brain regions.

KEY WORDS: Glutamate; aspartate; glutamine; γ-aminobutyrate; malate dehydrogenase; sensory systems.

INTRODUCTION

Glutamate and aspartate may serve as neurotransmitters in many brain regions, but final proof has been difficult to obtain, partly because of their ubiquitous presence and multiple other functions in neurons, including energy metabolism, protein synthesis, nucleic acid synthesis, and formation of di- and tripeptides (1).

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Although there are many potential synthetic pathways for glutamate and aspartate (1–6), available evidence points to two enzymes as particularly important for their synthesis in neurons (2,4). These are aspartate aminotransferase (EC 2.6.1.1, L-aspartate:2-oxoglutarate aminotransferase, AAT), which catalyzes interconversion of glutamate plus oxaloacetate with aspartate plus α-ketoglutarate, and glutaminase (EC 3.5.1.2, L-glutamine amidohydrolase), which catalyzes synthesis of glutamate from glutamine. By analogy with transmitters such as γ-aminobutyrate (GABA), which have no major roles in neurons besides neurotransmission, localization of glutamate and/or aspartate neurotransmitter function has been attempted by localization of AAT and glutaminase, especially by immunohistochemistry (7). In such studies, it has been hypothesized that neurons accumulating

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glutamate and/or aspartate for use as transmitters should have enhanced capability for their synthesis, reflected in elevated amounts of AAT and glutaminase (7). It has also generally been assumed that neurons using glutamate and/or aspartate as transmitters should have somewhat higher concentrations of these amino acids than other neurons, especially at their terminals (2). As support for these ideas, previous studies have looked for similarities in the distributions of immunoreactivity for AAT and/or glutaminase and immunoreactivity for glutamate and/or aspartate (8–10), as well as localizations of enzyme immunoreactivities to neurons for which there is much evidence for aspartate or glutamate as transmitter (7,11–16). The collective results of these studies, together with those of some quantitative chemical studies (17–19), have generally supported AAT and glutaminase as markers for neurons using glutamate and/or aspartate as transmitters. However, sometimes these enzymes, especially AAT, appear to be associated with neurons thought to use GABA as transmitter (13,16). This is not entirely surprising since GABA is synthesized from glutamate through the activity of glutamate decarboxylase (EC 4.1.1.15, L-glutamate 1-carboxy-lyase, GAD).

Despite the high spatial resolution of immunohistochemical labeling methods, their limited quantification makes difficult the interpretation of different labeling densities among neurons. Further, there are always concerns about cross-reactivity (20). Thus, quantitative assay of the distributions of AAT and glutaminase is needed to compare with conclusions from immunohistochemistry. We employed quantitative histochemistry (21), involving microdissection and direct chemical assay, to plot the distributions of AAT and glutaminase. This approach is complementary to immunohistochemistry in providing more precise quantitation, but generally at the resolution of small samples containing both neurons and glia rather than at subcellular resolution. Also, activities of enzymes were measured rather than concentrations of enzyme molecules. The main hypothesis tested was similar to that of the immunohistochemical studies: that the distributions of AAT and glutaminase should correlate with those of glutamate and aspartate to the extent that they are involved in synthesis of these amino acids as terminal products, such as neurotransmitters. The enzyme activities and amino acid concentrations should be elevated in regions where there is much evidence for glutamate and/or aspartate as transmitter.

Glutamate and aspartate have been implicated as transmitters in early stages of sensory processing (7,8,11,12,20,22–45). We measured the distributions of AAT and glutaminase specific activities in the olfactory bulb, cochlear nucleus, and retina, which contain the first central synapses of the olfactory, auditory, and visual system, respectively. The activities of the cytosolic and mitochondrial isoenzymes (46) were measured in addition to total AAT since there is some uncertainty about whether either or both of these may function in neurotransmitter metabolism (23,29,43). The distributions of these enzyme activities were compared with the distributions of their amino acid substrates and products: glutamate and aspartate for AAT and glutamine and glutamate for glutaminase. To provide some perspective about the correlations obtained, they were compared to those between GAD and GABA, based on previously published data for these same sensory centers (22,47–50). The distributions of AAT and glutaminase were also compared with that of GABA because of the correlations found in the immunohistochemical studies. Finally, the activity of AAT was compared to that of malate dehydrogenase (EC 1.1.1.37, L-malate:NAD^+ oxidoreductase, MDH) since correlations have previously been found (51–53). Although the retina data have been published (28–30,53), they are included here in a reorganized form that enables direct comparisons with the results for the other sensory centers.

EXPERIMENTAL PROCEDURE

All procedures used in this study have been described in detail (28,30,46,54) and so will only be summarized here. Treatment of animals was in accordance with regulations of the university animal care committee and of the National Institutes of Health.

Isolation of Tissue Samples. Olfactory bulb and cochlear nucleus samples were from Sprague-Dawley albino rats, retina samples from Long Evans hooded rats. Comparisons of retinal enzyme activities between these two rat stocks have generally shown small, statistically insignificant differences (53). Rats were decapitated and brain pieces frozen within 15–20 min in Freon (Curtin Matheson "Freeze-It")/liquid nitrogen (21). For cochlear nucleus and olfactory bulb, transverse sections 20 μm thick were cut in a cryostat at −20°C; every second or third was freeze-dried, the rest stained to aid identification of histological details (21,54). Retinal sections, 7.5 μm thick and tangential to the layers, were cut at −28°C and freeze dried. Freeze-dried sections were stored under vacuum below −20°C.

Dissection of freeze-dried sections into samples for assay was done at 25× magnification, at 50% or less relative humidity. Cochlear nucleus and olfactory bulb sample locations were recorded in section maps (54). Retinal samples were dissected from the centers of all sections in a series, thereby giving a superficial-to-deep progression across the layers. Samples were weighed on quartz-fiber microbalances (21), then loaded into 300 μl-capacity tube inserts for HPLC measurement of amino acids or into 3 ml-capacity glass tubes (10 × 75 mm) for fluorometric assay of AAT, glutaminase, or MDH activity.

Chemical Assays. Enzyme activities were assayed by fluorometric procedures in which the fluorescence of NADH was directly measured via a Farrand Ratio Fluorometer.

For assay of AAT, tissue was incubated for 30 min at 38°C with aspartate, a-ketoglutarate, MDH and NADH. The NAD^+ formed in

the reaction was measured by conversion to NADH through the alcohol dehydrogenase (EC 1.1.1.1, alcohol:NAD^+ oxidoreductase) reaction. The cytosolic and mitochondrial isoenzymes of AAT were sometimes assayed separately (46). Cytosolic AAT was assayed after destruction of the mitochondrial isoenzyme by heating. Mitochondrial AAT activity was measured by carrying out the incubation at reduced pH and aspartate concentration (in the earlier assays, on cochlear nucleus) or was calculated as total minus cytosolic AAT activity (olfactory bulb and retina). The former method gives activity which is approximately proportional to, but less than, mitochondrial AAT activity (46).

For assay of glutaminase, tissue was incubated for 60 min (90 min for retina) at 38°C with glutamine. The glutamate product was measured via its dehydrogenation to α-ketoglutarate by glutamate dehydrogenase (EC 1.4.1.2, L-glutamate:NAD^+ oxidoreductase (deaminating)), with concomitant reduction of NAD^+ to NADH (28,55).

For measurement of MDH, tissue samples were incubated at room temperature (25°C) with malate and NAD^+, and formation of NADH product was measured directly at timed intervals (52).

For HPLC assay of free amino acid concentrations, 0.01 M NaOH or 50% (vol/vol) methanol was added to the samples to extract the amino acids. Aliquots were derivatized with ortho-phthalaldehyde, and derivatized amino acids were separated by reverse-phase chromatography on a C-8 column (Dupont Zorbax), using gradient elution, and detected by their fluorescence (30,56). Some measurements of glutamate, aspartate, and GABA for olfactory bulb and cochlear nucleus were made via enzymatic assays (33,41). Data obtained by the two assay methods on the same sets of sections generally agreed within 20%.

Data Presentation. Since the enzymes and amino acids should be located in the non-lipid portions of the tissue, data are expressed per lipid-free dry weight to compensate for regional variations in lipid content (54). Lipid contents of olfactory bulb layers and cochlear nucleus regions of rats were measured in previous studies (57,58). Lipid contents of retinal layers of rat are difficult to measure by the same procedures because of the very small sizes of the individual layers, but values have been published for monkey (51). Lipid contents of most layers of rat retina have been determined by a different method (59). The non-lipid proportions in rat with this method are 84–95% of the proportions for comparable layers in monkey. The monkey retina values will be used here to adjust the retina data to a lipid-free dry weight basis, because they are based on the same method as used for olfactory bulb and cochlear nucleus, and because they have been measured for all the retinal layers.

The detailed maps of enzyme activity distributions showed that the major trends occurred across more than within regions of olfactory bulb and cochlear nucleus. Therefore, the data were summarized by first obtaining average values in each rat for each layer of olfactory bulb and each region of cochlear nucleus, then the overall means and standard errors of the mean across rats were calculated for each region. Olfactory bulb data are based on approximately 400 samples from 5 rats for total AAT (75 from 3 rats for the isoenzymes), 300 samples from 5 rats for MDH, 170 samples from 3 rats for glutaminase, and 160 samples from 3 rats for each amino acid except glutamine, for which there were 60 samples from 3 rats. Cochlear nucleus data are based on approximately 220 samples from 5 rats for total AAT (90 from 2 rats for mitochondrial AAT), 220 samples from 5 rats for MDH, 120 samples from 2 rats for glutaminase, and 180 samples from 6 rats for each amino acid except glutamine, for which there were 110 samples from 4 rats. The previously published retina data were also averaged to obtain mean values for each layer. An estimate of the overall correlation between pairs of chemicals in each sensory center, and across all 3 centers, was obtained by calculating correlation coefficients for the average regional values. This underestimates the actual reliability of the data because of the relatively large number of measurements averaged.

RESULTS

Aspartate Aminotransferase (AAT) Isoenzymes and Malate Dehydrogenase (MDH). For cochlear nucleus, the distributions of mitochondrial (reduced pH and aspartate) and total AAT activities were measured and found to be similar across regions (Fig. 1); the ratio of mitochondrial to total AAT activity was relatively constant (coefficient of variation of ratios = 0.07). This suggests that the ratio of cytosolic to total AAT activity would also be relatively constant across cochlear nucleus regions, so that the plot for total AAT can be taken as representative, with an appropriate scale adjustment, of that for cytosolic AAT. Based upon measurements on homogenates of cochlear nucleus, the cytosolic isoenzyme activity represents 40.8% of the total AAT activity. In the retina, the layers with higher total AAT activity (inner segments and plexiform layers) had relatively high proportions of mitochondrial AAT activity, so that the distribution of cytosolic AAT activity was rather different from that of the mitochondrial isoenzyme. The olfactory bulb presents an intermediate situation. The mitochondrial isoenzyme comprised a larger proportion of the total AAT activity in the more superficial layers, while the cytosolic isoenzyme predominated slightly in the deeper layers. In all three sensory structures, there was a close similarity between the distributions of total AAT and MDH activities.

Aspartate Aminotransferase and Its Substrate and Product Amino Acids. Generally, the distributions of aspartate and glutamate differed from each other in the primary sensory centers, and the distribution of AAT tended to parallel that of one or the other amino acid.

In the olfactory bulb, the distribution of total AAT activity closely paralleled the distribution of aspartate (Fig. 2). Both AAT activity and aspartate concentration peaked in the external plexiform layer and were less than half as much in the olfactory nerve fiber layer and periventricular layer.

In the cochlear nucleus, by contrast, the distribution of AAT activity was more similar to that of glutamate than to that of aspartate. Highest values of AAT activity were in the molecular layer of the dorsal cochlear nucleus, which also contained high glutamate concentration.

In the retina, the distribution of cytosolic AAT was

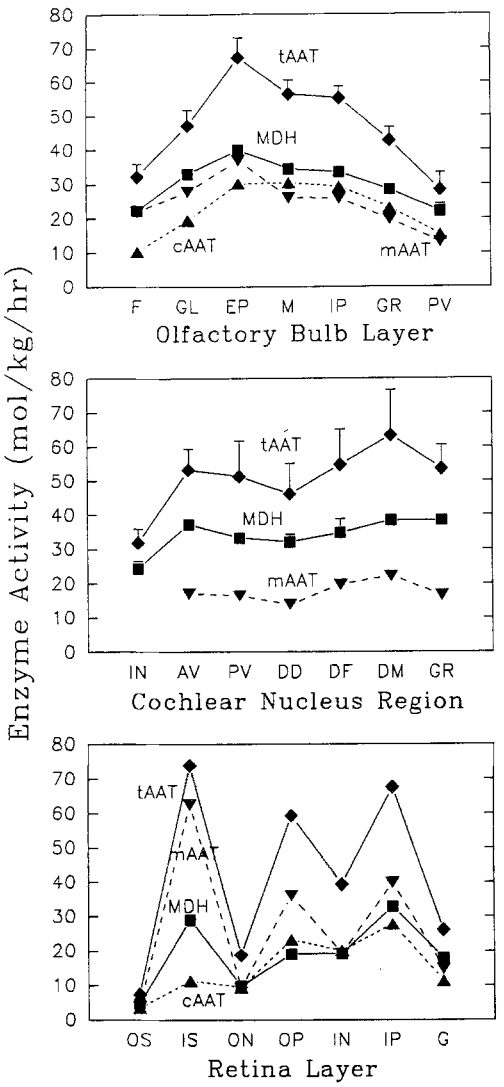

Fig. 1. Aspartate aminotransferase and malate dehydrogenase activities across regions of olfactory bulb, cochlear nucleus and retina. Standard errors of the mean are shown for olfactory bulb and cochlear nucleus. Error bars for MDH were usually smaller than the symbol size. The relatively large standard errors for tAAT in the cochlear nucleus resulted from large inter-animal variations in enzyme activity, although relative regional distributions were similar among rats. Chemical abbreviations: tAAT, total aspartate aminotransferase; cAAT, cytosolic aspartate aminotransferase; mAAT, mitochondrial aspartate aminotransferase; MDH, malate dehydrogenase. The somewhat lower values of mAAT activity for the cochlear nucleus than for the retina or olfactory bulb reflect the different method of measurement. Correlation coefficients for the correlations between the distributions of tAAT and MDH activities were 0.98 for olfactory bulb, 0.93 for cochlear nucleus, and 0.92 for retina. Anatomical abbreviations for all figures are, for olfactory bulb: F, olfactory nerve fiber layer; GL, glomerular layer; EP, external plexiform layer; M, mitral cell body layer; IP, internal plexiform layer; GR, granular layer; PV, periventricular layer; for cochlear nucleus: IN, interstitial nucleus (auditory nerve root); AV, anteroventral cochlear nucleus; PV, posteroventral cochlear nucleus; DD, DF, and DM, deep layer, fusiform soma layer, and molecular layer of dorsal cochlear nucleus; GR, granular layer dorsolateral to anteroventral cochlear nucleus; for retina: OS, outer segments layer; IS, inner segments layer; ON, outer nuclear layer; OP, outer plexiform layer; IN, inner nuclear layer; IP, inner plexiform layer; G, ganglion cell layer.

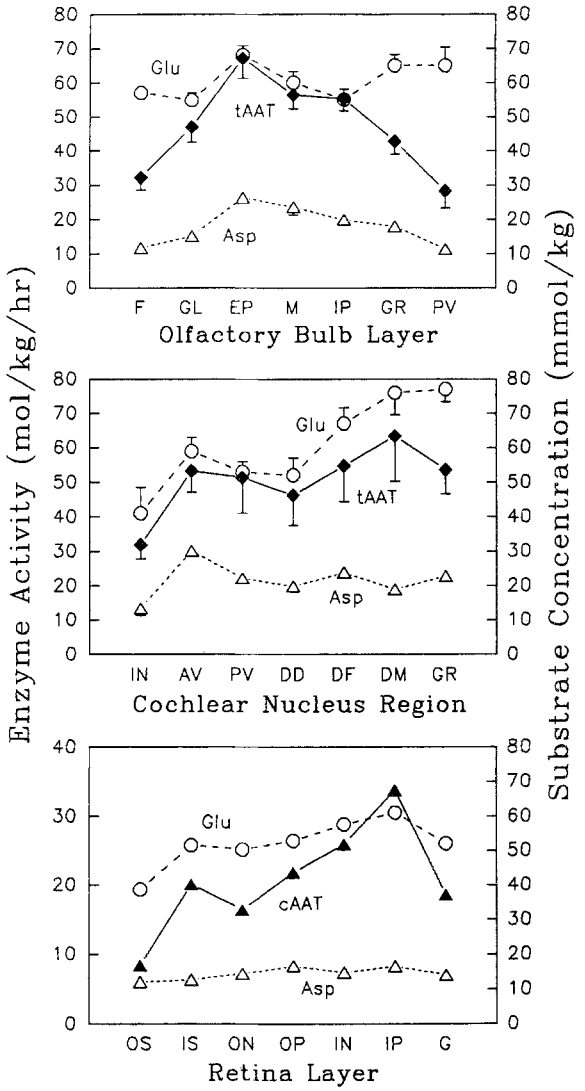

Fig. 2. Aspartate aminotransferase activity and glutamate and aspartate concentrations across regions of olfactory bulb, cochlear nucleus and retina. Standard errors of the mean are shown for olfactory bulb and cochlear nucleus. Abbreviations: tAAT, total aspartate aminotransferase; cAAT, cytosolic aspartate aminotransferase; Asp, aspartate; Glu, glutamate. Anatomical abbreviations as in Fig. 1.

rather similar to that of glutamate, with maxima in the inner plexiform layer.

Excluding the inner segments layer of the retina, which contains unusually high mitochondrial AAT activity, the magnitudes of AAT activities were roughly similar among the three sensory structures. Glutamate concentrations were also similar, but aspartate concentrations were generally somewhat lower in retina than in olfactory bulb or cochlear nucleus.

Glutaminase and Its Substrate and Product Amino

Acids. Like total AAT activities, glutaminase activities were generally similar among olfactory bulb, cochlear nucleus, and retina. The distribution of glutaminase (Fig. 3) was similar in the olfactory bulb to that of total AAT. In the cochlear nucleus, there was a prominent difference between the distributions of these enzyme activities in that glutaminase activity was relatively lower in the dorsal cochlear nucleus molecular layer and higher in the granular layer than AAT activity. In the retina, the glutaminase and total AAT distributions differed, but both showed peaks in the inner segments layer.

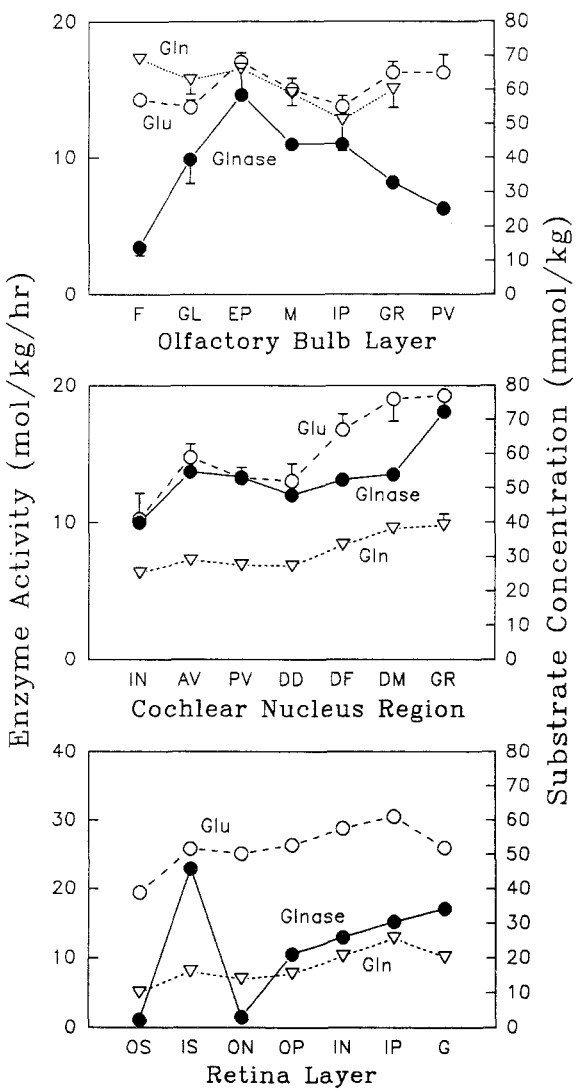

Fig. 3. Glutaminase activity and glutamate and glutamine concentrations across regions of olfactory bulb, cochlear nucleus and retina. Standard errors of the mean are included (where the number of rats exceeded 2) for olfactory bulb and cochlear nucleus. Abbreviations: Glnase, glutaminase; Gln, glutamine; Glu, glutamate. Anatomical abbreviations as in Fig. 1.

In the olfactory bulb, glutaminase activity peaked in the external plexiform layer and was less than half as high in the olfactory nerve fiber and periventricular layers. The activity for the fiber layer was particularly low, less than a fourth of that of the external plexiform layer. By contrast, the concentrations of both its substrate (glutamine) and its product (glutamate) were rather uniformly distributed across the layers of the bulb.

In the cochlear nucleus, the distributions of glutaminase, glutamate, and glutamine were fairly similar. However, glutamate and glutamine were high in both the granular region and dorsal cochlear nucleus molecular layer, while glutaminase was high only in the granular region. Since AAT was higher in the molecular layer than in the granular region (Fig. 2), the distributions of glutamate and glutamine concentrations resembled that of the sum of the normalized enzyme activities more closely than that of either enzyme alone.

In the retina, the distributions of glutamate and glutamine were parallel, but that of glutaminase differed in having a large peak in the inner segments and very low values in the outer segments and outer nuclear layers.

Although the relative distribution of glutamine concentration always paralleled that of glutamate, the absolute concentrations of glutamine differed strikingly among the 3 primary sensory centers, being lowest in retina and highest in olfactory bulb.

Comparisons Between AAT, Glutaminase, and GABA Distributions. The distribution of GABA concentration across the layers of the olfactory bulb resembled closely the distribution of cytosolic AAT activity (Fig. 4), as well as that of glutaminase (Fig. 3).

In the cochlear nucleus, the distribution of GABA concentration paralleled that of AAT, while in the retina the parallel was not as close (Fig. 4).

Correlations Between Enzyme and Amino Acid Distributions. As expressions of the correlations between the distributions of AAT and glutaminase activities and those of related amino acid concentrations, linear regression correlation coefficients were calculated (Table I). The glutaminase and AAT activities in the inner segments layer of the retina appear to be profoundly influenced by its unusually high concentration of mitochondria and high oxidative energy metabolism (51,60). The correlations with amino acid concentrations were therefore calculated without inclusion of the data for this layer as well as with them.

The correlation coefficients can be evaluated by comparison to those between GABA and GAD calculated from published data for the three sensory centers. These are 0.78 for rat olfactory bulb (48), 1.0 for guinea pig cochlear nucleus (only the 3 major subdivisions were

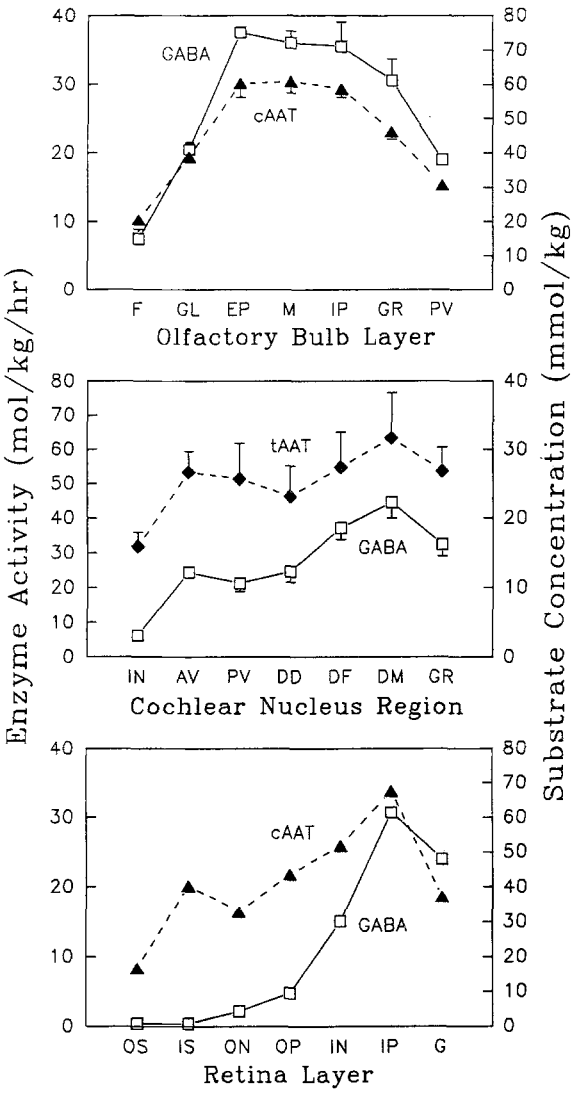

Fig. 4. Aspartate aminotransferase activity and γ-aminobutyrate concentration across regions of olfactory bulb, cochlear nucleus and retina. Standard errors of the mean are shown for olfactory bulb and cochlear nucleus. Abbreviations: tAAT, total aspartate aminotransferase; cAAT, cytosolic aspartate aminotransferase; GABA, γ-aminobutyrate. Anatomical abbreviations as in Fig. 1.

assayed) (47), 0.81 for rabbit retina and 0.95 for monkey retina (22,49,50). Such coefficients correspond to those significant at $p < 0.05$ or $p < 0.01$ in Table I. In olfactory bulb, high correlations were found between both aspartate and GABA and both glutaminase and AAT, particularly cytosolic AAT. In cochlear nucleus, high correlations were found between glutamate and both enzyme activities and between GABA and AAT. In retina exclusive of the inner segments, high correlations were found between both glutamate and aspartate and AAT, and between GABA and glutaminase. With the regions of all three sensory centers grouped together, correlations tended to be lower because of inter-system differences, but were still statistically significant because of the larger population size, for glutamate and aspartate with both glutaminase and AAT, and for GABA with cytosolic AAT.

Since both glutaminase and AAT activities can lead to formation of glutamate and aspartate and, through glutamate, GABA, correlations were also examined between the sum of the normalized glutaminase and AAT activities and the sum of glutamate, aspartate, and GABA concentrations. Correlation coefficients were 0.95 for olfactory bulb, 0.87 for cochlear nucleus, 0.86 for retina (excluding inner segments), and 0.61 for all 3 sensory centers combined, with all correlations statistically significant at $p < 0.01$. Somewhat higher correlations were obtained when only the cytosolic isoenzyme of AAT was summed with glutaminase without normalization (since the enzyme activities had similar magnitudes): 0.95 for olfactory bulb, 0.96 for cochlear nucleus, 0.94 for retina (excluding inner segments), and 0.75 for all 3 sensory centers combined. These high positive correlations of the summed enzyme activities with the sum of aspartate, glutamate, and GABA concentrations contrasted with a poor or negative correlation (correlation coefficient 0.39 for cochlear nucleus, −0.88 for olfactory bulb, −0.24 for combined data) with the concentration of alanine, an amino acid which is important in metabolism and participates in a transamination reaction with glutamate, but is not stored for neurotransmission (1,3,5).

DISCUSSION

Comparisons with Previous Histochemical Results for AAT and Glutaminase. The activities of AAT and glutaminase were often highest in plexiform and molecular layers, where there are high densities of synapses. The activities of mitochondrial AAT appeared to be particularly high in synaptic regions and in the retinal inner segments, which are all regions having high mitochondrial densities. On the other hand, for glutaminase and cytosolic AAT, there were exceptions to this trend. The glomerular layer of the olfactory bulb, with a very high density of synaptic terminals (42), had lower enzyme activities than some of the deeper layers, and the outer plexiform layer of the retina did not have high activities of cytosolic AAT or glutaminase. Thus, one can not make a simple correlation between cytosolic AAT or glutaminase activity and density of synaptic terminals;

Table I. Correlation Coefficients for Comparisons Between Enzyme and Amino Acid Distributions[a]

		Glutaminase	tAAT	cAAT	mAAT
Asp	OB	0.90*	0.96*	0.93*	0.78+
	CN	0.50	0.54		0.43
	Ret	0.54 (0.11)	0.94* (0.47)	0.83+ (0.76+)	0.88+ (−.12)
	All	0.57* (0.36)	0.68* (0.53+)	0.56* (0.56*)	0.51+ (0.01)
Glu	OB	0.25	0.11	0.18	0.01
	CN	0.80+	0.86+		0.83+
	Ret	0.75 (0.57)	0.81+ (0.66)	0.96* (0.96*)	0.83+ (0.28)
	All	0.49+ (0.35)	0.61* (0.51+)	0.53+ (0.53+)	0.53+ (0.10)
Gln	OB	−.33	−.31	−.61	0.17
	CN	0.77+	0.75+		0.74
	Ret	0.86+ (0.60)	0.72 (0.53)	0.92* (0.92*)	0.83+ (0.19)
	All	0.03 (−.09)	0.38 (0.25)	0.26 (0.27)	0.12 (−.17)
GABA	OB	0.88*	0.84+	0.98*	0.52
	CN	0.54	0.93*		0.94*
	Ret	0.87+ (0.40)	0.55 (0.22)	0.76 (0.72)	0.73 (−.11)
	All	0.31 (0.13)	0.43 (0.30)	0.71* (0.70*)	0.29 (−.11)

[a] Abbreviations: Asp, aspartate; Glu, glutamate; Gln, glutamine; GABA, γ-aminobutyrate; tAAT, cAAT, and mAAT, total, cytosolic, and mitochondrial aspartate aminotransferase; OB, olfactory bulb; CN, cochlear nucleus; Ret, retina; All, all 3 centers together. To obtain correlation coefficients in cochlear nucleus, a value of mAAT for interstitial nucleus was estimated as 59.2% of the value for tAAT, based on our unpublished measurements on homogenates. From these same measurements, cAAT activities for cochlear nucleus regions were estimated as 40.8% of tAAT activities to enable the calculations of overall correlation coefficients for amino acids vs. cAAT. For retina and overall correlations, the primary numbers do not include the inner segments because of their unusually prominent energy metabolism, but the values with their inclusion are given in parentheses. Statistical significances of correlations: + for $p < 0.05$ and * for $p < 0.01$.

the nature of the transmitter in the terminals should also be an important factor.

The high correlation between glutaminase and total AAT in the olfactory bulb suggests the possibility that the activities of both may be especially high in the same groups of cells. We are unaware of immunohistochemistry for glutaminase in the olfactory bulb, but an immunohistochemical study of AAT isoenzymes (43) reported a particular association of mitochondrial AAT with mitral and tufted cell bodies and with glomeruli, of cytosolic AAT with some periglomerular cell bodies, and of both with some granule cell bodies. Our results agree with these observations for glomerular and granular layers, but show more enzyme activity in the external plexiform layer and more cytosolic isoenzyme activity in the mitral cell body layer than appears in the immunohistochemistry.

In the cochlear nucleus, some immunohistochemical studies have reported very similar localizations of AAT and glutaminase, in auditory nerve terminals and granule cells (7,11,12). Our results do not entirely agree with this impression in that they do not show a high correlation between the distributions of the two enzymes. The main disagreement concerns the granular region, where glutaminase activity, but not AAT activity, was particularly high. Another immunohistochemical study (40) reported localization of glutaminase especially in many of the larger (non-granule) somata in the ventral cochlear nucleus and the neuropil around the granule cells. Since the two immunohistochemical studies localized glutaminase to the same cochlear nucleus regions, despite being in different neuronal elements, our results can not help to resolve the discrepancy.

The quantitative data for AAT and glutaminase in the retina have been compared with immunohistochemical results (28,29). Recent slide histochemistry for AAT (31) compares well with our results in finding some reaction product in all retinal layers, but especially in the photoreceptor inner segments and outer and inner plexiform layers, which were the three layers with highest total AAT activities in our study. Slide histochemical results for glutaminase (32) do not compare well with our data in that no reaction product was found in the inner parts of the retina, where we found relatively high glutaminase activities.

Comparisons With Previous Evidence Concerning

Amino Acid Transmitters. GABA has been implicated as a neurotransmitter of granule cells and some periglomerular cells in the olfactory bulb (42). The granule cells have their somata in the granular layer and processes extending through the internal plexiform, mitral cell body, and external plexiform layers. The periglomerular cells have their somata and processes virtually confined to the glomerular layer. In agreement with a high density of GABA-rich neurons in the olfactory bulb, its GABA concentrations are among the highest in the central nervous system (41). They are especially high in the layers containing the granule cell somata and processes, but also quite high in the glomerular layer. The distributions of both cytosolic AAT and glutaminase in the olfactory bulb correlated highly with that of GABA, and a substantial portion of these enzyme activities might be directed toward synthesis of GABA. Some evidence suggests a particular association of aspartate with mitral cells, possibly as a transmitter (42,61). Aspartate concentrations were highest in the mitral cell body layer and in the external plexiform layer, where there are many reciprocal synapses between mitral and granule cell dendrites (42). The high correlation between the distributions of AAT and glutaminase and that of aspartate suggests that it, as well as GABA, might be accumulated as a terminal product of these enzyme activities in the olfactory bulb.

There is evidence that glutamate and/or aspartate may function as transmitters of granule cells in the cochlear nucleus (36,38). Many of these neurons have their somata in granular regions and axons and terminals in the molecular layer of the dorsal cochlear nucleus (62). The relatively higher concentrations of glutamate and glutamine than of aspartate in the granular layer and dorsal cochlear nucleus molecular layer suggest that glutamate is more likely than aspartate as a transmitter in these neurons. Since the cochlear nucleus granule cells may use glutamate as transmitter and those of the olfactory bulb GABA, it is of interest to note that the total AAT activity in the cochlear nucleus granular region is 25% higher and glutaminase more than twice as high as in the olfactory bulb granular layer. This would be consistent with a more prominent association of these enzymes with glutamatergic than with GABAergic neurons. There is evidence that glutamate and/or aspartate are transmitters of auditory nerve fibers (11,12,34–38), which terminate extensively in all regions of the cochlear nucleus except the granular regions and the molecular layer of the dorsal cochlear nucleus (63). The distributions of AAT and glutaminase activities and glutamate, glutamine, and aspartate concentrations in our study could be consistent with this evidence if an additional prominent association of glutamate, glutamine, AAT, and glutaminase with granule cells is accepted. There is evidence for a transmitter role of GABA in the cochlear nucleus (33,37,39,47), but in a smaller proportion of the synapses than in the olfactory bulb (64,65), consistent with the much lower GABA concentrations in the cochlear nucleus. The distribution of GABA, with highest values in the molecular and fusiform soma layers of the dorsal cochlear nucleus and in the granular region, agrees with the major localizations of GABA terminals and somata based on immunohistochemical studies (66–68). Recently, extensive co-localization of cytosolic AAT with GABA and GAD immunoreactivity in the cochlear nucleus has been reported (69). The high correlation between GABA and AAT distributions may here again implicate some AAT activity in eventual formation of GABA.

In the retina, there is evidence for glutamate as a transmitter in the circuit from receptor to bipolar to ganglion cells, and for GABA as a transmitter in a few horizontal and many amacrine cells (22,24–27,30,49,50). Consistent with this evidence are the moderately high glutamate concentrations in all retinal layers except the photoreceptor outer segments and the peak of GABA concentration extending across all retinal layers deep to the inner segments. The distribution of cytosolic AAT correlated highly with that of glutamate across the retinal layers.

AAT and Glutaminase as Markers for Excitatory Amino Acid and/or GABA Neurotransmission. The consistently high correlation of AAT and moderate-to-high correlation of glutaminase distribution with that of MDH reflect the close association between glutamate and aspartate metabolism and energy metabolism (2,4,70). Additionally, the correlations with amino acid concentrations may reflect involvement of AAT and glutaminase in neurotransmitter metabolism of glutamate and aspartate, as well as GABA. Some of these correlations were remarkably high in view of the multiple metabolic involvements of AAT and glutaminase. A notable finding was the consistently high correlation between combined glutaminase and AAT activities and the sum of aspartate, glutamate, and GABA concentrations. This finding suggests that elevated activities of either or both of these enzymes may be directed toward storage of any one or more of these amino acids for neurotransmission. However, there is no way to know, solely from measurement or visualization of one or both enzymes, which of the three amino acids is being accumulated at a particular location. Combined studies of the enzymes and amino acids are necessary for such conclusions.

Overall, cytosolic AAT and glutaminase tended to

be highest in regions where other evidence has implicated glutamate and/or aspartate and/or GABA as neurotransmitters. Also, although not measured here, the acetylated dipeptide N-acetylaspartylglutamate has been implicated in retinal ganglion cells (71) and olfactory bulb mitral cells (72), two of the cell types in which glutamate and aspartate have been considered as transmitters. The possibility must be accepted that AAT and glutaminase could be involved in eventual formation of such molecules from aspartate, glutamate, and/or GABA. However, the high correlation between the combined enzyme activities and the combined amino acid concentrations suggests that elevated activities of AAT and glutaminase may be particularly directed toward accumulation of these amino acids themselves.

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