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GABA—2-OXOGLUTARATE TRANSAMINASE, GLUTAMATE DECARBOXYLASE AND THE HALF-LIFE OF GABA IN DIFFERENT AREAS OF RAT BRAIN

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Abstract—The activity of the enzymes GABA—2-oxoglutarate transaminase (GABA-T) and glutamate decarboxylase (GAD), the endogenous GABA content and the half-life of GABA have been estimated in whole rat brain, cerebellum, pons/medulla, midbrain, cerebral cortex/hippocampus, striatum and hypothalamus. The efflux of ^{3}H-GABA in all brain areas consists of at least two components, a fast phase having a half-life of between 0·5 and 1 hr and a slower phase with a half-life exceeding 1 hr. In addition, significant differences in the calculated rates of GABA turnover among brain regions were found. Endogenous GABA and also the faster turnover phase are proportional to GAD activity but are not dependent on concomitant GABA-T activity. The possible significance of these results are discussed in relation to the postulated role of GABA as a neurotransmitter in the CNS.

THE identification of GABA as a constituent of mammalian brain tissue is relatively recent[1,2] but was soon followed by the realisation that the amino acid might have a neurotransmitter function within the CNS.[3,4] Both the formation of GABA from glutamate and its subsequent metabolism have been extensively studied.[4–6] The concentration of GABA in neural tissues appears to depend on the glutamate decarboxylase (GAD: L-glutamate 1-carboxylyase; EC 4.1.1.15) activity,[5,7] whereas the major catabolic pathway for GABA, the transamination of GABA with 2-oxoglutarate in a reaction catalysed by a transaminase (GABA-T; 4-aminobutyrate: 2-oxoglutarate aminotransferase; EC 2.6.1.19) does not regulate the steady-state concentration of the amino acid.[8] However, although investigations of these two enzyme systems have led to a greater understanding of the neurochemical aspects of the GABA system it must be remembered that the tissue concentration of any compound is not static but reflects a dynamic equilibrium between its rate of formation and rates of utilization. In this report, an attempt has been made to estimate the half-life of GABA in six areas of rat brain and relate this to the corresponding GAD and GABA-T enzyme activities. Part of this work has been reported in preliminary form.[9]

MATERIALS AND METHODS

Chemicals. 2,3-^{3}H-GABA (3·5 c/mmole) was obtained from the New England Nuclear Corp., Dreieichenhain, West Germany; 1-^{14}C-DL-glutamic acid (23·5 mc/mmole) from the Radiochemical Centre, Amersham, England. All chemicals used in this study were of the highest purity obtainable.

Animals. White male Wistar rats weighing 140–175 g were used throughout. They were allowed free access to food and water.

Determination of free GABA levels. GABA levels were measured using the enzymic method originally described by Jakoby and Scott[10] as modified by Kravitz and Potter.[11] Rats were killed by cervical dislocation, the brains rapidly removed and placed in glass petri dishes which were standing in ice. The chilled brains were dissected (Glowinski and Iversen[12]) into six areas; cerebellum, pons/medulla, midbrain, cerebral cortex/hippocampus, striatum and hypothalamus, after which the tissue fragments were weighed and immediately homogenised in 0·01 N HCl to give a final concentration of 100 mg wet weight of tissue per ml. Samples (10 μl) were added to 100 μl of an incubation mixture containing final concentrations of 4·6 mmole 2-mercaptoethanol, 0·5 mmole NADP, 2·5 mmole 2-oxoglutarate and 5 μl of an enzyme preparation of GABASE (for preparation of this enzyme, Scott and Jakoby[13]) in Tris–HCl buffer (0·1 M, pH 7·9). Incubation was carried out for 30 min at room temperature after which 1·5 ml of distilled water was added to each sample and the fluorescence of the reduced NADP measured in a spectrophotofluorometer. Standard solutions containing between 1 and 6 $\times$ 10^{-9} mole of GABA were added to some extracts and also carried through the entire procedure. Tissue blanks containing 10^{-5} M aminooxyacetic acid (AOAA) to inhibit GABA-T activity were also included. Results have been expressed as μmole/g wet weight of tissue.

Measurement of enzyme activities. The activity of GAD was estimated using a modification of the method described by Roberts and Simonsen.[14] Tissues were removed immediately after sacrifice of the rats, dissected as previously described, weighed and homogenized using an all glass homogenizer in 0·3 M sucrose containing 0·5% v/v of the non-ionic detergent Triton X-100, 0·01 M 2-mercaptoethanol and 10 mg/l. of EDTA. The final concentration of wet weight of tissue was 50 mg/ml of sucrose solution. The homogenate was stored at 4° for at least 1 hr in order to allow the detergent to release all occluded enzyme activity.[15] Enzyme activity was estimated by measuring the radioactive CO_2 released from 1-^{14}C-DL-glutamic acid. The incubation mixture contained 800 μl of phosphate buffer (0·05 M, pH 6·4) 200 μl of pyridoxal-5′-phosphate (to give a final concentration of 1 mmole) and 500 μl of the homogenate. After addition of the substrate (0·9 μc of 1-^{14}C-glutamate and cold glutamate to give a final concentration of 5 mM in respect of the L-isomer) incubation was carried out at 37° under an atmosphere of nitrogen in sealed flasks for 60 min. The reaction was stopped by the addition of 200 μl of 4 N HCl and the incubation continued for a further 90 min during which $^{14}CO_2$ evolved was trapped on a piece of filter paper soaked in Hyamine 10-X solution. The radioactivity was eluted into 10 ml of a 1% w/v solution of the scintillator butyl-PBD [2-(4′-tertbutylphenyl)-5-(4″-biphenylyl)-1,3,4-oxadiazole] in toluene containing 2 ml of 2-ethoxyethanol and counted in a scintillation spectrometer. Results have been expressed as mM L-glutamate decarboxylated/kg wet weight of tissue/hr.

The activity of GABA-T present in tissue extracts was measured using the method described by Hall and Kravitz.[16] Tissues were homogenized in 0·3 M sucrose containing 0·5% v/v Triton X-100, 0·01 M 2-mercaptoethanol and EDTA (10 mg/l.) to give a final concentration of 10 mg wet weight of tissue/ml. Aliquots containing 1 mg of tissue were used. The substrate was 2,3-^{3}H-GABA (0·085 μc) to give a final concentration of 10 mM. The experimental procedure was the same as that described

by Hall and Kravitz.[16] Results have been expressed as mM GABA metabolized/kg wet weight of tissue/hr.

Estimation of the half-life of GABA in brain tissue. Two methods have been used in order to obtain an estimate of the *in vivo* half-life ($t_{\frac{1}{2}}$ value) of GABA. In the first, groups of six rats were injected intraperitoneally with either 25 or 40 mg/kg of a solution of AOAA in buffered saline (0·9% w/v, pH 6·4). This drug is a potent inhibitor of the enzyme GABA-T[17,19] and therefore causes a rise in the cerebral GABA content. An estimate of the half-life of GABA was made by measuring the maximum rate of increase in GABA content and calculating the time required for the content to double.

In the second method, the rate of disappearance of intracisternally administered ^{3}H-GABA was measured. Groups of eight rats were lightly anaesthetised with ether and either 5 or 20 μc of radioactive GABA in 40 μl of 0·9% w/v saline injected into the cisterna magna. Animals were killed at 0·5, 1, 2, 4, 6 and 8 hr after injection, the brains removed and, where applicable, dissected and then weighed. Whole brains were homogenized in 10 ml of 0·2 N HCl whereas dissected areas were homogenized in only 5 ml. The samples were stored at 4° overnight then heated in a boiling water bath for 5 min and the precipitated proteins removed by centrifugation at 2000 *g* for 15 min. The supernatant was reserved and a 0·2 ml aliquot counted to determine the total radioactivity (10 ml of 1% w/v butyl PBD in toluene plus 3 ml of 2-ethoxyethanol). The amino acids were isolated from the rest of the extract by ion exchange chromatography.[19] The extracts were passed through 0·5 × 5 cm columns of Amberlite CG-120, Type II, 200 mesh, H^+ form. The resin was washed with 10 ml of distilled water and the amino acids eluted with 10 ml of 2·0 N ammonium hydroxide through the resin bed. The eluate was evaporated to dryness under *vacuo* and redissolved in 5 ml of distilled water. An aliquot of this was also counted to estimate total radioactive amino acids. The GABA plus glutamine present were separated from the radioactive acidic amino acids (glutamate and aspartate) by passing the extract through a 0·5 × 4 cm column of Dowex-AG-1X2 resin, 100–200 mesh, acetate form. Glutamine and GABA passed through the resin whereas glutamate and aspartate were retained. The column was washed with 5 ml of distilled water, the washings added to the eluate (GABA fraction) and mixed with 10 ml of "Instagel" (Packard Instrument Co.). When known amounts of radioactive GABA were added to brain homogenates and passed through the entire procedure a recovery of 77·8 ± 3·6 (mean of ten experiments ± S.E.M.) was obtained. All results have been corrected for recovery and expressed as dis./min/gram wet weight of tissue.

In order to assess the purity of the final extracts paper chromatography was carried out in fifteen experiments. Using Whatman No. 1 paper, 30 μl of the GABA fraction was spotted on together with appropriate standard amino acids (20 μg each of glutamate, aspartate, GABA and glutamine) and ascending chromatography carried out overnight using a solvent system of *n*-butanol–acetic acid–water (4:1:1 by vol.). After drying the chromatogram, the amino acid spots were visualized using ninhydrin reagent, the paper cut into strips and the radioactivity estimated using an Actigraph III Radiochromatograph (Nuclear Chicago). In all experiments, more than 90 per cent of the total radioactivity in the "GABA fraction" was GABA; no correction for the other 10 per cent, which was probably glutamine, has been made.

The dis./min/gram for ^{3}H-GABA were expressed in $\log_{10}$ form and the regression

coefficients of the lines calculated. Half-lives were estimated from the slopes using the formula $t_{\frac{1}{2}} = (\log_{10}2)/\text{slope}$. Significant differences between the slopes were measured by applying the Student's *t*-test to the regression coefficients. An estimate of turnover was made using the endogenous GABA concentration and appropriate $t_{\frac{1}{2}}$ value.

RESULTS

Dissection of rat brain. The brains were dissected into six areas; cerebellum, pons/medulla, midbrain, cerebral cortex/hippocampus, striatum and hypothalamus. In order to check the reproducibility of the dissection procedure, 20 rat brains were dissected and the mean weight of each calculated (Table 1).

TABLE 1. THE REPRODUCIBILITY OF THE DISSECTION PROCEDURE, THE ENDOGENOUS GABA LEVELS AND THE GAD AND GABA-T ACTIVITIES OF WHOLE RAT BRAIN AND THE DIFFERENT AREAS INVESTIGATED

Brain area	Mean weight (mg) ± S.E.M. $n = 20$	Endogenous GABA content (μmole/g ± S.E.M.) $n = 5$	GAD activity (mmole substrate metabolized/kg tissue/hr incubation; $n = 6$)	GABA-T activity
Cerebellum	230 ± 5	1·26 ± 0·089	36·0 ± 1·0*	119·4 ± 10·2
Pons/medulla	207 ± 5	1·55 ± 0·031	29·4 ± 2·4*	81·8 ± 10·0
Midbrain	155 ± 9	3·21 ± 0·063	52·4 ± 4·8	87·4 ± 14·3
Cerebral cortex/hippocampus	687 ± 11	1·82 ± 0·054	38·0 ± 2·9*	72·2 ± 6·6†
Striatum	177 ± 8	2·39 ± 0·044	46·3 ± 3·6	98·4 ± 8·3
Hypothalamus	55 ± 1	4·49 ± 0·330	58·0 ± 4·4	87·0 ± 10·1
Whole brain	1562 ± 21	3·14 ± 0·168	47·8 ± 4·2	90·6 ± 10·0

The endogenous GABA contents were measured in unfrozen tissue samples by the method described by Kravitz and Potter.[11]

* Significantly different ($P < 0·05$) compared with the midbrain and hypothalamus.

† Significantly different ($P < 0·05$) compared with cerebellum.

Endogenous GABA levels. The free GABA levels in the brain areas investigated and in whole rat brain are also shown in Table 1. The content varies from 4·49 μmole/g in the hypothalamic region to 1·26 μmole/g in the cerebellum. These contents are similar to those reported by other workers.[20]

Enzyme activities in the rat brain. Table 1 also shows the GAD and GABA-T activities present in whole rat brain and in the various areas investigated. The GABA-T activities were invariably greater than those of GAD. The activities of the latter enzyme were greatest in the hypothalamic region and lowest in the area of the pons and medulla. On the other hand, the highest GABA-T activity occurred in the cerebellum whereas the lowest was in the cerebral cortex. The relationship between GAD activities and endogenous GABA concentrations is shown in Fig. 1; the GABA content of tissues appears to be a function of GAD activity but is not related to GABA-T activities.

Half-life of GABA in rat brain. The effect of injection of AOAA (25 and 40 mg/kg) on GABA content of rat brain is shown in Fig. 2. The initial rate of increase in tissue content—that is until 1 hr after injection—was similar for both dose levels and gave a mean $t_{\frac{1}{2}}$ value of 0·86 hr (95 per cent confidence limits of 0·68–1·00). The rate of

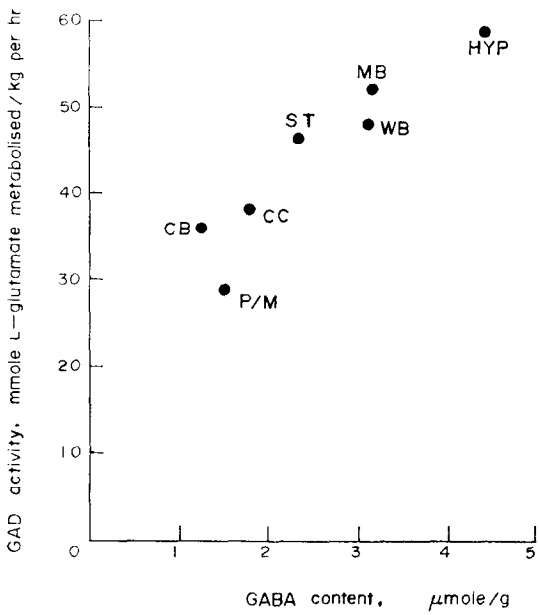

FIG. 1. The relationship between GAD activity and the GABA content of whole rat brain (WB) and its constituent areas (HYP, hypothalamus; MB, midbrain; ST, striatum; CC, cerebral cortex/hippocampus; CB, cerebellum; P/M, pons/medulla).

increase in GABA content then fell and reached zero between 3 and 4 hr after injection. As both dose levels produced an equal initial rate of rise in GABA content it may be assumed that GABA-T activity was fully inhibited. Assuming a whole brain GABA content of 3·14 μmole/g (see Table 1), the turnover of GABA is approximately 1·83 μmole/g/hr.

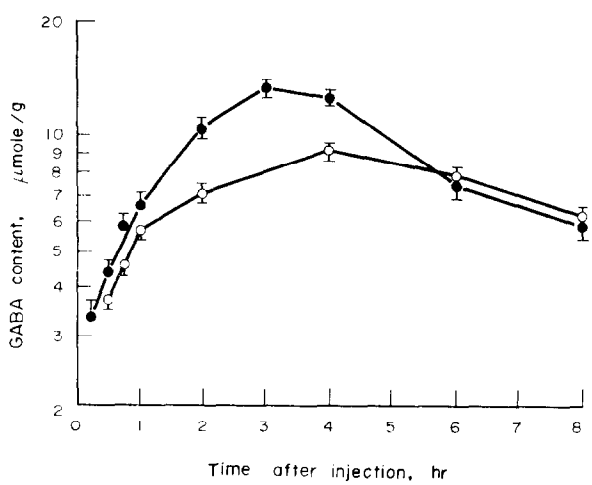

FIG. 2. The effect of AOAA (25 mg/kg (○) and 40 mg/kg (●) intraperitoneally) on the GABA content of whole rat brain. Each point is the mean of six experiments ± S.E.M. From the initial maximum rate of increase in GABA concentration, the mean $t_{\frac{1}{2}}$ value was calculated as 0·86 hr.

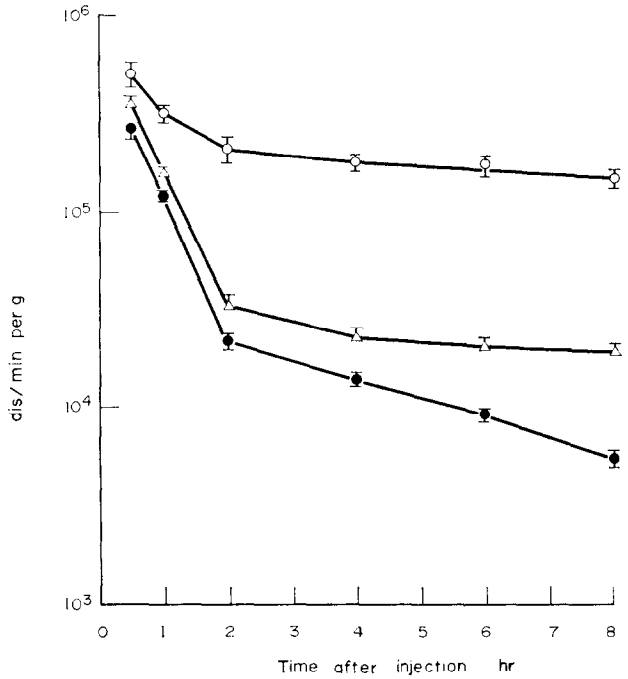

FIG. 3. Changes in radioactivity in whole rate brain after the intracisternal injection of 5 μc of ^{3}H-GABA. Each point is the mean of eight experiments $\pm$ S.E.M. Total radioactivity, (○) total amino acids, (△); ^{3}H-GABA (●). The $t_{\frac{1}{2}}$ value for GABA between 0·5 and 2 hr is 0·45 hr whereas that between 2 and 8 hr is 3·10 hr (see Table 2).

TABLE 2. THE $t_{\frac{1}{2}}$ VALUES AND CALCULATED GABA TURNOVER RATES FOR WHOLE RAT BRAIN AND ITS CONSTITUENT AREAS

Area	0·5–2 hr after 20 μc of 2,3-^{3}H GABA			2–8 hr after 20 μc of 2,3-^{3}H GABA		
	$t_{\frac{1}{2}}$ (hr)	Confidence limits (95%)	Turnover (μmole/g/hr)	$t_{\frac{1}{2}}$ (hr)	Confidence limits (95%)	Turnover (μmole/g/hr)
Cerebellum	0·86	0·68–0·95	0·733	1·25†	1·09–1·25	0·504
Pons/medulla	1·10*	0·89–1·36	0·704	1·92§	1·59–2·35	0·405
Midbrain	0·50	0·38–0·82	3·21	2·70	2·43–2·98	0·596
Cerebral cortex/hippocampus	0·80	0·66–0·98	1·14	3·24	2·87–3·76	0·281
Striatum	0·51	0·31–0·76	2·34	4·70	3·54–5·89	0·256
Hypothalamus	0·51	0·38–0·69	4·40	4·72	4·03–5·81	0·475
Whole brain	0·45	0·29–0·66	3·49	3·10	2·92–3·33	0·506

Groups of eight rats were injected intracisternally with 20 μc of 2,3-^{3}H-GABA and the $t_{\frac{1}{2}}$ values measured by following the rate of disappearance of radioactive GABA (see Materials and Methods). Turnover rates were calculated from estimated $t_{\frac{1}{2}}$ values and size of endogenous GABA pool (see Table 1).

*Significantly different ($P < 0·05$) compared with midbrain.

†Significantly different ($P < 0·05$) compared with midbrain, cerebral cortex, striatum and hypothalamus.

§Significantly different ($P < 0·05$) compared with cerebral cortex, striatum and hypothalamus.

The second method of estimating the half-life of GABA involved measuring the efflux of radioactive GABA after intracisternal administration. The fall in total radioactivity, total radioactive amino acids and also of ^{3}H-GABA in whole brains of rats injected with 5 μc of ^{3}H-GABA is shown in Fig. 3. Between 0·5 and 2 hr after administration there was a rapid exponential fall in labeled GABA having a $t_{\frac{1}{2}}$ of 0·45 hr. Thereafter, the efflux of GABA slowed and between 2 and 8 hr had a turnover time of 3·10 hr (see also Table 2). Less than 5 per cent of the injected dose remained in the brain after 0·5 hr and 2 hr after injection, of the radioactivity extracted from the brain, only 10 per cent was present as ^{3}H-GABA. From the endogenous GABA contents of the brains (Table 1), an estimate of the turnover of GABA during both the fast and slow efflux curves was made (Table 2). Table 2 also shows the $t_{\frac{1}{2}}$ values and calculated turnover rates of GABA in various areas of rat brains. As in the whole brain, each area showed a biphasic fall in radioactivity. The fast phase had a range of $t_{\frac{1}{2}}$ values from about 0·5 hr in the midbrain, striatum and hypothalamus to 1·1 hr in the region of the pons and medulla. On the other hand, the $t_{\frac{1}{2}}$ values of the second phase were lowest in the cerebellum ($t_{\frac{1}{2}}$ = 1·25 hr) and highest at about 4·7 hr in the striatum and hypothalamus. The calculated turnover rates were invariably faster in the initial efflux (0·733–4·40 μmole/g/hr) than in the slower phase (0·256–0·596 μmole/g/hr).

DISCUSSION

In contrast to the catecholamines, and in addition to its putative role as a neurotransmitter substance in the vertebrate CNS,[4] GABA has long been known to have a role in intermediary metabolism.[21] Moreover, much accumulated evidence now suggests that GABA exists in at least two pools within the CNS.[22–24] The uptake, synthesis, metabolism and hence turnover of GABA within the compartments probably differs[23] thereby making interpretation of both dynamic and biochemical investigations somewhat difficult. However, an attempt has been made in this study to relate GABA content and turnover to GAD and GABA-T activities.

Two methods have been employed to estimate the half-life of GABA *in vivo*. The first ideally depended on the complete and specific inhibition of GABA-T activity by AOAA. In order to achieve this, high doses of the drug were required and it is likely that at such concentrations many other enzyme systems were affected:[18] as both GAD and GABA-T are pyridoxal-5′-phosphate dependent enzymes[25] AOAA would almost certainly inhibit both. The rapid fall in GABA content 3–4 after drug administration (see Fig. 2) may be the consequence of inhibition of GAD. Furthermore, so far as is known, the most important source of GABA is by glutamate decarboxylation and so drugs altering glutamate levels or turnover may in turn affect the rate of formation of GABA. It appears that AOAA has a strong activating activity on the enzyme glutamate dehydrogenase (G. G. S. Collins, unpublished results) which might itself interfere with glutamate and hence GABA metabolism. The second method by which $t_{\frac{1}{2}}$ values were measured was by the use of radioactively labelled GABA. At no time did the amount of ^{3}H-GABA accumulated exceed 4 per cent of the endogenous GABA stores and so although not a true tracer dose, the injected amino acid did not significantly elevate tissue concentrations. In addition, the avid uptake mechanism of brain tissue for GABA[26] together with the rapid equilibration of exogenous GABA with endogenous stores[27,28] makes such an experimental approach feasible.

However, it is unlikely that the ^{3}H-GABA penetrated far into the brain tissues[29] and so the calculated turnover rates should only be used for comparative purposes.

After AOAA administration, the increase in GABA levels is initially exponential although this is followed by a slower rate of rise. Over the time period investigated, the rate of disappearance of ^{3}H-GABA from whole rat brain and from the areas investigated was also biphasic in nature. These two findings provide added evidence for the existence of at least two storage compartments for GABA. There is also the possibility that the rapid efflux phase represents the loss of as yet unbound amino acid, but the rapid equilibration of ^{3}H-GABA with endogenous stores[27,28] makes this seem unlikely. The observation that less than 5 per cent of the initially accumulated GABA is present in the brain 2 hr after injection suggests that the initial phase is probably of major importance. Further evidence for the significance of the faster efflux phase is suggested by the close similarity between these $t_{\frac{1}{2}}$ values and those calculated from the experiments involving AOAA. Although both the $t_{\frac{1}{2}}$ values and calculated turnover rates of GABA are only the composite of many processes occurring within the neural tissue, clearly differences exist between the various brain areas studied. In the fast efflux phase, brain areas may be divided into two groups; those having turnovers exceeding 2 μmole/g/hr (midbrain, striatum and hypothalamus) with corresponding $t_{\frac{1}{2}}$ values of about 0·5 hr and those areas with turnovers less than

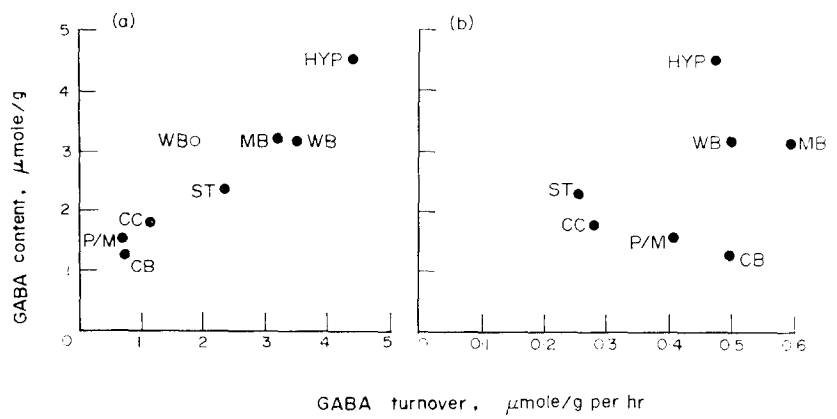

FIG. 4. The relationship between GABA content and the initial fast turnover (A) and secondary slower turnover (B) in whole rat brain and constituent parts. The filled circles are from experiments using radioactive GABA whereas the open circle represents the turnover calculated after administration of AOAA. For explanation of abbreviations, see legend of Fig. 1.

2 μmole/g/hr and $t_{\frac{1}{2}}$ values of approximately 1 hr (cerebellum, pons/medulla and cerebral cortex/hippocampal areas). Similarly, in the slower phase, two types of areas are discernable; those with a turnover of less than 0·3 μmole/g/hr (striatum and cerebral cortex/hippocampus) and the other areas whose turnovers exceed 0·4 μmole/g/hr. These values compare with a figure of 1·0 μmole/g/hr for the GABA flux in isolated slabs of guinea-pig cerebral cortex.[24] It seems that the faster turnover rates are related to the endogenous GABA contents of the brain areas (see Fig. 4A) whereas this is clearly not so for the slower turnovers (Fig. 4B). It has been known for some time that

the endogenous GABA contents are a function of tissue GAD activities[5,8] and this is also true for rat brain (see Fig. 1). We therefore have the interesting possibility that at least in part, GABA turnover is dependent on GAD activity. Neither the fast or slower turnover components are dependent on GABA-T activities. (See Tables 1 and 2).

What is the significance of the two efflux phases? The most likely brain areas in which GABA plays a role as an inhibitory transmitter substance are the cerebellum and cerebral cortex. Since these areas have neither the highest GABA content, $t_{\frac{1}{2}}$ values or turnovers, perhaps the measured half-lives are those of "metabolic" rather than "transmitter" GABA? The smaller pool of GABA constitutes between about 10 and 30 per cent [22,23]of the total and its half-life is in the order of a few minutes. On the other hand, turnover times in the order of hours have also been reported[22,30] suggesting that the larger GABA pool has a much longer turnover time. It is probable that the smaller compartment both of GABA and other amino acids is located in the nerve endings[28,31] and could represent that fraction concerned with neurotransmission. Unfortunately, interpretation of the present results is further complicated by the fact that metabolism of GABA varies depending on the route of administration being slowly metabolized when added to slices, more rapidly when injected into the CSF and most rapidly when precursors are injected into the CSF.[31] Comparison of the present $t_{\frac{1}{2}}$ values with those previously published,[22,23] suggests that they represent the turnover times of "metabolic" rather than "transmitter" GABA. Measuring the rate of disappearance of ^{3}H-GABA at earlier times—between 5 and 30 min after injection—gave $t_{\frac{1}{2}}$ values in the range of 2–22 min. Because of the strong possibility that at this time ^{3}H-GABA was still equilibrating with endogenous stores it is difficult to assess the significance of these results but they may represent the faster turnover of "transmitter" GABA.

As transamination of GABA to succinic semialdehyde is by far the most important mechanism for the metabolic degradation of GABA, why is the activity of GABA-T not the controlling factor regulating GABA turnover? Re-uptake of transmitter-released GABA may be an important factor in conserving the amino acid in GABA-releasing neurones.[26] However, "metabolic" GABA is presumably not released but confined to the intraneuronal environment. Several explanations suggest themselves to account for the non-correspondence of GABA turnover and GABA-T activity. First, transamination of GABA is only the first step in the metabolic pathway and although unlikely, it may be that a later reaction is rate limiting and not the formation of succinic semialdehyde. Second, the localization of GABA probably differs from that of GABA-T. It is thought that GABA is present throughout the neurone[32] whereas GABA-T is present in the mitochondria of the post synaptic cell.[33] Moreover, as has already been mentioned, the localization of the "transmitter" and "metabolic" components probably differs. Third, the turnover times measured using radioactive GABA are only approximations of the true values. After transamination of GABA, the succinic semialdehyde formed is oxidised to succinate which, of course, enters the tricarboxylic acid cycle and is converted to 2-oxoglutarate. Transamination of 2-oxoglutarate to glutamate followed by decarboxylation reforms GABA. The identification of radioactive glutamate, aspartate and glutamine in the present series of experiments suggests that this pathway may be of significance. This effective reversal of glutamate decarboxylation would tend to under-estimate GABA turnover.

In conclusion, the present results emphasize the limitations of the methods available for measuring GABA turnover; unfortunately, the use of labelled glutamate to estimate flux is equally unsatisfactory because of the compartmentation of glutamate itself.[34] The variation in turnover between brain areas does not contradict the possibility of GABA having a role as an inhibitory transmitter in the CNS but probably reflects differences in the importance of its metabolic role.

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