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Inhibition of GABA Metabolism in Rat Brain Slices by Halothane

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Based on studies with rat cerebral cortex slices, it was previously hypothesized that halothane anesthesia may result from increased GABA (γ-aminobutyric acid) content in the synapses. Since GABA is an inhibitory neurotransmitter, such increases may cause a reduction in synaptic activity. The increase in GABA content could arise from several possible causes which are examined in this study using rat cerebral cortex slices as a model. The effects of halothane on uptake, release, and catabolism of GABA were determined. Uptake was studied by the amounts of radioactive GABA accumulated by the slices, and release studied by that discharged into the medium from slices preloaded with radioactive GABA. Catabolism was assessed by preloading the slices with radioactive GABA and then followed by measuring the amount of radioactivity found in unmetabolized GABA or in pooled GABA metabolites. Since CO_2 was established as a major metabolite, it was subsequently used alone to measure the inhibition of GABA catabolism in the presence of varying amounts of halothane. Halothane (3 per cent) did not affect the high-affinity uptake or the release of GABA but did inhibit the catabolism of GABA. Using $^{14}CO_2$ production as an index of catabolism, the inhibition of GABA catabolism by halothane was dose-related (8.79 per cent inhibition/per cent halothane). Such results support the hypothesis that halothane anesthesia may result at least in part from an inhibition of GABA catabolism which, in turn, causes increased GABA level in the synapse with resultant synaptic inhibition. (Key words: Anesthetics, volatile: halothane. Brain: gamma-aminobutyric acid; neurotransmitters; synapses. Theories of anesthesia.)

WE PREVIOUSLY HYPOTHESIZED that halothane produces anesthesia because of the accumulation of GABA (γ-aminobutyric acid) in synaptic areas of the brain.[1–4] This hypothesis is based on observations that GABA content increased in the rat cerebral cortex slices upon incubation in the presence of halothane. Synaptic GABA accumulation might be caused by several possible mechanisms such as reduced uptake, increased release, reduced catabolism, or increased synthesis.

We have now studied the accumulation of GABA in rat brain slices exposed to different halothane concentrations and some of the possible mechanisms underlying the accumulation of GABA in these slices. Factors studied included uptake, release, and catabolism of GABA. Increased synthesis was ruled out in previous experiments.[1–3,5]

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Materials and Methods

White, male Wistar rats, 275–300 g body weight, fed *ad libitum*, were used for the study. Cerebral cortex slices were prepared as described previously[6] and were incubated at 30°C in either Krebs-bicarbonate- or Krebs-phosphate-Ringer's solution, *p*H 7.4, as indicated below. Slices were always incubated in the bicarbonate medium but were processed or washed in the phosphate medium. This variation is dictated by practical difficulty in maintaining a number of bicarbonated solutions at the same time in uptake and release experiments described below. The concentrations of halothane were ascertained to within ±5 per cent of the desired value for each experiment by gas chromatography using a Porapak® column 1 m in length in a F and M 700 Series gas chromatograph.

GABA CONTENTS IN CORTEX SLICES

After 15, 30, or 60 min of incubation in Krebs-bicarbonate-Ringer's solution, equilibrated with 5 per cent CO_2 and O_2 containing 0, 1.5, or 3.0 per cent halothane, the slices were homogenized in 5 per cent trichloroacetic acid. The separation and determination of GABA from this homogenate are described in a later section.

UPTAKE EXPERIMENTS

Cortex slices from Wistar rats were trimmed, weighed, and transferred to special holders (fig. 1, part 1B). The differences between these holders and those previously described[7] were: 1) these were made of polyethylene frames and nylon screen, and 2) the inside dimensions of the oblong chamber were ¼ in × ⅜ in (0.635 cm × 0.953 cm). Four trimmed cerebral cortex slices were able to fit into each holder. Draining of liquid was easy with the coarse nylon screen although it was never complete.

Paired slices in holders were temperature equilibrated at 30° C in beakers containing 5 ml of Krebs-phosphate-Ringer's solution which were placed in a water bath for 5 min. The holder was drained quickly and transferred to 5 ml of Krebs-bicarbonate-Ringer's solution in a Warburg-type flask (fig. 1, part 1A) containing [1-^{14}C]GABA (32 μM and 0.1 μCi/ml) for another 5, 10, or 15 min of incubation. The medium was equilibrated with 5 per cent CO_2 and O_2 containing

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0 or 3.0 per cent halothane. After incubation, the slices were drained, rinsed, and redrained twice to clear GABA from the surface of the slices. This required about 30 s. They were then plunged into hot 5 per cent trichloroacetic acid on a steam bath for extraction of GABA.

Release and Catabolism Experiments

Cortex slices (four per sample) from Wistar rats were weighed before transferring to another type of slice holder.[8] This holder (fig. 1, part 2) facilitated slice rinsing and was more suited for release studies. The slices in these holders were temperature equilibrated for 5 min at 30° C in a Krebs-phosphate-Ringer's solution. Liquid was then quickly drained and the holder with slices was further incubated for another 5 min in 5 ml of Krebs-phosphate-Ringer's solution containing [1-^{14}C]GABA (160 μM and 0.5 μCi/ml).

During this preincubation time, the uptake of radioactive GABA by the slices was in excess of 6 per cent (6.09 ± 0.33 per cent) of the GABA present in the medium and there was no significant difference between paired samples. After two quick rinses in fresh GABA-free Krebs-phosphate-Ringer's solution, the slices were transferred to Krebs-bicarbonate-Ringer's solution without added GABA. Paired samples were incubated for another 15 or 30 min in the presence of 5 per cent CO_2 and O_2 containing 0 or 3.0 per cent halothane. The holders containing the slices were then quickly drained and plunged into hot 5 per cent trichloroacetic acid. Aliquots of incubation medium and of the trichloroacetic acid extract of the incubated tissue were counted for radioactivity. Duplicate pairs of experiments were performed at the same time and the results averaged. In some experiments, the final incubation was omitted to obtain zero time values.

Modified Catabolism Experiments

Two cortex slices from Sprague-Dawley rats were incubated per flask (fig. 1, part 1A) in Krebs-phosphate-Ringer's solution in a paired fashion. (Preliminary results showed no difference between those rats and Wistar rats used earlier.) The slices were not placed in any holder and radioactive $^{14}CO_2$ was measured as an index of the catabolism of [1-^{14}C]GABA (160 μM and 0.5 μCi/ml). A suspended well (Kontes Item No. 882320) containing filter paper dampened with 0.05 ml 2 N NaOH served as a trap for this $^{14}CO_2$. Halothane was flushed through the closed flasks at various concentrations in a gas mixture containing 50 per cent O_2. Nitrogen, water vapor, and halothane

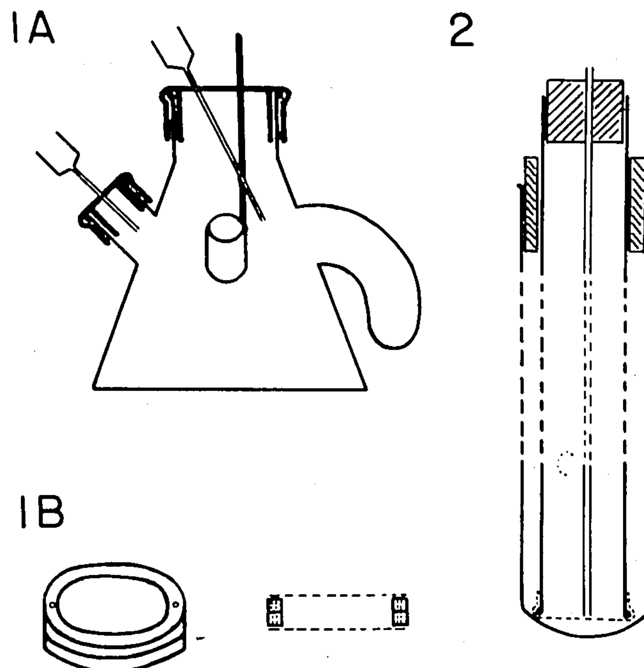

FIG. 1. Two types of incubation vessels. Type 1 consists of a 25-ml flask (part 1A) and a slice holder (part 1B). The 25-ml flask (part 1A) is provided with a sidearm for substrate, a suspended centerwell for NaOH, and a small sidevent. Serum bottle caps over the side-vent and flask opening can be punctured by hypodermic needles for gaseous equilibration. The holder (part 1B) for brain cortical slices is similar to those described by McIlwain.[6] Two frames are fastened together at ends and each frame is covered with nylon screen for fluid passage. A holder containing slices is placed in the flask. Type 2 is similar to those described by Hertz.[7] The innter tube which serves as the slice holder is capped at the bottom end with nylon screen for liquid drainage. The holder can be quickly removed from the main tube and passed through several changes of medium in a short time. The inner capillary tube is for aeration.

made up the remaining 50 per cent. The concentration of halothane was determined as described above. The equilibration was allowed for 20 min after which the system was closed and [1-^{14}C]GABA in the sidearm was mixed with the medium containing the slices. After another hour of incubation, acid was added with a hypodermic needle to the closed system to kill the slices and to distill the $^{14}CO_2$ into the NaOH trap. The durations of incubation and preincubation were varied during preliminary tests (see results).

Treatment of Killed Tissue

A similar technique[5,6] was employed for all the experimental conditions described above. The trichloroacetic acid extract was separated from precipitated protein and pooled with water washes of the precipitate. Trichloroacetic acid was removed by ether extraction and the excess ether vaporized with N_2 bub-

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TABLE 1. Effects of Halothane Concentration on GABA Contents in Rat Cerebral Cortex Slices

Time	Halothane (Per Cent)			b ± 95 Per Cent C.L.	r	P_b
	0	1.5	3.0			
15 min	19.0 ± 1.3	20.6 ± 1.1	19.2 ± 1.4	0.09 ± 1.19	0.03	0.88
30 min	18.8 ± 1.0	19.9 ± 1.2	22.9 ± 1.4	1.34 ± 1.08	0.42	0.015
60 min	19.9 ± 0.9	21.6 ± 1.3	25.2 ± 1.7	2.06 ± 1.18	0.55	<0.01

The number of experiments in each group (N) is 10. Contents (means ± SEM) are given in nmol/mg protein. Regression coefficients (b) and their 95 per cent C.L. are given in nmol/mg protein/per cent halothane. Correlation coefficients (r) for the regression lines are statistically significant at 30 and 60 min. Analysis of covariance shows that regression with halothane concentration is significant at ${}_{86}{}^{1}F = 13.42$ ($P < 0.005$) and with duration of incubation at ${}_{86}{}^{1}F = 5.05$ ($P < 0.05$).

bling. The clear aqueous extract was diluted to less than 5 mM in ionic concentration and chromatographed for GABA and its metabolites (see below). The trichloroacetic acid precipitate was dissolved in hot 0.1 N NaOH which was later diluted to less than 50 mM. Protein in this solution was determined with phenol reagent.[9]

In some experiments, both the medium and the cleared extract were first acid hydrolyzed and dried before chromatography. Acid hydrolysis (2 N HCl for 2 hours at 100° C) caused complete conversion of glutamine to glutamate. Anionic metabolites in the reconstituted hydrolysate were absorbed on an AG-1-acetate column and eluted together with 2 N HCl. The unabsorbed initial effluent and water wash were further chromatographed on a BioRex-70® column where GABA could be recovered in a single fraction without contamination. The other fractions were pooled representing other cationic metabolites. The initial effluent from this latter column contained neutral metabolites.

The GABA-containing fraction from the BioRex® column was dried and redissolved in 2 ml H_2O. GABA content was determined based on the color developed with ninhydrin.[10]

The radioactivity in dissolved protein, in collected CO_2, and in the cleared extract and its chromatographed fractions were determined with a scintillation counter (Nuclear Chicago Model Mark I®) with 70 per cent counting efficiency which was continuously monitored. The filter paper strip and the cut-off center-well did not interfere with counting efficiency.

In uptake experiments, the sum of the radioactivity in the cleared extract and in the protein was taken as a measure of GABA uptake. Metabolic products of GABA lost in the medium or into the gas phase as CO_2 were not collected. The quantity of these metabolites was small in relation to total GABA uptake and probably did not influence interpretation of results.

In release and retention experiments, the radioactivity in GABA isolated from the cleared extract represented the amount of GABA remaining unmetabolized in the slices, and that isolated from the medium represents the amount of GABA released from the preloaded slices into fresh GABA-free medium. The sum of the radioactivity in collected ${}^{14}CO_2$, in AG-1 column eluents (anionic metabolites), in BioRex-70® pooled eluents (cationic metabolites), in effluent of these columns without adsorption (neutral metabolites), and in the protein was taken to represent the radioactivity in GABA metabolites. In later experiments only ${}^{14}CO_2$ was collected as an indicator of GABA catabolism since it is the major metabolite of GABA for incubations longer than 15 min.

Statistical comparisons using *t* tests, F tests, and 95 per cent confidence limits (C.L.) were performed according to standard equations.[11]

Results

GABA Content in Cortex Slices

In the absence of halothane, the GABA content remained constant over 60 min of incubation (table 1). After 30 or 60 min, halothane caused significant dose-

TABLE 2. Effects of 3 Per Cent Halothane on Uptake of [1-^{14}C]GABA by Rat Cerebral Cortex Slices

Duration of Incubation (min)	Control (N = 5)	Halothane (N = 5)	Difference	*P*
5	32,950 ± 5660	31,820 ± 4320	1,130 ± 2550	0.67
10	66,040 ± 8700	66,760 ± 3870	−610 ± 6040	0.92
15	86,960 ± 6120	108,700 ± 5900	−21,720 ± 6860	0.035

Data (cpm/mg protein) are given in means ± SEM. Difference (in cpm/mg protein) and *P* values are based on mean differences between paired samples.

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TABLE 3. Effects of 3 Per Cent Halothane on the Release of Preloaded [1-^{14}C]GABA by Rat Cerebral Cortex Slices

Duration of Incubation (min)	Control	Halothane	Difference	*P*
0 (est)	88	88	—	—
15	185 ± 28	239 ± 59	−28 ± 25	0.32
30	112 ± 17	214 ± 55	−102 ± 40	0.065

Radioactivity in the incubation medium (cpm/mg protein) is taken as a measure of GABA release, and is reported as means ± SEM (N = 5). Difference refers to mean difference between paired samples. The *P* values are based on comparison between paired differences. Zero-time value is calculated by pooling data from 126 experiments which indicate 1.7 ± 0.1 per cent carry-over of radioactivity into the first rinse, and 9.9 ± 0.7 per cent into the second rinse. Total radioactivity in the second rinse was 12,900 cpm with an average of 14.5 mg of tissue protein. A similar 9.9 per cent carry-over into the incubation medium from the second rinse provides the zero-time estimate of 88 cpm/mg protein.

related increases in GABA content; 15 min were apparently insufficient to demonstrate this change. Analysis of covariance showed that the regression was significant with halothane concentration and with time.

Uptake of [1-^{14}C]GABA into Cerebral Cortex Slices

Radioactivity was rapidly accumulated into the cortex slices (table 2). In brain slices exposed to 3 per cent halothane, only the 15-min incubation experiments showed statistically significant increases in GABA uptake based on paired analysis.

Release of [1-^{14}C]GABA from Preloaded Slices

Labeled GABA preloaded into the slices was released very slowly, if at all (table 3). The amounts released were insignificant when compared either to GABA uptake (table 2) or to the amount of GABA remaining in the slices (table 4). After 15 or 30 min of incubation, there was no statistical difference in GABA release between the control and halothane-exposed slices. In addition, there was no significant difference from the zero-time value indicating that there was no GABA release.

Unmetabolized [1-^{14}C]GABA Remaining in Preloaded Slices

In control slices, about half of the preloaded [1-^{14}C]GABA was metabolized in 30 min (table 4). Halothane significantly inhibited this catabolism after 15 or 30 min of incubation based on differences between paired samples.

Nonvolatile Metabolites Derived from Preloaded [1-^{14}C]GABA

The amounts of nonvolatile metabolites derived from [1-^{14}C]GABA were small (table 5) in comparison to the amounts of GABA remaining in the slices (table 4), and these metabolites did not accumulate beyond 15 min. Halothane-exposed slices had lower metabolite content than paired nonexposed controls. These data indicated a need to examine CO_2 production (fig. 2) which could not be done in these experiments conducted in a bicarbonate buffered medium and a 5 per cent CO_2 atmosphere.

$^{14}CO_2$ Derived from [1-^{14}C]GABA

CO_2 was confirmed to be a major metabolite in control slices (table 6). Preliminary experiments showed that the production of $^{14}CO_2$ from labeled GABA was directly proportional to the duration of incubation, and a minimum period of 10 min of preincubation in halothane was required to reach a constant level of inhibition. Subsequent experiments were conducted routinely with 20 min of preincubation in halothane, and 1 hour of incubation with radioactive substrate. The dose-related inhibitory effect of various concentrations of halothane on the production of $^{14}CO_2$ from [1-^{14}C]GABA (10 μM, 0.5 μCi/ml) is shown in figure 3.

Discussion

Our hypothesis[1–4] that halothane anesthesia causes an excess accumulation of GABA (an inhibitory neurotransmitter) in the synaptic cleft cannot be approached by direct measurements at this time because of its submicroscopic nature. However, experimental

TABLE 4. Effects of 3 Per Cent Halothane on [1-^{14}C]GABA Remaining in Rat Cerebral Cortex Slices

Duration of Incubation (min)	Control (N = 5)	Halothane (N = 5)	Difference	*P*
0	14,250 ± 3,070	14,250 ± 3,070	—	—
15	11,510 ± 1,160	13,710 ± 2,180	−2,200 ± 590	0.020
30	7,710 ± 1,140	11,230 ± 1,570	−3,510 ± 1,810	0.012

Data (cpm/mg protein) are given in means ± SEM. Difference (in cpm/mg protein) and *P* values are based on mean differences between paired samples.

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TABLE 5. Effects of 3 Per Cent Halothane on Nonvolatile Metabolites Derived from [1-^{14}C]GABA in Rat Cerebral Cortex Slices

Duration of Incubation (min)	Control (N = 5)	Halothane (N = 5)	Difference	P
0	340 ± 45	340 ± 45	—	—
15	1,698 ± 215	1,013 ± 110	686 ± 106	<0.005
30	1,710 ± 295	834 ± 168	875 ± 163	<0.005

Data (cpm/mg protein) are given in means ± SEM. Difference (in cpm/mg protein) and P values are based on mean differences between paired samples.

models of this submicroscopic structure, such as brain slices or synaptosomes, can be utilized for such investigation. This communication deals with data obtained with brain cortex slices. Another study presented in this issue[12] will deal with the synaptosomal model.

Cortex slices studied at 37° C rapidly lose structural and metabolic integrity, but they remain intact longer at 30° C. At this lower temperature, metabolic events are slowed nearly half since the brain tissue has a temperature coefficient (Q_{10}) of approximately 2.[13] At 37° C, these metabolic findings would have occurred more rapidly but it is unlikely that metabolite concentrations would have been altered significantly. A companion study[12] deals with synaptosomes at 30° C and earlier work on slices[5] performed at 30° C provide direct comparisons of results.

The uptake, release, or metabolism of GABA by the cortex slices are thought to represent processes similar to those at the synaptic level (fig. 4). An increase in GABA concentration in slices may result from any of the following: 1) increased synthesis, which has previously been ruled out;[1-3,5] 2) reduced catabolism; 3)

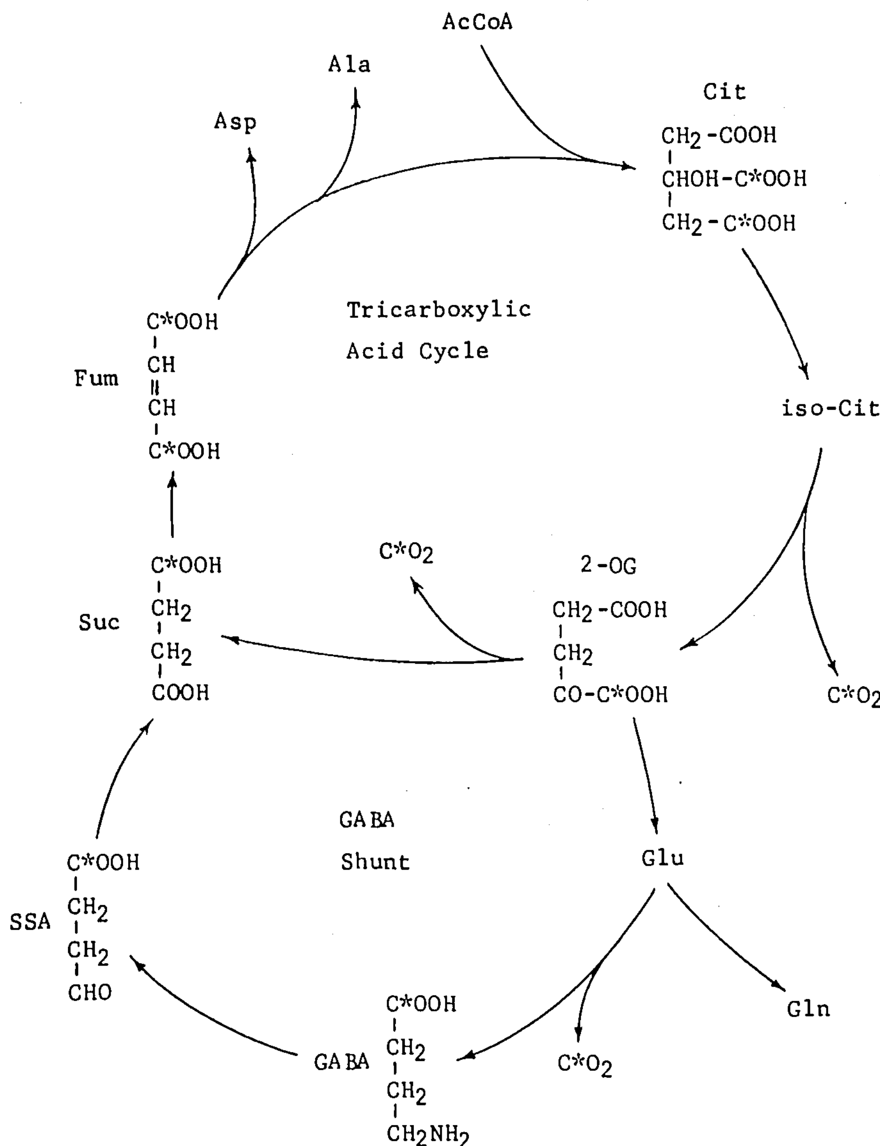

FIG. 2. Metabolism of [1-^{14}C]GABA via the tricarboxylic acid cycle. Abbreviations: AcCoA = acetyl coenzyme-A; Ala = alanine; Asp = aspartate; Cit = citrate; Fum = fumarate; GABA = γ-aminobutyrate; Gln = glutamine; Glu = glutamate; iso-Cit = isocitrate; 2-OG = 2-oxoglutarate (α-keto-glutarate); SSA = succinic semialdehyde; and Suc = succinate. C* indicates the radioactive carbon (^{14}C). The radioactivity in C-1 of GABA enters succinate via C-1 but randomizes into C-4 since succinate is a symmetrical molecule. This is shown with C-1 labeled succinate and C-1, C-4 labeled fumarate. The carboxyl labeled oxaloacetate enters the lower portion of citrate. One carboxyl group appears as CO_2 during the formation of 2-oxoglutarate, and the other one as CO_2 during the decarboxylation of 5-carbon acid to 4-carbon acid. All the radioactivity from [1-^{14}C]GABA appears as $^{14}CO_2$ in one cycle. No radioactivity re-enters GABA.

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reduced uptake; or 4) increased release. These may act singularly or in combination and can be considered to be similar to the process of uptake, release, or metabolism of GABA occurring at the synaptic level. High-affinity uptake by an active process is considered to be the mechanism for the removal of GABA from active sites. Its time scale is measured in seconds or milliseconds. Its K_M is 8 μM, in contrast to the much larger K_M of 35 μM for the nonactive, diffusion-dependent, slow component.[14,15]

In this study, we first demonstrated that halothane causes both a dose- and time-related increase in GABA content in brain slices (table 1). Conflicting reports on the effect of halothane on cerebral GABA content exist; halothane decreases whole brain GABA content *in vivo*[16] and causes no change in cultured nerve cells.‡ Neither of these studies evaluates the effect of anesthetics on the synaptic pool of GABA which is critical to its neurotransmitter function. This pool represents less than one-third of the total brain GABA content.[17] Cortex slices used in this study are mixed tissue, but they are enriched in synapses and cell bodies as compared to whole brain. The companion articles,[12,18] using synaptosomes, offer stronger support to this hypothesis since these particles represent pinched-off nerve endings and are, in a sense, a highly enriched synaptic preparation. The findings with the synaptic preparations are in full agreement with the data from the slice experiments and lend further credence to these data.

‡ Hess WH, Nahrwold ML, Bethel DR: Halothane-induced alterations of gamma-aminobutyric acid levels and efflux from cultured cells of nervous system. Abstracts of Scientific Papers. Annual meeting of the American Association of Anesthesiologists, 1976, pp. 77–78.

TABLE 6. Distribution of GABA Metabolites in Rat Cerebral Cortex Slices (N = 7)

Metabolites	15 min Mean ± SEM	15 min Per Cent	30 min Mean ± SEM	30 min Per Cent
CO_2	1,142 ± 116	41.8	3,386 ± 503	58.7
Anionic metabolites				
in slices	799 ± 107	29.3	1,486 ± 367	25.8
in medium	41 ± 6	1.5	79 ± 10	1.4
Cationic metabolites				
in slices	354 ± 116	13.0	331 ± 72	5.7
in medium	17 ± 5	0.6	37 ± 11	0.6
Neutral metabolites				
in slices	131 ± 41	4.8	164 ± 29	2.8
in medium	13 ± 4	0.5	24 ± 5	0.4
Protein	234 ± 30	8.6	264 ± 36	4.6
TOTAL	2,730 ± 289	100	5,771 ± 906	100

Values are given in cpm/mg protein at 30°C. Definitions of metabolites: CO_2 = acid-labile and NaOH absorbed; anions = absorbed by AG-1; cations = absorbed by BioRex-70®; neutrals = not absorbed by either; protein = trichloroacetic acid precipitated.

Halothane has no effect on GABA uptake measured as radioactivity in the slices (table 2) until 15 min of incubation has passed at which time there is an increase in radioactivity in the halothane-treated slices. This increase probably does not represent altered GABA uptake since halothane inhibits CO_2 formation from GABA (fig. 3) and more radioactive CO_2 is liberated and removed from the control slices than from the halothane-treated slices. Under any circumstance,

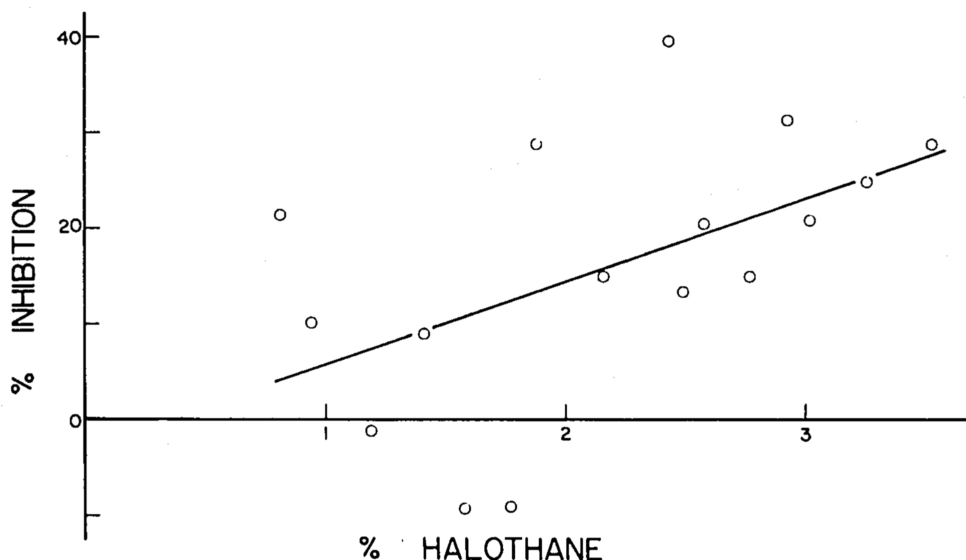

FIG. 3. Halothane inhibition of GABA catabolism in rat cerebral cortex slices. Incubation was for 1 h at 30° C. Per cent inhibition (Y) was calculated from duplicate, paired experiments. The data were represented by Y = 8.79X − 2.90 where X was per cent halothane and r = 0.53 (N = 16). The Y-intercept (a = −2.90) was not different from the origin and the slope (b = 8.79) has a P value of <0.05.

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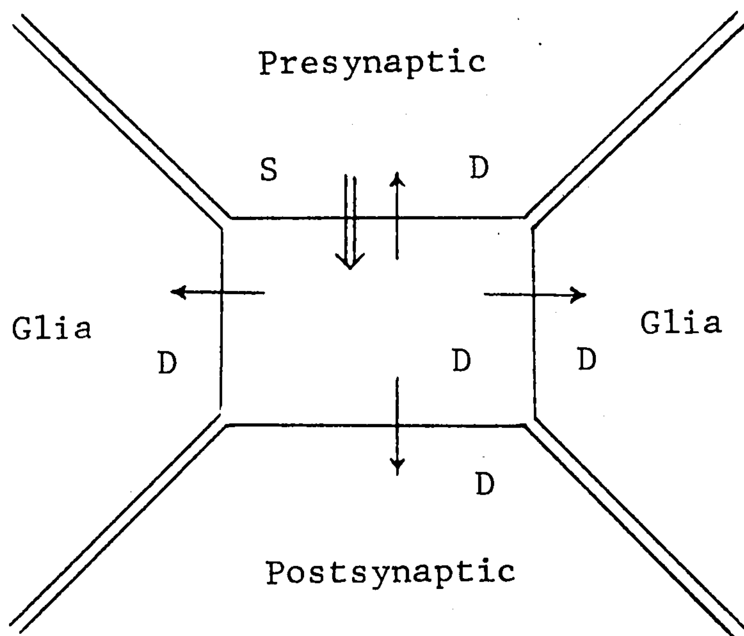

⟶ Uptake S - Synthesis & Storage

⟹ Release D - Degradation & Removal

FIG. 4. Events associated with the functioning of a neurotransmitter (GABA) in a simplified-diagramatic synapse. S = synthesis and storage; D = degradation; → = uptake; and ⇒ = release. Reproduced with the permission of International Anesthesia Research Society.

the physiologic function of an uptake mechanism at a synapse to terminate transmitter function occurs in a small fraction of a second and not on a time scale of 15 min.

Catabolism or breakdown of GABA is assessed in two ways: 1) by the amounts of GABA remaining unmetabolized in the slices (table 4), and 2) by the amounts of GABA metabolites produced (table 5). Significantly less GABA remains in the control preloaded slices after incubation than in the halothane-treated slices (table 4). This means that halothane inhibits the catabolism of GABA. Difficulties were encountered in collecting $^{14}CO_2$ in a continuously aerated system utilizing Krebs-bicarbonate-Ringer's medium. The breakdown of [1-^{14}C]GABA through the tricarboxylic acid cycle (fig. 2) should give $^{14}CO_2$ as the principal product provided that the tricarboxylic acid cycle related metabolite pools reached isotopic equilibration, which required 15 min of incubation. Two different levels of isotopic equilibration were probably reached in the control and halothane-treated slices suggesting that, in the latter, the steady-state rate of tricarboxylic acid cycle metabolism was inhibited (*i.e.*, reduced rate). Therefore, halothane inhibition of GABA catabolism implies also a GABA turnover inhibition, a concept under current investigation.

In order to trap the $^{14}CO_2$ from the catabolism of [1-^{14}C]GABA, Krebs-phosphate-Ringer's medium was employed and CO_2 equilibration was eliminated to provide a closed incubation system. This system works surprisingly well when compared to an aerated, open system. The amounts of radioactivity in metabolites in the closed system after 15 and 30 min of incubation, when corrected for CO_2, are 1588 and 2385 cpm/mg protein respectively (table 6). There is no statistical difference between these and their corresponding values of 1698 and 1710 cpm/mg protein in the open, aerated system (table 5). Using the closed system, a dose-related inhibition of GABA catabolism by halothane can be shown (fig. 3). At the MAC for halothane in rats, 0.9–1.1 per cent,[19] the inhibition of GABA catabolism in cortex slices amounts to 5.0–6.8 per cent. Similar data with rat brain synaptosomes[12] indicate 13–15 per cent inhibition at MAC for halothane.

The physiologic effects of this degree of inhibition by halothane on GABA catabolism have not been correlated in behavioral terms. The inhibition by anesthetics of impulse transmission in nervous tissue may

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be mediated through GABA. Although this has not been directly demonstrated, evidence obtained from the cuneate nucleus[20] and from the olfactory bulb[21] strongly support such a possibility. Our data suggest that the GABA effect may depend on reduced catabolism but not on altered uptake, release, or synthesis.

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References

1. Cheng S-C: Metabolic compartmentation of the GABA system; relationship of GABA metabolism to anesthesia, GABA—Biochemistry and CNS Function. Edited by Mandel P, deFeudis FV. New York, Plenum Publishing Corp., 1979, pp 161–175
2. Cheng S-C, Brunner EA: Two neurotransmitters in brain slices, Molecular Mechanisms of Anesthesia. Edited by Fink BR. New York, Raven Press, 1975, pp 509–518
3. Cheng S-C, Brunner EA: A neurochemical hypothesis for halothane anesthesia. Anesth Analg (Cleve) 54:242–246, 1975
4. Cheng S-C, Brunner EA: Thiopental inhibition of γ-aminobutyrate transaminase in rat brain synaptosomes. Biochem Pharmacol 28:105–109, 1979
5. Cheng S-C, Brunner EA: Alteration of tricarboxylic acid cycle metabolism in rat brain slices by halothane. J Neurochem 30:1421–1430, 1978
6. Nakamura R, Cheng S-C, Naruse R: A study on the precursors of the acetyl moiety of acetylcholine in brain slices. Observations on the compartmentalization of the acetylcoenzyme A pool. Biochem J 118:443–450, 1970
7. McIlwain H, Rodnight R: Practical Neurochemistry. Boston, Little Brown and Company, 1962, pp 163–166
8. Arnfred T, Hertz L, Lolle L, et al: An improved holder for transfer of brain slices during *in vitro* incubation. Exp Brain Res 11:373–375, 1970
9. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275, 1951
10. Moore S, Stein WH: A modified ninhydrin reagent for the photometric determination of aminoacids and related compounds. J Biol Chem 211:907–913, 1954
11. Snedecor GW, Cochran WG: Statistical Methods. Ames, The Iowa State University Press, 1967
12. Cheng S-C, Brunner EA: Effects of anesthetic agents on synaptosomal GABA disposal. ANESTHESIOLOGY 55:34–40, 1981
13. Siesjö BK: Brain Energy Metabolism. New York, John Wiley and Sons, 1978, pp 327–328
14. Levi G, Raiteri M: Detectability of high and low affinity uptake systems for GABA and glutamate in rat brain slices and synaptosomes. Life Sci 12:81–88, 1973
15. Iversen LL, Johnston GAR: GABA uptake in rat CNS: comparison of uptake in slices and homogenates and the effect of some inhibitors. J Neurochem 18:1939–1950, 1971
16. Biebuyck JF, Dedrick DR, Scherer YD: Brain cyclic AMP and putative amino acids during anesthesia, Molecular Mechanisms of Anesthesia. Edited by Fink BR. New York, Raven Press, 1975, pp 451–470
17. Balázs R, Machiyama Y, Hammond BJ, et al: The operation of the γ-aminobutyrate bypath of the tricarboxylic acid cycle in brain tissue *in vitro*. Biochem J 116:445–467, 1970
18. Cheng S-C, Brunner EA: Inhibition of GABA metabolism in rat brain synaptosomes by midazolam (RO-21-3981). ANESTHESIOLOGY 55:41–45, 1981
19. Eger EI II: Anesthetic Uptake and Action. Baltimore, Williams and Wilkins, 1974, p 5
20. Galindo A: Effects of procaine, pentobarbital and halothane on synaptic transmission in the central nervous system. J Pharmacol Exp Ther 169:185–195, 1969
21. Nicoll RA: The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. J Physiol (Lond) 223: 803–814, 1972

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