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Conversion *in vivo* of γ-aminobutyric to γ-hydroxybutyric acid in the rat

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GAMMA-BUTYROLACTONE (GBL) and its hydrolytic cleavage product, gamma-hydroxybutyric acid (GHB), can produce unconsciousness and anesthesia in a variety of animals, including man.[1–5] Despite a close chemical structural similarity to gamma-aminobutyric acid (GABA), the symptoms of central nervous system depression elicited by these compounds do not seem to be related to an increase in brain levels of GABA.[6, 7] (However, see also Wollemann and Dévényi[8] and della Pietra *et al.*[9]) In fact it has been demonstrated that administration of GHB inhibits the synthesis of GABA in rat brain.[10] On the other hand, rat brain can convert ^{14}C-GHB to ^{14}C-GABA *in vitro*, but the mechanisms of this conversion remains obscure. The enzymatic reduction of succinic semialdehyde (a metabolite arising from the transamination of GABA with α-oxoglutarate, cf. Fig. 1) to GHB by mammalian brain *in vitro* has been clearly demonstrated by Fishbein and Bessman.[11] These investigators believe that GHB or GBL or both are endogenous constituents of mammalian brain in a combined concentration of 10^{-3} M[12]. That this is unlikely in the conscious, alert animal may be deduced from the finding of Giarman and Roth[13] who have shown that, after administration of an anesthetic dose of GHB or GBL, at a time when rats are behaviorally anesthetized, the brain level of GHB is 1 to 3 × 10^{-3} M. In point of fact, GHB has not been rigorously identified as an endogenous component of the brain of the alert rat, mouse and cat in excess of 10^{-6} M[13] This, however, does not preclude the possibility of the natural occurrence of GHB at levels of 10^{-6} M or less in mammalian brain. In fact, Roth[14] has found a compound in cat and rat brains at a concentration of 2 to 3 × 10^{-6} M which, when analyzed by gas chromatography, had a retention time identical to that of GBL. (GHB is converted to GBL in preparing samples for gas chromatography.[13–15]

In view of the possibility that GHB may be a normal metabolite of mammalian brain, the experiments in this report were conducted to determine whether GABA might serve as a precursor of GHB in the rat brain *in vivo*. Fig. 1 illustrates the postulated relationship between these compounds.

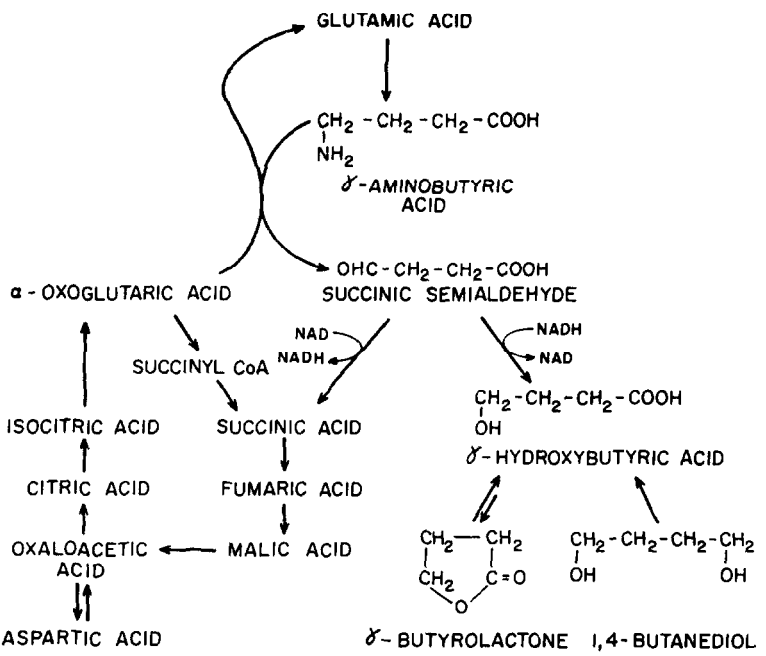

FIG. 1. Metabolic interrelationship of γ-hydroxybutyric acid and other brain constituents.

Since GABA does not readily cross the blood–brain barrier, ^{3}H-GABA was administered intracisternally, according to the procedure of Jeffers and Griffith[16] as modified by Roth.[14] Radioactive ^{3}H-GABA (Calbiochem. sp. act., 3·5 c/m-mole) was dissolved in 0·9 % sodium chloride to give a final concentration of 1·0 mc/ml. Fifty μl ^{3}H-GABA was administered via a 27 gauge needle into the cisterna magnum and the animals were killed 20 min later by decapitation. The brains were rapidly removed and immediately homogenized in 10 ml of 20% trichloracetic acid containing 1 mg/ml of the sodium salt of GHB. For controls, the brains of noninjected rats were removed and homogenized in 10 ml of 20% trichloracetic acid containing 50 μc ^{3}H-GABA and 1 mg/ml of sodium GHB. A 10-μl aliquot was taken for counting in Bray's solution.[17] The trichloroacetic acid extracts were heated in a water bath at 90° to convert the GHB to GBL, and the GBL was extracted and assayed by gas chromatography as described previously.[13–15] However, in this case the gas chromatograph was equipped with an effluent splitter, which conducted 1 part of the column effluent to the detector and 16 parts to a heated collection port. During the collection period, the collection port was connected to a 9 in. piece of teflon tubing and the effluent gas was bubbled through 20 ml of a scintillation mixture composed of 4 g of 2,5-diphenyloxazole + 50 mg of 1,4-*bis*-2(5-phenyloxazolyl) per liter of toluene. At the end of the collection, the teflon tubing was rinsed with scintillation fluid to remove any compounds which might have condensed in the tubing. The samples were counted for 20 min in a Packard

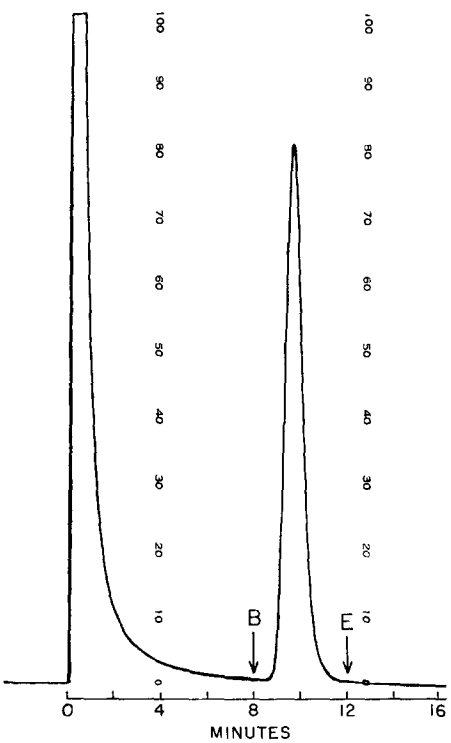

FIG. 2. Typical gas chromatogram of an extract from rat brain. The column was equipped with an effluent splitter which conducted 1 part of the effluent gas to the detector and 16 parts to a collection port. B, beginning of the collection period; E, end of the collection.

Tri-carb liquid scintillation spectrometer and quenching was monitored with internal standards. Preliminary experiments with ^{14}C-labeled GHB indicated that the recovery by means of this collection procedure was 84 per cent. Fig. 2 illustrates a typical gas chromatogram of a brain sample prepared from a rat injected intracisternally with ^{3}H-GABA.

When ^{3}H-GABA was administered intracisternally, about 16 per cent of the administered radioactivity was found in the brain after 20 min. Since the control samples had the labeled GABA added directly to the precipitated brain, they contained approximately 6 times more radioactivity than the experimental brains. Despite this weighted bias in favor of the control samples, the amount of radioactivity isolated in the GHB peak was only minimal and probably represents some volatile decomposition products of GABA produced during gas chromatography which raised the background activity of the carrier gas. However, the possibility could not be ruled out that some of this radioactivity may represent a small trace of GHB present in the commercially available GABA or more likely formed nonenzymatically during manipulation of the brain extracts. In spite of the presence of a small amount of radioactivity in the control samples, it can be clearly observed from the data in Table 1 that rat brain *in vivo* is capable of converting ^{3}H-GABA to GHB, although this pathway under normal conditions does not appear to be exceptionally active.

TABLE 1. FORMATION OF ^{3}H-GHB FROM ^{3}H-GABA IN THE RAT

Rats	^{3}H in brain extract (cpm × 10^{-6})	^{3}H in GHB peak (cpm)
Control		
1	17·04	1600
2	16·56	1550
3	17·67	1750
4	16·40	2125
Experimental		
1	2·46	9450
2	2·68	9725
3	4·06	28,050
4	1·92	9525
5	2·61	12,425

* GHB, gamma-hydroxybutyric acid; GABA, gamma-aminobutyric acid.

It is, of course, possible that the normal condition of alertness in an animal does not represent the physiological state most favorable to the production of GHB *in vivo*. There is also the possibility that GHB is derived biosynthetically from another source. In view of the finding that 1,4-butanediol (BD) may exist among lipid-diols in the rat[18] and the evidence that BD is converted in the rat to GHB,[19] it is conceivable that endogenous BD may serve as a depot-form of precursor of GHB.

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REFERENCES

1. H. LABORIT, J. M. JOUANY, J. GERARD and P. FABIANI, *Neuropsychopharmacology*, **2**, 490 (1961).
2. B. A. RUBIN and N. J. GIARMAN, *Yale J. Biol. Med.* **19**, 1017 (1947).
3. N. J. GIARMAN, *Ph.D. Dissertation*, Yale University (1948).
4. P. BENDA and P. PERLES, *C.r. hebd Seanc Acad. Sci., Paris* **251**, 1312 (1960).
5. H. LABORIT, J. M. JOUANY, J. GERARD and F. FABIANI, *Presse med.* **68** 1867 (1960).
6. N. J. GIARMAN and K. F. SCHMIDT, *Br. J. Pharmac. Chemother.* **20**, 563 (1963).
7. CH. MITOMA and S. E. NEUBAUER, *Experientia* **24**, 12 (1968).
8. M. WOLLEMANN and T. DÉVÉNYI, *Agressologie* **4**, 593 (1963).
9. G. DELLA PIETRA, G. ILLIANO, V. CAPANO and R. RAVA, *Nature, Lond.* **210**, 733, 1966.
10. Y. GODIN and J. MARK, *C.r. Séanc. Soc. Biol.* **161**, 1392 (1967).
11. W. N. FISHBEIN and S. P. BESSMAN, *J. biol. Chem.* **239**, 357 (1964).
12. S. P. BESSMAN and W. N. FISHBEIN, *Nature, Lond.* **200**, 1207 (1963).
13. N. J. GIARMAN and R. H. ROTH, *Science* **145**, 583 (1964).
14. R. H. ROTH, *Ph.D. Dissertation*, Yale University (1965).
15. R. H. ROTH and N. J. GIARMAN, *Biochem. Pharmac.* **15**, 1333 (1966).

16. W. A. JEFFERS and J. Q. GRIFFITH, JR., in *The Rat in Laboratory Investigation* (Eds. E. J. FARRIS and J. Q. GRIFFITH, JR.), p. 196. J. P. Lippincott, Philadephia (1949).
17. G. BRAY, *Analyt. Biochem.* **1**, 279 (1960).
18. L. D. BERGELSON, V. A. VAVER, N. V. PROKAZOVA, A. N. USHAKOV and G. A. POPKOVA, *Biochim. biophys. Acta* **116**, 511 (1966).
19. R. H. ROTH and N. J. GIARMAN, *Biochem. Pharmac.* **17**, 735 (1968).

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N^6-(Δ^2-isopentenyl) adenosine interference with methionine metabolism in axenic cultures of mammalian cells

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N^6-(Δ^2-ISOPENTENYL) ADENOSINE (IPA) is located adjacent to the presumed anticodon in t-$RNA^{Ser,Tyr}$ of yeast, plants and mammalian tissues.[1] It has biological activity as a cytokinin,[2] is toxic to some cultured mammalian cells,[3] dogs and rodents,[4] and has exhibited therapeutic activity in one case of human promyelocytic leukemia.[5] Evidence will be presented herein that IPA interferes with methionine metabolism in a line of cultured mammalian cells.

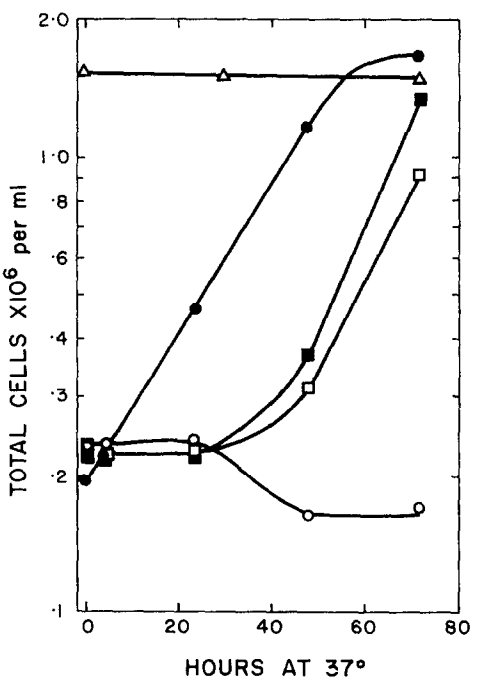

FIG. 1. Growth of RPMI No. 2402 cells in RPMI medium No. 1379 supplemented with 2% by volume calf serum in the presence of the following levels (μM) of IPA: (0), 15; (□) 3; (■), 1·5; (●), zero. The triangles represent a culture in the absence of IPA inoculated with $1{\cdot}5 \times 10^6$ cells per ml.