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The comparative effect of γ-hydroxybutyrate and phenobarbital on brain energy metabolism*

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γ-HYDROXYBUTYRATE (GHB) has been the subject of recent investigation because of its depressant properties on central nervous system. Although it can be formed through the reduction of succinic semialdehyde catalyzed by lactic dehydrogenase, its concentration in brain is apparently vanishingly small.[1] However, upon injection it produces in animals and man a state described as similar in some ways to that of normal sleep, and recently it has been used as an anesthetic adjuvant in man.[2]

The following study was undertaken to determine whether the depressant properties of GHB could be related to specific changes in brain energy metabolism, and if such changes occurred, whether they differ from those produced by a chemically unrelated cerebral depressant—phenobarbital.

MATERIAL AND METHODS

Female Charles River CD-1 mice (26 days old) were injected i.p. with sodium GHB (500 mg/kg), sodium phenobarbital (200 mg/kg), or saline and, in each case, sacrificed 1 hr later. "Zero-time" animals were frozen whole in a mixture of Freon 12 and 22, kept at its freezing point (−150°) in liquid nitrogen; "15-second" animals were decapitated and their heads frozen after this interval. Brains were dissected, powdered, and weighed in a cold room at −20°. Extraction of brain powders in 3 M perchloric acid, subsequent dilution, buffering, and enzymatic assay of ATP, creatine phosphate, glucose, and lactate followed procedures described by Lowry *et al.*[3] Malate was assayed in a hydrazine hydrate buffer as has been described by Hohorst,[4] with the exception that 3-acetylpyridine NAD^+ rather than NAD^+ was used together with malic dehydrogenase. Citrate was assayed by means of a preparation of aconitase from hog heart, isocitric dehydrogenase, and $NADP^+$.

Activity of creatine phosphokinase from rabbit skeletal muscle was assayed spectrophotometrically with an imidazole buffer, pH 7·1, 150 mM potassium acetate, 5 mM K_2HPO_4, 1 mM ADP, 1·5 mM glucose, 4 mM creatine phosphate, 1 mM $NADP^+$, 12 μg glucose-6-phosphate dehydrogenase/ml, and 25 μg hexokinase/ml. When production of NADPH reached a steady velocity after the addition of creatine phosphokinase, GHB was added to a final concentration of 1 mM. This is close to the estimated concentration of GHB in brain 1 hr after this dose of the drug.[1]

Creatine phosphokinase activity in brains of animals given 500 mg GHB/kg and of controls were measured fluorometrically by means of a similar system. Rates were not maximal, since concentrations of ADP had to be kept low because of its contamination with ATP.

Enzymes were obtained from California Corp. for Biochemical Research; 3-acetylpyridine NAD^+ was obtained from Pabst Laboratories.

RESULTS AND DISCUSSION

In animals given phenobarbital, zero-time brain glucose, ATP, and creatine phosphate were elevated, and zero time lactate and malate were depressed (Table 1). These changes have been observed by other workers.[3, 5, 6] Similar results were produced in the animals treated with GHB, with the exception of creatine phosphate, which was significantly lower at zero time than in the animals with phenobarbital. Brain glucose/blood glucose ratios were elevated in both groups of animals receiving drugs, as compared to controls. Mayman *et al.*[6] noted this increase in brain glucose with a variety of anesthetics and postulated that anasthesia alters parameters of glucose transport or compartmentation in brain.

After 15 sec of ischemia, the accumulation of lactate is less in the animals treated with either drug than in controls. The animals depressed with GHB differ from the other two in the considerably slower fall of creatine phosphate over this 15-sec period, accompanied by a faster decrease in ATP. Prompted by this difference we investigated the influence of GHB on the activity of creatine phosphokinase activity in a system simulating intracellular fluid. The drug did not alter the activity of commercial rabbit muscle creatine phosphokinase or the activity of the enzyme in brains of animals given 500 mg

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TABLE 1. LEVELS OF BRAIN SUBSTRATES IN ANIMALS GIVEN PHENOBARBITAL OR γ-HYDROXYBUTYRATE

Values are given in millimoles per kilogram wet brain ± S.E.M. Each value is the average from 6 animals.

	Creatine phosphate	ATP	Glucose	Lactate	Citrate	Iso-citrate	α-Keto-glutarate	Malate	Blood glucose
Zero-time animals									
Controls	4·15 ±0·40	2·41 ±0·07	1·25 ±0·04	2 12 ±0·10	0 31 ±0·04	0·011 ±0·001	0·09 ±0·001	0·42 ±0·002	10·1 ±0·9
Phenobarbital	4·72 ±0·09	2·66 ±0·08	2·78 ±0·03	1·02 ±0·01	0·33 ±0·04	0·010 ±0·006	0·09 ±0·002	0·28 ±0·004	10·9 ±0·7
GHB	4·04 ±0·12	2·87 ±0·04	3·14 ±0·24	0·96 ±0·01	0·37 ±0·08	0·009 ±0·002	0·08 ±0·001	0·24 ±0·001	13·3 ±1·3
15-Second animals									
Control	1·36 ±0·03	1·90 ±0·08	0·78 ±0·02	4·82 ±0·01	0·30 ±0·04	0·010 ±0·006	0·03 ±0·001	0·40 ±0·001	
Phenobarbital	1·88 ±0·04	2·22 ±0·11	2·22 ±0·11	2·87 ±0·04	0·33 ±0·10	0·008 ±0·001	0·03 ±0·001	0·51 ±0·001	
GHB	2·24 ±0·08	1·98 ±0·06	2·62 ±0·11	3·25 ±0·07	0·34 ±0·06	0·012 ±0·001	0·04 ±0·001	0·52 ±0·002	

Zero-time animals were frozen whole; 15-sec. animals were decapitated and their heads frozen after that interval.

TABLE 2. DIFFERENCES IN ATP, CREATINE PHOSPHATE, AND LACTATE BETWEEN ZERO TIME AND 15 SECONDS*

	ATP phosphate	Creatine	Lactate	$\Delta \geqslant P$
Control	0·51	2·79	2·70	6·00
Phenobarbital	0·44	2·84	1·85	5·13
GHB	0·89	1·80	2·29	4·98

* The sum of these differences may be taken as a measure of the production and utilization of high-energy ($\geqslant$) phosphate during this period.

of drug/kg, as compared to controls. Thus, studies *in vitro* did not clarify events observed *in vivo*.

By adding the differences in ATP, creatine phosphate, and lactate between zero time and 15 sec, a measure of production and utilization of high-energy phosphate over this period of ischemia may be obtained (Table 2). This amount was very close in the phenobarbital and GHB animals. Krebs cycle substrate concentrations were very similar as well. The anesthetized animals differed from controls in their diminished malate concentration at zero time with a rise after 15 sec of ischemia. Similar results have been noted by Goldberg *et al.*[7] after treatment with a variety of anesthetics.

Although the rate of glycolysis in brain during 15 sec of ischemia was depressed as much in the GHB mice as in those given phenobarbital, the appearance of the animals differed considerably. The animals on phenobarbital were deeply depressed, limp, and flaccid. The animals after GHB were more lightly anesthetized at the dosage chosen and some retained a righting response. They had a marked exophthalmos and showed occasional brief, bilaterally synchronous myoclonic twitching. Thus, although the magnitude of fall in overall cerebral metabolic activity was similar in the two groups it was concluded that GHB affects to a greater or lesser extent discrete and different portions of brain compared to those affected by phenobarbital, or affects differently some metabolic site not examined in the present investigation.

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Decarboxylase inhibitors and histamine in guinea pigs*

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THE experiments reported here were performed to study several histidine decarboxylase inhibitors for their effects upon tissue histamine concentration and anaphylaxis in guinea pigs.

METHODS

Young guinea pigs of both sexes were used. In an effort to deplete the animals of any easily releasable histamine they were first subjected to passive anaphylactic shock with a supralethal amount

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