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EVIDENCE THAT CENTRAL NERVOUS SYSTEM DEPRESSION BY 1,4-BUTANEDIOL IS MEDIATED THROUGH A METABOLITE, GAMMA-HYDROXYBUTYRATE

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Abstract—Gas chromatographic analysis of blood and brain extracts from rats anesthetized with 1,4-butanediol (BD) revealed that BD was metabolized to γ-hydroxybutyrate (GHB), since the latter compound was found in both blood and brain. Further, there appeared to be a correlation between the brain concentration of GHB and the "sleep" induced by BD. β-hydroxybutyrate, a known antagonist of GHB-induced "sleep" was found also to antagonize BD-induced "sleep" as well as to cause a reduction in the brain concentration of GHB. This evidence is taken to support the view that GHB is the active metabolite involved in the production of the "sleep" induced by BD.

BUTANEDIOLS possess a number of chemical and physical properties which make them of potential interest for use in polyester synthesis, solvents and plasticizers.[1] In addition, one of these compounds, 1,4-butanediol, possesses some depressant activity on the central nervous system. A recent report has indicated that the neuropharmacological effects exerted by 1,4-butanediol (BD) are largely similar to those produced by γ-butyrolactone (GBL) and γ-hydroxybutyric acid (GHB).[2] There were two striking differences: 1) it was observed that GBL and GHB had a significantly shorter onset of action than BD when given in equal molar doses by the i.p. route; and 2) it was observed that GBL and BD had a longer duration of action than GHB. This discrepancy in action could be explained as a result of the metabolism of BD to an active metabolite, which then subsequently exerted its characteristic pharmacological action, thus causing a longer onset of action as well as a longer duration of effect. Since GHB is a closely related congener of BD and has been demonstrated to be the active pharmacologic form of the lactone, GBL,[3] it seemed reasonable to expect that BD might be oxidized to GHB which would then be responsible for the anesthetic-like properties of BD. The structural relationships among these agents are illustrated in Fig. 1.

MATERIALS AND METHODS

Sprague-Dawley rats (200–300 g) were injected i.v. with 5·8 m-Equiv. BD/kg body wt. In some cases rats were pretreated with 3-hydroxybutyrate (2 g/kg, i.p.; neutralized to pH 7·0 with NaOH) 20 min prior to the administration of the BD. Gross behavioral effects were observed and the "anesthetic" effects were recorded as: 1) the interval from the injection of the drug to the loss of the righting reflex referred to as sleep induction time, and 2) the sleeping time. The sleeping time was taken as the interval from the loss of the righting reflex until the righting reflex was regained.

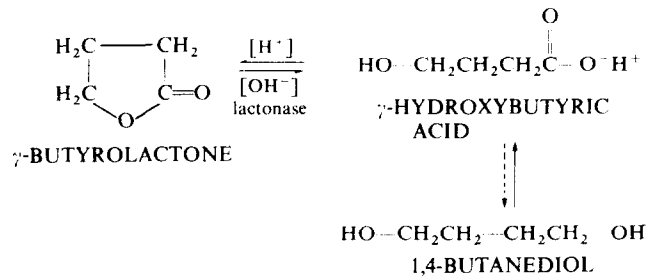

FIG. 1. Postulated interrelationships of GBL, GHB and BD.

Assay for GHB. Rats were killed by decapitation at appropriate intervals after injection of BD. The blood was collected in 100 units of heparin sodium and the brain was quickly excised, weighed and homogenized in 5 ml of ice-cold 20% trichloroacetic acid (TCA). Five ml of blood was then precipitated with an equal volume of 20% TCA. The tissue extracts were centrifuged at 30,000 *g* to remove precipitated proteins, and the precipitate was re-extracted with 2 ml of TCA. The supernatant fractions were combined and the concentration of GHB was determined by the gas chromatographic method described by Giarman and Roth.[4] This method, it should be noted, depends upon the conversion of GHB to GBL, and the final estimation of GBL. The method used to identify and quantitate GHB was essentially the same as that reported earlier,[5] with the minor modification that the Dowex column step was omitted. Instead, after heating the extract, 1 ml of 1 M phosphate buffer, pH 7, was added and the extract was adjusted to pH 6·0 prior to the benzene extraction step. This served the same purpose as the Dowex column used previously to remove small amounts of extracted TCA by preventing the extraction of TCA, since the acid is ionized at pH 6·0 and is therefore not readily extractable into the benzene phase.

In some cases, 50 mg BD (about ½ the total dose received by a 200-g rat) was added to 5 ml of 20% TCA and carried throughout the entire extraction procedure to determine if any GHB was present in the commercially available BD or if the chemical manipulations involved in the preparation of the tissue could be responsible for the conversion of BD to GHB. With this procedure, no trace of GHB could be detected either in the BD used or from chemical manipulation of the BD.

RESULTS

Initial experiments demonstrated that the rat was capable of the biotransformation of BD to GHB, which was subsequently found in the blood and brain 1½ hr after the i.v. injection of BD. Fig. 2 shows a comparison of a gas chromatogram of authentic GBL and the extract from a rat's brain and blood after administration of BD. The retention time on the gas chromatographic column of the compound isolated from rat brain and blood is identical with that of authentic GBL (in the processing of tissue extracts GHB is converted quantitatively to the lactone form, GBL; see Methods). In view of this ability of the rat to convert BD to GHB, it was of interest to determine if indeed the GHB was formed to the extent that it could be the active metabolite responsible for induction and maintenance of sleep. Therefore, we examined a time course of the blood and brain concentrations of GHB after administration of BD and compared this with the anesthetic-like effects on the rats (Fig. 3). From this figure

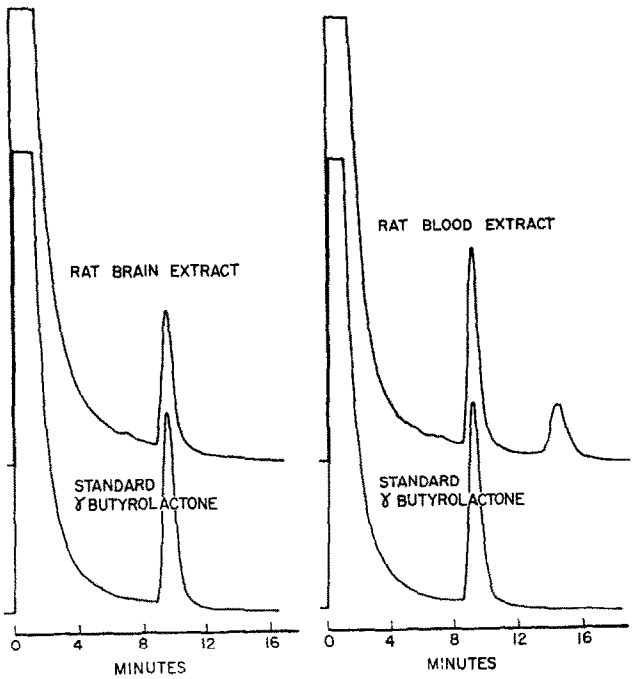

FIG. 2. Left panel: gas chromatogram of authentic GBL (3·3 μg) compared with an extract from the brain of a rat 90 min after the administration of BD (5·8 mEquiv./kg). Right panel: gas chromatogram of authentic GBL (3·3 μg) compared with an extract from the blood of a rat 90 min after the administration of BD (5·8 mEquiv./kg).

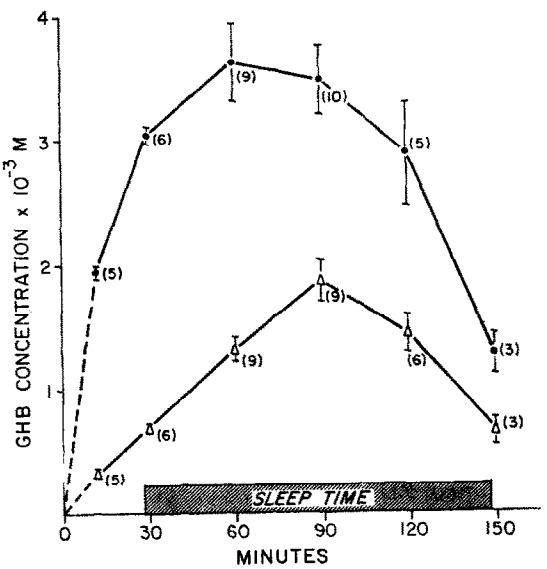

FIG. 3. Time course of the blood (●—●) and brain (△—△) concentrations of GHB after i.v. administration of BD (5·8 mEquiv./kg). Values in parentheses indicate the number of rats used at each point. The vertical bars depict the S.E.M.

it can be observed that when the amount of GHB in the brain reaches a critical level of about $0{\cdot}7 \times 10^{-3}$ M, the animal loses the righting reflex and a state of sleep ensues. Also, when the brain level falls below this same critical level, the animal awakens and regains the righting reflex.

Although this experiment demonstrates that the levels of GHB in the brain correlate well with the anesthetic-like action of BD, this finding does not rule out the possibility that the correlation is fortuitous and that the induced "sleep" is unrelated to the brain levels of GHB. Therefore, it was desirable to use an approach known to alter the brain levels of GHB and determine if this in any way influenced the behavioral effects exerted by BD. β-Hydroxybutyrate, a substance previously shown to antagonize the central nervous system depressant activity of both GHB and GBL[5] by increasing their rate of disappearance, was administered to rats prior to injection of an anesthetic dose of BD. The results of this experiment are summarized in Table 1. It is clearly seen

TABLE 1. REVERSAL WITH β-HYDROXYBUTYRIC ACID OF SLEEP INDUCED BY 1,4-BUTANEDIOL*

Treatment (dose)	Sleep time (min)	Brain level GHB† (μg/g)	Blood level GHB (μg/g)
1,4-Butanediol (5·8 mEquiv./kg, i.v.)	147·9 (14)‡ ± 7·5	161·2 (9) ± 15·8	299·3 (10) ± 24·1
β-Hydroxybutyric acid (2 g/kg, i.p.) followed by 1,4-butanediol	93·2 (6) ± 15·7 $P < 0{\cdot}001$	79·1 (6) ± 17·6 $P < 0{\cdot}02$	260·6 (6) ± 47·8

* Values are expressed as mean ±S.E.M.
† GHB is expressed as GBL equivalents.
‡ Number of experiments.

that β-hydroxybutyrate significantly reduced the level of GHB in the brain 1½ hr after administration of BD. In addition, β-hydroxybutyrate also significantly reduced the sleeping time, as well as increasing the sleep induction time.

DISCUSSION

Previous experiments have indicated similarities in the neuropharmacological action of GHB, GBL and BD.[2] However, the molecular basis for the action of these compounds still remains obscure, although it has been conclusively demonstrated in the case of GBL and GHB that the acid structure (GHB) rather than the lactone (GBL) is the active species in exerting the anesthetic-like action of these two drugs.[3] In addition, the longer duration of action attributed to GBL[4, 6, 7] has been clarified by a study of the tissue distribution of this compound, which demonstrated a large pool in muscle that provides a slow release of the drug and thus maintains the blood and brain concentration of GHB.[5]

After the administration of BD the blood level of GHB never exceeds about 4 μmole/ml, while the brain concentration reaches a maximum level of about 2 μmole/g 90 min after administration of BD. This finding can be contrasted to the results obtained after administration of equimolar amounts of GHB and GBL where it was observed that at 90 min ,when the brain level is only about 1 and 1·5 μmole/g respectively, the blood concentration of GHB is equal to or greater than that observed after treatment with

BD.[4] This might suggest that some of the brain GHB is derived directly from local metabolism of BD rather than solely from blood-borne GHB. Experiments are now in progress to determine if brain tissue can convert BD to GHB. However, an alternative explanation may be that a blood concentration of GHB maintained for a prolonged period of time at 2–4 μmole/ml by a constant rate of peripheral metabolism of BD may be sufficient to explain the resultant brain concentrations of GHB.

The fact that GHB is indeed formed biosynthetically from BD in the rat has been verified by the coincidence between the gas chromatograms obtained from the blood and brain of BD-treated rats and that of authentic GBL. In addition, no GBL peak is observed when large amounts of BD are carried through the acidification and extraction technique used to analyze tissues for GHB. The experiments with β-hydroxybutyrate have also given further support to the conclusion that GHB is the active metabolite involved in the central nervous system depression exerted by BD. Thus, in a previous report β-hydroxybutyrate has been shown to antagonize the sleep time induced by both GBL and GBH as well as to cause a reduction in blood and brain concentrations of GHB. In addition, β-hydroxybutyrate appeared to inhibit the metabolism of GHB to CO_2. In the investigation mentioned above, β-hydroxybutyrate was also found to antagonize both the sleep time and the brain levels of GHB induced by i.v. administration of BD. This finding again suggests that GHB is probably the active metabolite in the induction and maintenance of "sleep" in rats treated with BD.

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