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The effect of sodium γ-hydroxybutyrate on the metabolism of dopamine in the brain

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Summary

1. Sodium gamma hydroxybutyrate (GHB-Na), when given to rats and mice, caused a sleep-like state and a fall in body temperature of about 10° C.

2. GHB-Na produced a dose-dependent increase in the concentration of dopamine (DA) in the brains of mice kept at an environmental temperature of 18–20° C or 30–32° C.

3. The concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA), the metabolites of DA, were increased in the striatal tissues of rats and mice, after the administration of GHB-Na. However there was a delay of 60 min before the concentration of HVA increased whereas there appeared to be little delay before the concentration of DOPAC increased.

4. When GHB-Na was administered to reserpine-treated animals, no increase occurred in the concentration of dopamine in the brains of mice or rats, or of DOPAC in the rat brain.

5. Reserpine did not prevent the induction of the sleep-like state by GHB-Na.

6. The results presented suggest that the effect of GHB-Na in increasing the concentration of DA requires unimpaired storage mechanisms for the amine.

Introduction

γ-Hydroxybutyric acid (GHB) occurs naturally in the mammalian brain (Bessman & Fishbein, 1963a & b; Roth & Giarman, 1970), possibly as a metabolite of γ-aminobutyric acid (GABA) (Roth & Giarman, 1968; Roth, 1970). When administered to animals, sodium γ-hydroxybutyrate (GHB-Na) has a depressant action on the central nervous system (CNS) causing a sleep-like state. GHB-Na also causes an increase in the concentration of dopamine (DA) in the brain but does not change the cerebral concentrations of GABA, noradrenaline or 5-hydroxytryptamine (Giarman & Schmidt, 1963; Gessa, Vargiu, Crabai, Boero, Caboni & Camba, 1966). The CNS depression and the increase in the concentration of DA show a close temporal correlation but it is not clear whether the altered metabolism of DA is responsible for the sleep-like state. In this study, the effect of GHB-Na on the concentrations of DA and its metabolites 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the brains of rats and mice has been investigated.

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Methods

Albino mice (20–30 g) or rats (200–300 g) of either sex were used. The animals were killed by decapitation, the brains were removed and cooled on a glass plate in an ice bath. The corpora striata of the rats were dissected out. The striatal tissue of the mouse was prepared as described by Sharman (1966). In some experiments DA was determined in the fore-brain of the mouse. DA was estimated fluorimetrically (Laverty & Sharman, 1965) after adsorption from tissue extracts onto a column of Dowex 50-X 8 cation exchange resin and elution with 2 M hydrochloric acid. The tissue from one animal was used for each estimation. Recovery of 100 ng DA added to brain tissue homogenates averaged 63 ± 4 (S.E.M.) ng; $n=35$. DOPAC and HVA were estimated by the method of Murphy, Robinson & Sharman (1969), except that each acid was extracted from a separate tissue sample. The tissue from 1 or 2 rats, or 2–4 mice, was used for each estimation of HVA or DOPAC. The recovery of 100 ng of each of the acids added to tissue homogenates was 60 ± 3 (S.E.M.) ng, $n=18$, for HVA and 44 ± 3 (S.E.M.) ng, $n=16$, for DOPAC. The results are given uncorrected for recoveries. GHB-Na was prepared from γ-butyrolactone by reacting with sodium hydroxide solution and then crystallizing out. It was administered dissolved in distilled water. Reserpine base was dissolved in 5% w/v ascorbic acid solution. The drugs were injected intraperitoneally. Rectal temperatures were measured by means of a thermistor probe.

Results

When administered in doses that resulted in a depression of the CNS, GHB-Na caused a dose-dependent increase in the concentration of DA in the forebrain of mice (Table 1). At room temperature (18–20° C) there was also a fall in body

TABLE 1. *Effect of sodium γ-hydroxybutyrate (GHB-Na) on the concentration of dopamine in the forebrains of mice maintained at an environmental temperature of* 18–20° *or* 30–32° *C*

Treatment	Dose (mg/kg)	Duration of treatment (h)	Dopamine concentration (μg/g ± S.E.M.) 18–20° C	30–32° C
Untreated control	–	–	1·04 ± 0·06 (17)	0·96 ± 0·04 (9)†
GHB-Na	375	2	1·24 ± 0·05 (4)	
GHB-Na	750	2	1·66 ± 0·20 (4)*	1·18 ± 0·01 (6)
GHB-Na	1500	2	1·76 ± 0·12 (8)**	1·41 ± 0·04 (4)**

Difference from corresponding control values * $P<0.01$; ** $P<0.001$, Student's *t*-test. † The control animals at the higher temperature were kept at 30–32° C for 2 h. Number of observations is indicated in parentheses.

temperature of about 10° C. When the mice were kept at an environmental temperature of 30–32° C GHB-Na did not produce a fall in body temperature but an increase in the concentration of cerebral DA still occurred (Table 1).

DA was depleted from the forebrain of the mouse and the striatum of the rat, by reserpine (5mg/kg), for up to 7 h (Table 2). The administration of GHB-Na (1,500 mg/kg) to mice or rats, previously treated with reserpine (5 mg/kg), did not cause an increase in the concentration of DA in the brain (Table 2). However, reserpine did not prevent the induction of the sleep-like state by GHB-Na. Twenty-four hours after an injection of reserpine (5 mg/kg), DA had re-accumulated in the striatum of the rat to a concentration of 0·23 μg/g but GHB-Na still did not produce a significant increase in the concentration of DA (Table 3).

TABLE 2. *The effect of sodium γ-hydroxybutyrate (GHB-Na) on the concentration of dopamine in the brains of mice and rats treated with reserpine and maintained at an environmental temperature of* 30–32° *C*

	Treatment	Dose (mg/kg)	Duration of treatment (h)	Dopamine concentration (μg/g)
				Forebrain
A. Mouse	Untreated control	—	—	0·96±0·04 (9)
	GHB-Na	1500	1·0	2·04±0·02 (6)**
	Reserpine	5	4·0	<0·03 (5)
	Reserpine+	5	4·0	
	GHB-Na	1500	1·0	<0·04 (8)
				Corpus striatum
B. Rat	Untreated control	—	—	6·58±0·39 (16)
	GHB-Na	1500	1·5	13·36±1·76 (6)*
	Reserpine	5	3·5	<0·07 (7)
	Reserpine+	5	3·5	
	GHB-Na	1500	1·5	<0·12 (8)
	Reserpine	5	7·0	<0·05 (10)
	Reserpine+	5	7·0	
	GHB-Na	1500	1·5	<0·07 (11)

Dopamine values are means (±S.E.M. where applicable). The number of estimations is indicated in parentheses. Difference from the control value: * $P<0·005$; ** $P<0·001$, Student's *t*-test.

The concentrations of DOPAC and HVA were increased in the striatum of rats and mice after the administration of GHB-Na (Tables 3, 4 and 5). In the mouse the increases in the concentrations of the two acids were dose dependent and occurred whether the animals were kept at room temperature (18–20° C) or at

TABLE 3. *Effect of sodium γ-hydroxybutyrate (GHB-Na) on the concentration of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in the corpora striata of rats treated with reserpine and maintained at an environmental temperature of* 30–32° *C for* 24 *hours*

Treatment	Dose (mg/kg)	Duration of treatment (h)	Dopamine concentration (μg/g)	DOPAC concentration (μg/g)
Untreated control	—	—	7·26±1·10 (4)	0·35±0·02 (14)
GHB-Na	1500	1	16·00±1·80 (4)*	0·73±0·05 (4)**
Reserpine	5	24	0·23±0·07 (4)**	0·37±0·03 (6)
Reserpine+	5	24		
GHB-Na	1500	1	0·51±0·26 (4)	0·32±0·02 (6)

Difference from control value: * $P<0·005$; ** $P<0·001$, Student's *t*-test. Number of observations is indicated in parentheses.

30–32° C (Table 4). Twenty-four hours after treatment with reserpine (5 mg/kg) the concentration of DOPAC in the striatum of the rat did not differ from the control value. However, the increase in the cerebral concentration of DOPAC produced by GHB-Na was abolished in these reserpine-treated rats (Table 3). The

TABLE 4. *Effect of sodium γ-hydroxybutyrate (GHB-Na) on the concentration of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatal tissue of mice maintained at an environmental temperature of* 18–20° *C or* 30–32° *C*

Dose of GHB-Na (mg/kg)	Time (h)	HVA concentration (μg/g±S.E.M.) 18–20° C	HVA concentration (μg/g±S.E.M.) 30–32° C	DOPAC concentration (μg/g±S.E.M.) 18–20° C	DOPAC concentration (μg/g±S.E.M.) 30–32° C
—	–	0·26±0·01 (10)	0·29±0·01 (6)†	0·29±0·01 (6)	0·30±0·01 (6)†
750	2	0·49±0·02 (4)*		0·56±0·03 (4)*	0·95±0·06 (6)*
1500	2	0·67±0·01 (4)*	0·92; 0·76	0·71±0·01 (4)*	1·33±0·03 (6)*

Difference from corresponding control values: * $P<0·001$, Student's *t* test. † The control animals at the higher temperature were kept at 30–32° C for 2 h. Number of observations is indicated in parentheses.

TABLE 5. *Effect of sodium γ-hydroxybutyrate (GHB-Na) on the concentration of homovanillic acid (HVA) in the corpora striata of rats*

Treatment	Dose (mg/kg)	Duration of treatment (h)	HVA concentration (μg/g±S.E.M.)
Untreated control	—	–	0·32±0·03 (10)
GHB-Na	750	2	0·70±0·06 (4)*
GHB-Na	1500	2	1·13±0·12 (6)*

Difference from the control value: * $P<0·001$, Student's t-test. Number of observations is indicated in parentheses.

effect of pretreatment with reserpine on the increase in HVA concentration produced by GHB-Na was not investigated.

The greatest increase in the concentration of DOPAC in the striatum of the rat was observed 2 h after the injection of GHB-Na (1,500 mg/kg) (Fig. 1). The concentration of DOPAC then declined reaching the control value after a further 2 hours. There were two aberrant observations. In one rat which awoke before the others the concentration of DOPAC 2 h after GHB-Na was about 50% of the mean value found in the other treated animals at this time and in one rat which was still sleeping 4 h after GHB-Na, when the other animals were awake, the concentration of DOPAC was twice as large as the control value.

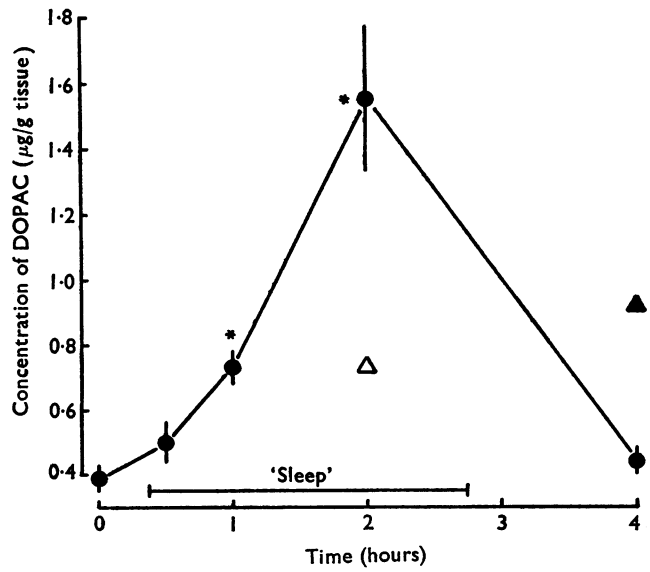

FIG. 1. The effect of sodium γ-hydroxybutyrate (1,500 mg/kg) (●——●) on the concentration of DOPAC in the corpus striatum of the rat. Each point is the mean of at least 4 observations. Vertical bars indicate the S.E.M. Difference from control value, *$P<0·01$, Student's t test. Two hours after the injection one rat awoke before the other rats (△) and at 4 h one rat continued "sleeping" (▲).

Figure 2 shows the effect of GHB-Na on the concentrations of DA, HVA and DOPAC in the striatum of the mouse. There were similar proportionate increases in the concentrations of DA and DOPAC one hour after GHB-Na (1,500 mg/kg) with no increase in the concentration of HVA. During the second hour after GHB-Na the concentration of DA decreased towards the control value. The

concentration of DOPAC continued to increase during this period and the concentration of HVA was almost trebled.

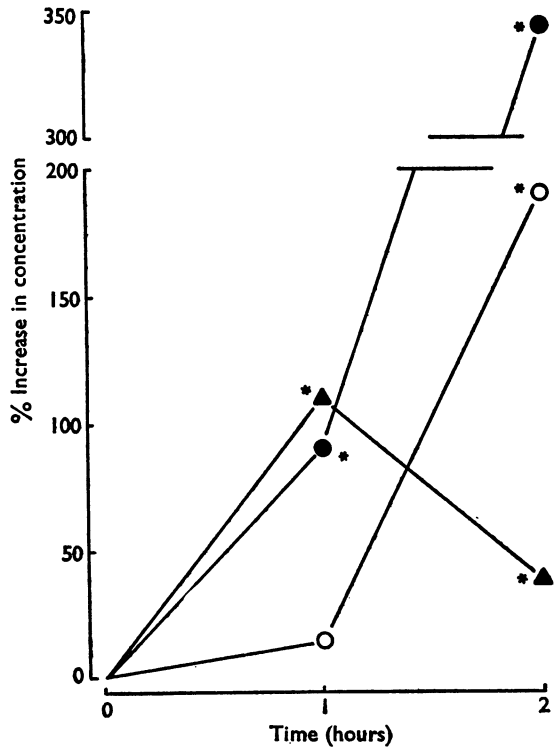

FIG. 2. The effect of sodium γ-hydroxybutyrate (1,500 mg/kg) on the concentrations of dopamine (DA, ▲——▲), homovanillic acid (HVA, ○——○) and DOPAC (●——●) in the striatal tissue of mice maintained at an environmental temperature of 30–32° C. Each point is the mean of at least 4 observations. Difference from control value, $*P<0.001$, Student's *t* test.

Discussion

The present results confirm the finding that GHB-Na causes a dose-dependent increase in the concentration of DA in the striatum, first made by Gessa *et al.* (1966). Gessa, Crabai, Vargiu & Spano (1968) suggested that γ-hydroxybutyrate might protect DA from enzymic breakdown possibly by a reaction between DA and γ-hydroxybutyrate or a metabolite of this substance. This suggestion was based on the observation that the administration of γ-hydroxybutyrate to rats in which the cerebral DA had been depleted by the administration of reserpine resulted in the restoration of the concentration of DA. We have been unable to confirm this result in either the rat or the mouse. Even 24 h after treatment with reserpine, when the DA was starting to re-accumulate in the striatum of the rat, the administration of GHB-Na did not increase the concentration of striatal DA. It is thus unlikely that a combination of γ-hydroxybutyrate, or one of its metabolites, with DA to protect the amine from enzymic destruction, plays any part in the effect of GHB-Na in increasing the concentration of DA in the striatum.

That there is a delayed increase in the concentration of HVA after γ-butyrolactone or γ-hydroxybutyrate has been observed previously by Roth (1971) and Spano, Tagliamonte, Tagliamonte & Gessa (1971). Roth (1971) obtained evidence for a

reduction in the rate of formation of HVA in the rat brain during the first 1·5 h after administering γ-butyrolactone, and suggested that an action of the drug is to prevent the release or utilization of DA. In addition Roth & Suhr (1970) and Spano *et al.* (1971) concluded that γ-hydroxybutyrate stimulated the synthesis of DA, but the former authors described the increase in synthesis as transient.

The present results are compatible with an increase in synthesis of DA. Since γ-hydroxybutyrate has been shown to have no effect on monoamine oxidase or catechol-*O*-methyl transferase (Gessa *et al.*, 1966), the enzymes which metabolize DA in the brain, the increase in DOPAC during the first hour after treatment with GHB-Na might reflect an increased metabolism of DA to this acid. Thus the total amount of DA formed during this time would be larger than that indicated by the observed increase in the concentration of DA. It has been shown that in the mouse brain DOPAC is not normally metabolized to HVA to any great extent (Murphy *et al.*, 1969 ; Roffler-Tarlov, Sharman & Tegerdine, 1971), and the latter authors have suggested that the DOPAC in the striatum is formed within the DA-containing neurones. However, the posibility that γ-hydroxybutyrate prevents the efflux of DOPAC as well as DA from the nerves must not be ignored. Whether γ-hydroxybutyrate does prevent the release or utilization of DA in the brain cannot be decided from the evidence of the present experiments. It is obvious that during the second hour after giving GHB-Na there is a large increase in the rate of metabolism of DA. The further large increase in the concentration of DOPAC suggests that much of the DA might be metabolized within the DA-containing nerves.

The effect of GHB-Na in increasing the concentration of DA requires unimpaired storage mechanisms for the amine. When the storage of DA is prevented by reserpine, no DA accumulates after the administration of GHB-Na. If GHB-Na has an effect on the synthesis of DA, then it no longer occurs after treatment with reserpine because no increase in the concentration of DOPAC could be observed after giving GHB-Na to reserpine-treated rats. When reserpine is given to animals there is an increase in the concentration of DOPAC in the striatum as the concentration of DA is reduced (Andén, Roos & Werdinius, 1964 ; Guldberg & Broch, 1971 ; Roffler-Tarlov *et al.*, 1971). If the action of GHB-Na was simply to increase the rate of synthesis of DA, then it might be expected that in reserpine-treated animals any DA formed in response to GHB-Na would be metabolized immediately to DOPAC. Twenty-four hours after treatment with reserpine, DOPAC was still present in the striatum of the rat indicating that some metabolism of DA to DOPAC was taking place. However, the administration of GHB-Na to rats given reserpine 24 h previously did not increase the concentration of DOPAC.

An injection of GHB-Na into reserpine-treated rats still induces a sleep-like state. This occurs apparently in the absence of any effect on DA metabolism and thus it is unlikely that the two effects are causally related.

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