Biochemical Pharmacology, Vol. 23, pp. 879–885. Pergamon Press, 1974. Printed in Great Britain.

STUDIES ON THE BIOCHEMICAL MECHANISMS OF THE CENTRAL EFFECTS OF GAMMA-HYDROXYBUTYRIC ACID

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(*Received* 27 *September* 1972; *accepted* 10 *July* 1973)

Abstract—In mice, administration of a 15 mg/kg dose of d-amphetamine sulfate (d-amph)., a 10 mg/kg dose of chloropromazine hydrochloride (CPZ) or a 50 mg/kg dose of tetrabenazine methane sulfonate (TBZ) caused marked elevation of homovanillic acid (HVA) levels in the caudate nucleus. Gamma-hydroxybutyric acid (GHB) given alone did not change the level of HVA, but blocked the rise caused by d-amph and CPZ. GHB was not only ineffective in blocking the elevation of HVA levels brought about by TBZ, but in TBZ-pretreated mice GHB also failed to block the HVA-elevating effects of d-amph and CPZ. Apparently, GHB blocks the release of dopamine (DA) by acting at the storage granule membrane and, if the functional integrity of these granules is disrupted, as after TBZ, GHB is no longer effective. Using a thin-layer chromatographic method, it also was shown that GHB does not alter the binding or metabolic pattern of DA by caudate nucleus.

GAMMA-HYDROXYBUTYRIC ACID (GHB), reported to be a normal constituent of mammalian brain,[1,2] produces general "anesthesia" in most experimental animals and in man.[3–6] One interesting biochemical effect of GHB is its ability to cause a significant increase of brain dopamine (DA) without concomitant changes in the levels of either serotonin (5-HT) or norepinephrine (NE).[7,8] It has been postulated that this compound blocks the release of DA from storage sites.[9] Indirect evidence for this was obtained by the observation that the akinesia and rigidity caused by GHB in mice was antagonized by drugs, like *d*-amphetamine, which cause a release of DA from storage sites.[10] The present study was undertaken to provide direct evidence for this possibility, and to investigate the possible site of action of GHB. This was accomplished by investigating the interaction between GHB and certain drugs which enhance the formation of homovanillic acid (HVA) as a result of their effects on the release and metabolism of DA. Using a thin-layer chromatographic method, an attempt also was made to see if GHB alters the binding and metabolism of DA by the caudate nucleus. This work has been reported in preliminary form.[11,12]

EXPERIMENTAL

Male albino Swiss mice (25–32 g, Hilltop Labs) were used for the present studies. All the injections were made intraperitoneally (i.p.) and the experiments were performed at a room temperature of 23 ± 1°. Details of experimental procedures and

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sequences of drug injections are described below and, for clarity, the latter are represented schematically, in Table 1.

The sodium salt of GHB (Aldrich Chemical Co.) was used in all experiments.

Influence of GHB on the enhanced formation of HVA caused by various drugs

d-Amphetamine sulfate (d-amph). Groups of mice treated with different doses of d-amph. (7·5 or 25 mg/kg) were sacrificed 1 hr after injection. Other groups of mice were pretreated with a 350 mg/kg dose of GHB 10 min before d-amph and were sacrificed 1 hr after the second injection. In all experiments, after d-amph treatment the animals were placed in individual cages until the time of sacrifice.

Chlorpromazine hydrochloride (CPZ). Groups of mice were treated with CPZ (10 mg/kg) and were sacrificed 30 min later. Other groups of mice received GHB (350 mg/kg) 10 min before CPZ and were sacrificed 30 min after the second injection.

Tetrabenazine methane sulfonate (TBZ). Groups of mice were treated with a 50 mg/kg dose of TBZ and were sacrificed 1 hr later. Other groups received a 350 mg/kg dose of GHB 10 min before TBZ and were sacrificed 1 hr after the second injection.

In all experiments, control animals received injections of an equal volume of saline at appropriate times and were sacrificed 1 hr later.

In another series of experiments, the interaction of GHB with either d-amph or CPZ was studied in TBZ-pretreated mice. In this case the animals were pretreated with a 50 mg/kg dose of TBZ and received GHB (350 mg/kg) 15 min later. The animals then were divided into two groups, the first group was treated with a 15 mg/kg dose of d-amph and sacrificed 60 min later, while the second group was treated with a 10 mg/kg dose of CPZ and sacrificed 30 min later. The results were compared with those in mice treated similarly, but which had received saline in place of GHB.

Determination of HVA level of caudate nuclei of mice

In all the above experiments the caudate nuclei of the animals were dissected out as described and diagrammatically represented by Westfall *et al.*[13] The tissues from two mice were pooled and the HVA content was estimated according to the method of Murphy *et al.*[14] The average weight of the pooled caudates (n = 94) was 38·5 mg (25- to 60-mg range).

Influence of GHB on the uptake and metabolism of ^{14}C-DA by the mouse caudate nucleus in vivo

DA-2-^{14}C hydrochloride (^{14}C-DA) of sp. act. 57·3 mCi/m-mole (Amersham Searle Corp.) was dissolved in 0·01 N HCl. Appropriate aliquots were diluted in isotonic saline to give 0·1 μCi ^{14}C-DA/μl solution.

Mice were injected with saline or GHB (350 mg/kg), i.p., and were divided into two groups. The first group received ^{14}C-DA 15 min after GHB and the second group received ^{14}C-DA 30 min after GHB treatment. One μl (0·1 μCi) of the ^{14}C-DA solution was administered into the lateral ventrical by freehand injection[15] under light ether anesthesia. The two groups were sacrificed 30 and 15 min, respectively, after the intraventricular injection. The caudate nucleus of two animals were pooled and analyzed for ^{14}C-DA and its metabolites. Aliquots were taken from the homo-

genate to determine the uptake of the ^{14}C-DA and the metabolites of DA were analyzed by our thin-layer chromatographic method.[16]

RESULTS

Influence of GHB on the elevation of HVA level caused by d-amph, CPZ and TBZ

The results are given in Fig. 1 and Table 1. Since various schedules of saline treatment did not cause any alteration in the HVA levels, the control HVA values have

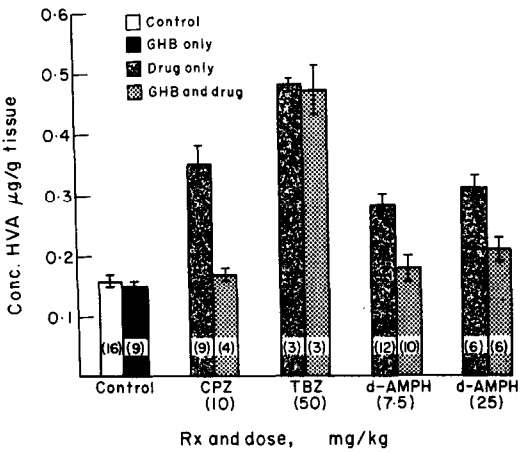

FIG. 1. Effect of GHB on caudate HVA. Influence of GHB (350 mg/kg) pretreatment on the elevation of homovanillic acid (HVA) in the caudate nucleus of mice caused by chlorpromazine HCl (CPZ), tetrabenazine methane sulfonate (TBZ) and *d*-amphetamine sulfate (d-amph).

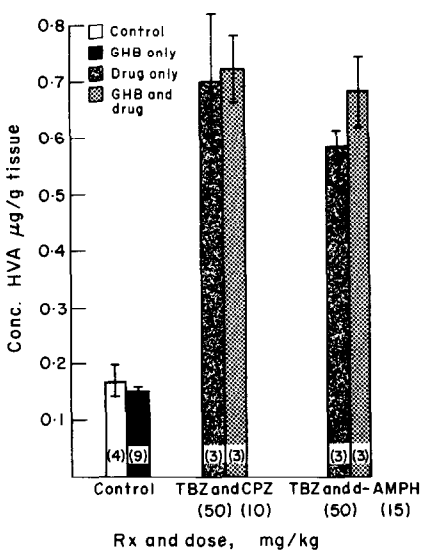

FIG. 2. Effect of GHB on caudate HVA after TBZ or CPZ. Influence of GHB (350 mg/kg) on the elevation of homovanillic acid (HVA) in the caudate nucleus of mice caused by either a combination of tetrabenazine and chlorpromazine (TBZ and CPZ) or by a combination of tetrabenazine and *d*-amphetamine sulfate (d-amph).

TABLE 1. SCHEMATIC REPRESENTATION OF THE SCHEDULE OF DRUG TREATMENTS AND SUMMARY OF DATA SHOWN IN FIGS. 1 AND 2

Schedule of drug treatment*	Caudate HVA level (% increase over control values†	Probability‡
0 — GHB 350 — 10 Saline — 40 Sacrifice	−6	NS
0 Saline — 10 CPZ 10 — 40 Sacrifice	+118	<0·001
0 GHB 350 — 10 CPZ 10 — 40 Sacrifice	+6	NS
0 Saline — 10 TBZ 50 — 70 Sacrifice	+200	<0·001
0 GHB 350 — 10 TBZ 50 — 70 Sacrifice	+194	<0·001
0 Saline — 10 d-amph 7·5 — 70 Sacrifice	+65	<0·001
0 GHB 350 — 10 d-amph 7·5 — 70 Sacrifice	+6	NS
0 Saline — 10 d-amph 25 — 70 Sacrifice	+93	<0·001
0 GHB 350 — 10 d-amph 25 — 70 Sacrifice	+31	<0·05
0 TBZ 50 — 15 d-amph 15 — 75 Sacrifice	+241	<0·001
0 GHB 350 — 10 TBZ 50 — 25 d-amph 15 — 85 Sacrifice	+300	<0·001
0 TBZ 50 — 15 CPZ 10 — 45 Sacrifice	+312	<0·001
0 GHB 350 — 10 TBZ 50 — 25 CPZ 10 — 55 Sacrifice	+323	<0·001

* Numbers above the horizontal lines indicate total time (min) elapsed from zero time. Numbers below the horizontal lines indicate doses of the drugs.

† Control value obtained from saline-treated mice is 0·16 ± 0·02 μg/g.

‡ Probability was calculated using Student's *t*-test. NS = not significant.

been combined. GHB itself did not cause any change in the caudate HVA level but d-amph, CPZ and TBZ caused many-fold increases in the level of HVA. Pretreatment with GHB significantly blocked these effects of d-amph and CPZ but failed to influence the TBZ effects.

The influence of GHB on the rise in HVA levels caused by either d-amph or CPZ on TBZ-treated mice is shown in Fig. 2 and Table 1. The caudate HVA levels of animals receiving a combination of TBZ and d-amph or TBZ and CPZ were considerably higher and pretreatment of GHB failed to reduce the accumulation of HVA in both cases. In fact, the GHB-treated mice gave a higher HVA level than the groups which did not receive GHB.

TABLE 2. RADIOACTIVITY RECOVERED IN MOUSE CAUDATE NUCLEUS AFTER INTRAVENTRICULAR INJECTION OF LABELED DA

Average cpm/100 mg tissue ± S.E.			
15 min*		30 min	
Control (n = 5)	GHB† (n = 4)	Control (n = 4)	GHB‡ (n = 5)
29,746 ± 5876	20,382 ± 3231	22,580 ± 4462	16,912 ± 3864

* Time of sacrifice after intraventricular injection of 0·2 μCi ^{14}C-DA (sp. act. 57·3 mCi/m-mole).

† GHB (350 mg/kg), i.p., given 30 min prior to ^{14}C-DA. Details given in text. Differences between control and GHB-pretreated mice are not significant when P value was calculated using Student's *t*-test.

‡ GHB (350 mg/kg), i.p., given 15 min prior to ^{14}C-DA. Details given in text. Differences between control and GHB-pretreated mice are not significant when P value was calculated using the Student's *t*-test.

TABLE 3. METABOLISM OF ^{14}C-DA BY MOUSE CAUDATE NUCLEUS *in vivo*

	Average per cent of recovered ^{14}C found in reference standards combined to total 100 per cent ± S.E.†			
	15 min‡		30 min	
Reference standard*	Control (n = 4)	GHB§ (n = 4)	Control (n = 3)	GHB‖ (n = 3)
NA				0·5 ± 0·5
DA	63·1 ± 2·7	65·2 ± 3·1	71·3 ± 1·4	67·5 ± 1·2
3-MT	2·2 ± 0·7	1·6 ± 0·5	0	0·2 ± 0·2
DOPAC	22·1 ± 1·2	22·5 ± 2·2	12·6 ± 2·6	16·9 ± 1·1
HVA	12·8 ± 3·1	12·6 ± 2·7	15·0 ± 0·6	15·1 ± 1·6

* NA, noradrenaline; DA, dopamine; 3-MT, 3-methoxytyramine; DOPAC, dopacetic acid (3,4-dihydroxyphenylacetic acid); and HVA, homovanillic acid.

† Per cent recovery of ^{14}C determined by TLC was 80·05 ± 0·95 (n = 8) for the 15-min experiment and 86·0 ± 3·1 (n = 6) for the 30-min experiment.

‡ Time of sacrifice after intraventricular injection of 0·2 μCi ^{14}C-DA (sp. act. 57·3 mCi/m-mole).

§ GHB (350 mg/kg), i.p., given 30 min prior to ^{14}C-DA.

‖ GHB (350 mg/kg), i.p., given 15 min prior to ^{14}C-DA.

Influence of GHB on the uptake and metabolism of ^{14}C-DA by mouse caudate nucleus in vivo

The results are shown in Tables 1 and 2. It may be seen that GHB pretreatment caused a slight inhibition of the uptake of ^{14}C-DA by the caudate nucleus of mice, but this was not found to be statistically significant (Table 2). No significant change was produced in the major metabolites of DA (Table 3).

DISCUSSION

The increase of brain DA level caused by GHB in rats has been confirmed by both biochemical [17] and histochemical[18] methods, but in these experiments it was found necessary to administer exceptionally high doses (four to six times higher than we used) in GHB, which causes the "anesthetic" effects in rats. In the present study, however, "non-anesthetic" doses of GHB have been used and both biochemical[11,12] and preliminary hitochemical studies showed that this dose of GHB does not cause any marked change in the brain DA level.

In the present study, three drugs which cause release of DA by different mechanisms were selected. It is known that d-amph releases DA from storage sites,[19,20] CPZ causes enhanced synthesis of DA by a feedback mechanism as a result of its blocking effect of DA receptors[21,22] and TBZ which, like reserpine, acts by disrupting the ability of the amine storage granules to store monoamines, including DA.[23] Due to these effects in animals treated with these drugs, the metabolism of DA in the brain occurs rapidly, and this is reflected in the increased formation of HVA in all cases.[20–22,24] It is interesting to note that GHB is capable of blocking the effects of only d-amph and CPZ, but not that of TBZ. These results could be explained on the basis of the fact that neither d-amph nor CPZ affects the functional integrity of the amine storage granules, whereas TBZ disrupts it. Apparently, GHB blocks the release of DA by acting at the storage granular membrane, a functionally intact membrane being essential for its action, and if its functional integrity is disrupted, as happens after TBZ, GHB cannot act any longer. This conclusion also is supported by the observation that even the effectiveness of GHB in blocking the DA-releasing effects of both d-amph and CPZ was nullified by pretreatment with TBZ. Another explanation could be that GHB prevents the entry of d-amph and CPZ into their site of action by a non-specific effect on the membranes and transport mechanisms. Pharmacological data do not support this possibility. For example, CPZ exerted a marked depressant effect in GHB-pretreated mice. Moreover, in the present studies GHB pretreatment did not significantly block the uptake of labeled DA. Above all, in TBZ-pretreated mice, the HVA-elevating effects of both d-amph and CPZ were not blocked by GHB, indicating that GHB as such does not block the entry of these drugs into their active sites. These data also indicate that GHB does not have an effect on the removal of HVA from its site of formation.

Blockade of DA release by GHB was expected to cause an increase in the caudate DA level and a corresponding decrease in the level of HVA. This was not found to occur in our studies on mice, nor in the work of Roth[25] on rats given very high doses of GHB. In order to explain this, TLC analyses were performed to demonstrate whether GHB alters the metabolic pathway of DA in the brain, but no evidence for this was obtained. It seems possible that some feedback mechanism is operative so

predominantly that the rate of synthesis of DA is possibly regulated by the rate of its release from storage sites.[26]

Acknowledgements—The authors wish to thank Smith Kline & French Laboratories for the gift of *d*-amphetamine sulfate and chlorpromazine hydrochloride, and Hoffman–La Roche, Inc. for the tetrabenazine methane sulfonate.

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