Biochemical Pharmacology, Vol. 19, pp. 3001–3012. Pergamon Press, 1970. Printed in Great Britain

MECHANISM OF THE γ-HYDROXYBUTYRATE-INDUCED INCREASE IN BRAIN DOPAMINE AND ITS RELATIONSHIP TO "SLEEP"

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(*Received* 9 *February* 1970; *accepted* 20 *April* 1970)

Abstract—The naturally occurring central nervous system depressant γ-hydroxybutyrate (GHB) and its lactone precursor, γ-butyrolactone (GBL) cause a marked and selective increase in brain dopamine. Concomitant with the increase in brain dopamine, homovanillic acid, a metabolite of brain dopamine, is decreased. GBL also causes a selective increase in the specific activity of brain dopamine but not of brain norepinephrine when rats are injected with ^{14}C-tyrosine. This is probably the result of a compensatory activation of dopaminergic neurons because of a blockade in dopamine release induced by GHB. When catecholamine biosynthesis is inhibited by administration of α-methyl-*p*-tyrosine, GBL completely blocks the disappearance of subcortical dopamine but exerts no antagonism on the disappearance of subcortical norepinephrine. These observations are suggestive that GBL increases brain dopamine primarily by selectively blocking the release of this monoamine from dopamine containing neurons. Indirect evidence is also presented which suggests that the central nervous system depressant properties of GHB may be related to this block in the release of brain dopamine.

γ-HYDROXYBUTYRATE (GHB) appears to be a rather unique central nervous system depressant for at least two reasons: (a) it occurs as a natural metabolite of mammalian brain in a concentration of 2–4 nmoles/g;[1] (b) parenteral administration of GHB or related compounds such as γ-butyrolactone (GBL) or 1,4-butanediol (which are converted to GHB *in vivo*) cause a marked increase in brain dopamine with little or no effect on other brain monoamines such as norepinephrine or 5-hydroxytryptamine or on brain GABA.[2–4*] Many other central nervous system depressants so far investigated do not have this unique and rather selective effect on brain dopamine.* The increase in brain dopamine induced by GHB and its analogues appears to have the same regional distribution as normal endogenous dopamine.[2] Recent experiments employing fluorescence microscopy have also indicated at a more refined level that GHB produces this selective increase in brain dopamine exclusively within the terminals or varicosities of the dopamine-containing neurons.[5] This selective localization after GHB contrasts with the generalized increase in fluorescence observed after administration of monoamine oxidase inhibitors.[5, 6] The GHB-induced increase in dopamine can be attributed to new formation of this monoamine since it does not occur after selective inhibition of tyrosine hydroxylase.[7]

The primary purpose of this paper is to investigate the mechanism of this GHB-induced increase in brain dopamine, as well as to determine if the action of this compound on dopamine-containing neurons is at all related to the "sleep"-inducing

*R. H. Roth, unpublished data.

properties of this agent. Experiments with pentobarbital were included to see if the reported observations were unique to GHB and related analogues or a general property of all central nervous system depressants.

METHODS

Norepinephrine analysis. Tissue levels of norepinephrine were determined essentially by a modification of the fluorimetric method of von Euler and Lishajko.[8] Tissues were homogenized in either 15 per cent trichloroacetic acid or 0·4 M perchloric acid. After centrifugation, EDTA (10 mg/ml) and 0·2 ml of 1 M Tris buffer, pH 8·2, were added to the extract. The extracts were then adjusted to pH 8·4 and applied to 1·4 × 0·4 cm columns of aluminium oxide (British Drug House) twice, as described previously.[9] The columns were eluted with 2 ml of 0·2 M perchloric acid plus 2 ml of distilled water. One-half of the eluate was taken for oxidation. Here the exception was made in that 0·3 ml of 0·5 M phosphate buffer, pH 7·0, was used to bring the pH to 6·5 prior to oxidation with ferricyanide. All values are corrected for an average recovery of 80 per cent.

Dopamine assay. Dopamine was determined essentially by the method of Anton and Sayre[10] after initial isolation from tissue extracts by alumina-column chromatography described above. Recovery was determined by addition of tracer amounts of ^{14}C-dopamine to the brain extracts or by oxidation of internal standards carried through the isolation procedure. A standard curve of external standards was run for each individual assay. All values are corrected for an average recovery of 81 per cent (81 ± 4·8 per cent, No.=74) or for recovery based on internal standards analyzed on the day of a given experiment.

Homovanillic acid (HVA) determination. All rats in this group received probenecid (200 mg/kg) 2 hr prior to decapitation. Experimental rats were killed 1½ hr after administration of GBL (750 mg/kg) or pentobarbital (60 mg/kg). The brains were precipitated with 4 vol. of 0·4 M perchloric acid and the extracts centrifuged to remove the precipitate. The precipitates were washed with an additional 2 ml of 0·4 M perchloric acid, recentrifuged, and the supernatants pooled. The pooled supernatants were neutralized to pH 6·5 with KOH, chilled, centrifuged, and the supernatant obtained extracted with 4 vol. of chloroform. The organic phase was discarded and the aqueous phase applied to alumina columns as described above. The column effluent plus water wash (10 ml) were collected and acidified to pH 2·0 with concentrated HCl. The aqueous phase was then saturated with NaCl and extracted with 3 vol. of ethyl acetate (Mallinkrodt, nanograde). The ethyl acetate phase was retained, 4 ml of 0·05 M Tris buffer, pH 8·5, added and the mixture shaken for 20 min. After centrifugation the aqueous phase was removed and assayed for HVA according to the procedure of Anden *et al.*[11] In most cases tracer amounts of ^{14}C-labeled HVA (see below) were added to the brain extracts and carried through the entire extraction procedure in order to determine the recovery. In instances where this step was omitted results were corrected for an average recovery of 68 per cent (68 ± 4 per cent, No.=8).

In all fluorimetric assay procedures, individual blanks, either reverse (oxidant after antioxidant) or nonoxidized blanks, were run for each sample ro rule out any possible interference in the assay by the drugs employed in these experiments. In addition, the drugs employed in these experiments when added in milligram amounts to internal

standards and carried through the above isolation procedures did not significantly alter the NE, DA, 5HT or HVA assays.

Preparation of labeled homovanillic acid (HVA). Rat liver was homogenized in isotonic KCl at 4° and a crude preparation of catechol-O-methyl transferase (COMT) was prepared essentially as described by Axelrod and Tomchick.[12] Labeled HVA was then prepared enzymatically by incubating COMT, with S-adenosyl-methionine-^{14}C and 3,4-dihydroxyphenyl acetic acid (DOPAC)[13] as follows: 2 ml of the COMT preparation was added to 0·5 M phosphate buffer, pH 7·8, 0·1 ml of 0·5 M $MgCl_2$, 1 mg of 3,4-dihydroxyphenyl acetic acid, and 50 μc of ^{14}C-S-adenosyl-methionine (sp. act., 42 mc/mole, New England Nuclear Corp.) in a final volume of 4·0 ml. The mixture was incubated at 37° for 1 hr. After this incubation, another 0·5 mg of DOPAC was added together with 2 additional ml of COMT and the incubation continued for another hour. The homogenate was then acidified with 1 ml of 3 N HCl, saturated with NaCl, and extracted with ethyl acetate. The ethyl acetate extract was flash evaporated to dryness and the residue taken up in about 0·5 ml of ethyl acetate and streaked on Whatman No. 3 MM. On one corner of the paper, a portion of the unlabeled HVA (10 μg) was also spotted. The chromatogram was developed overnight by ascending chromatography in isopropanol–ammonia–water (8:1:1). After drying, the strip containing the unlabeled HVA was cut off and sprayed with diazotized *p*-nitroaniline to visualize the HVA and calculate the R_f. The HVA band on the unsprayed chromatogram (labeled HVA) was then cut out and the labeled HVA eluted with

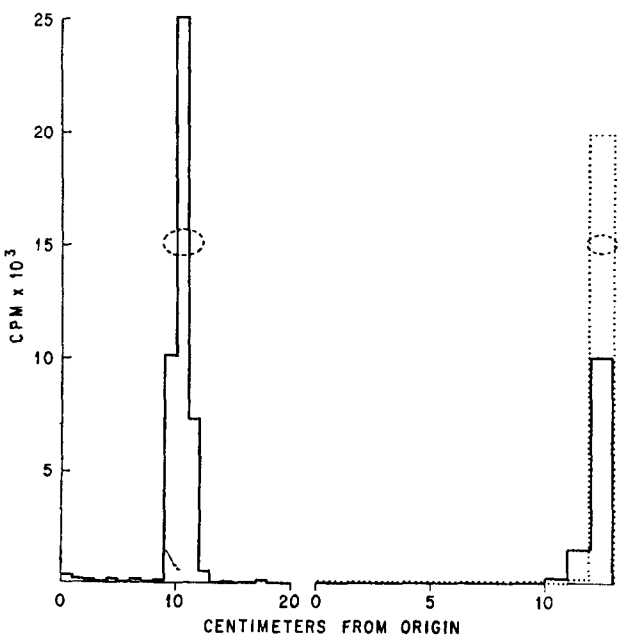

FIG. 1. Paper and thin-layer chromatography of enzymatically prepared ^{14}C-homovanillic acid. The left side of this Figure illustrates an ascending paper chromatographic analysis of ^{14}C-homovanillic acid in a solvent system of isopropanol, ammonia and water (8:1:1). The right side of this figure illustrates duplicate thin-layer chromatograms (solid and broken lines) on cellulose 300 mn of ^{14}C-homovanillic acid run in a solvent of butanol saturated with 3 M HCl. Spots indicate the movement of unlabeled HVA.

distilled water. The labeled HVA prepared in this manner was found to be chromatographically homogenous by both paper and thin-layer chromatography (see Fig. 1). The labeled HVA was stored frozen at about pH 4 in the aqueous solution.

5-Hydroxytryptamine assay. Brains were homogenized in 0·1 M HCl and assayed for 5-hydroxytryptamine essentially by the method of Bogdanski *et al.*,[14] with the exception that a saturated solution of Na_2CO_3 was used instead of crystals to neutralize the brain extract.

In vivo *synthesis of norepinephrine and dopamine from* ^{14}*C-tyrosine.* Male Sprague–Dawley rats (about 100 g) obtained from Charles River were injected with GBL (750 mg/kg/i.p.). Ten min later these same animals were injected intravenously with 25 μc of ^{14}C-tyrosine (sp. act., 406 mc/m-mole, New England Nuclear Corp., purified on alumina just prior to injection). Control rats received only the intravenous ^{14}C-tyrosine injection. One hr after the injection of tyrosine the rats were killed by decapitation and the brains rapidly removed and frozen on dry ice. The brains were weighed and then homogenized in 5 ml of 0·4 M perchloric acid and treated as described in the section on analysis of norepinephrine. The alumina columns in this case were eluted with 2 ml of 0·15 M perchloric acid followed by 2·5 ml of water. Four-tenths of a ml of the eluate was taken for determination of total radioactivity, 0·9 ml for analysis of dopamine, 2·0 ml for chromatographic analysis according to the procedure of Stjärne and Lishajko[15] (after addition of carrier dopamine, norepinephrine and DOPA, 50 μg each) and the remainder was retained for the fluorimetric analysis of norepinephrine.

Tyrosine analysis. Tyrosine was analyzed by a modification of the method of Weiner and Rabadjija.[16]

The alumina column effluents from brain, heart and blood were acidified to pH 2 with HCl and applied to 40 × 4 mm columns of Dowex 50 × 8. The columns were washed with 10 ml of 0·1 per cent EDTA, followed with 20 ml of distilled water. Tyrosine was then eluted with 5 ml of 1·0 M NH_4OH. A portion was taken for determination of radioactivity and the remainder was saved for the fluorimetric determination of tyrosine.

Drug administration. Drugs were given by intraperitoneal injection. In most cases GBL instead of the hydrolytic product GHB was given by this route since the lactone is more rapidly and uniformly taken up into the blood stream. Once in the blood stream, GBL is rapidly hydrolyzed to its active form GHB.[17,18] Dosage of GBL and GHB ranged from 350 mg/kg up to a total cumulative dose of 1500 mg/kg. Lower doses of 350 mg/kg of GBL (or equivalent doses of 500 mg/kg of GHB) were used in the behavioural studies since this dosage caused the animals to sleep for only 1 to 1½ hr and thus made it easier to follow the duration of "sleep". Larger and divided dosage schedules were employed only in cases where it was necessary to keep the animals asleep for longer periods of time. For the majority of the biochemical studies, a dosage of 750 mg/kg of GBL was chosen since this dose produced a maximal increase in brain dopamine and also maintained "sleep" for periods in excess of 3 hr. α-Methyl-*p*-tyrosine methyl ester was dissolved in water and injected in a dose of 250 mg/kg. α-Methyl-*p*-tyrosine (200 mg/kg) was dissolved in dilute base and then neutralized to pH 9·5 prior to injection. Probenecid (injectable) was given intraperitoneally in a dose of 200 mg/kg and the animals killed 2 hr after injection.

Behavioral studies. Gross behavioral effects were observed and the "anesthetic"

effects were recorded as "sleeping time". The "sleeping time" was taken as the interval from the loss of the righting reflex until the righting reflex was regained.

Statistical analyses were performed according to the procedure outlined by Snedecor.[19]

RESULTS

Biochemical studies. In our initial experiments we confirmed the recent reports by Gessa *et al.*[2,7] that GHB and GBL given in anesthetic doses to rats cause a marked increase in the brain levels of dopamine (Table 1). In addition, the effect of GBL and GHB on dopamine appeared to be rather selective in that only a small increase was observed in whole brain 5-hydroxytryptamine and no significant increase was noted in brain norepinephrine.

TABLE 1. EFFECT OF PENTOBARBITAL, γ-HYDROXYBUTYRATE AND γ-BUTYROLACTONE ON THE MONOAMINE CONTENT OF RAT BRAIN*

Treatment	No.	DA	No.	NE	No.	5 HT
None	18	628 ± 33	11	396 ± 16	5	518 ± 12
Pentobarbital (60 mg/kg)	14	867 ± 65‡	9	418 ± 15§	6	631 ± 20‡
None	4	674 ± 54	4	375 ± 18		
GHB (1000 mg/kg)	4	1046 ± 11‡	4	400 ± 20§		
None	5	664 ± 30	4	323 ± 15		
GBL (1000 mg/kg)	5	1354 ± 97‡	4	329 ± 22§		
None	12	615 ± 39	6	287 ± 18	12	491 ± 15
GHB‖ (500 mg/kg) 3 times	7	1213 ± 96¶	6	280 ± 12§	11	588 ± 14¶

*All values are expressed in ng/g ± S.E.M.

†Unless indicated otherwise, rats sacrificed 1½ hr after drug treatment and analysis conducted on whole brains.

‡Comparison of drug treated with control tissue: $P < 0.01$.

§Comparison of drug treated with control tissue: Not significant at 0·1 level.

‖Rats injected every hour and sacrificed 3 hr after first injection.

¶Comparison of drug treated with control tissue: $P < 0.001$.

In some experiments the effect of GBL on the conversion of ^{14}C-tyrosine to dopamine and norepinephrine *in vivo* was determined (see Methods). In these instances, GBL (750 mg/kg) in addition to increasing the brain levels of dopamine about 94 per cent also caused a 90 per cent increase in the specific activity of the dopamine isolated from the brain while only producing a slight increase in the specific activity of brain norepinephrine (Table 2). The final specific activity of brain tyrosine was also increased. However, this increase was not significant ($P < 0.1$).

Further analysis of regional changes in brain monoamines revealed that in subcortical regions (i.e. basal ganglia, diencephalon and midbrain), GBL had a completely selective effect in increasing brain dopamine since there was little or no effect on

TABLE 2. CATECHOLAMINE SYNTHESIS IN RAT BRAIN FROM ^{14}C-TYROSINE*

		Brain NE		Brain DA		Brain tyrosine
	No.	(ng/g)	Sp. act. (dis./min/μg)	(ng/g)	Sp. act. (dis./min/μg)	Sp. act. (dis./min/μg)
Control	5	222 ± 11	695 ± 79	458 ± 27	619 ± 98	1496 ± 80
GBL	5	219 ± 15†	768 ± 114†	890 ± 79‡	1183 ± 130‡	2083 ± 349†

*GBL given 10 min prior to intravenous administration of ^{14}C-tyrosine. Rat sacrificed 1 hr after ^{14}C-tyrosine administration. All values are expressed as the mean ± S.E.M.
†Comparison of drug treated with control tissue: Not significant at 0·1 level.
‡Comparison of drug treated with control tissue: $P < 0·01$.

subcortical norepinephrine or 5-hydroxytryptamine (Table 3). In addition, concurrent with the increase in subcortical dopamine, there was a marked reduction in subcortical homovanillic acid (Table 3). No significant increase in 5-hydroxytryptamine was observed in this brain region.

TABLE 3. EFFECT OF γ-BUTYROLACTONE AND PENTOBARBITAL ON THE LEVEL OF DOPAMINE 5-HYDROXYTRYPTAMINE AND HOMOVANILLIC ACID IN RAT BRAIN*

	No.	Dopamine	Homovanillic acid	No.†	5-Hydroxytryptamine
Control	12	1·71 ± 0·05	0·370 ± 0·025	5	0·88 ± 0·07
γ-Butyrolactone (750 mg/kg)	6	3·57 ± 0·26‡	0·199 ± 0·011‡	5	0·98 ± 0·07§
Pentobarbital (60 mg/kg)	6	1·71 ± 0·14§	0·432 ± 0·063§		

*Data expressed in terms of μg/g ± S.E.M. Brain region consisted of midbrain, diencephalon and basal ganglion.
†Number of experiments. For HVA and dopamine analysis three rat brain subcortical regions were pooled for each experiment.
‡Comparison of drug treated with control tissue: $P < 0·001$.
§Comparison of drug treated with control tissue: not significant at 0·01 level.

These results suggested that GBL might be increasing brain dopamine either by increasing dopamine biosynthesis, by interfering with dopamine catabolism, by antagonizing dopamine release, or by a combination of the above mechanisms. Previous studies by Gessa *et al.*[7] indicate that GHB and related analogues do not inhibit the enzymes involved in the degradation of dopamine (catechol-*O*-methyltransferase and monoamine oxidase). Thus, an inhibition of dopamine catabolism seemed unlikely. One way to rule out a selective effect on biosynthesis is to inhibit this process and then see if the agent in question has an effect on the disappearance or turnover of catecholamines.

Experiments in which α-methyl-*p*-tyrosine methyl ester (250 mg/kg) was used to block catecholamine biosynthesis showed that GBL antagonized the α-methyl-*p*-tyrosine methyl ester-induced disappearance of dopamine in whole brain while it had little or no effect on norepinephrine depletion (Fig. 2). Similar experiments with pentobarbital indicated that this central nervous system depressant antagonized to a

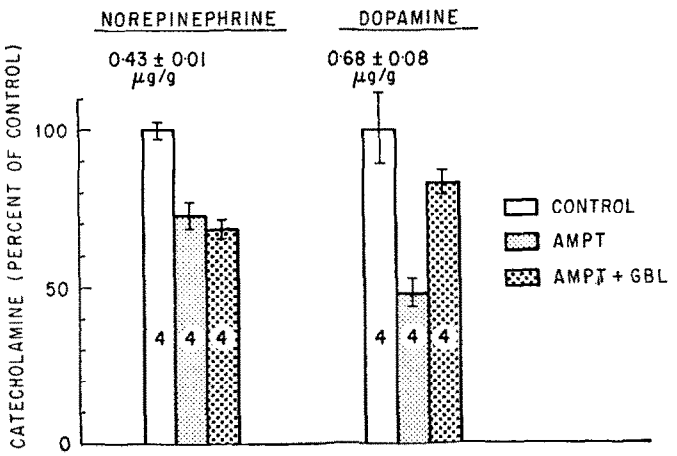

FIG. 2. The effect of γ-butyrolactone on the utilization of whole brain catecholamines. α-Methyl-*p*-tyrosine methyl ester (AMPT, 250 mg/kg) given 10 min after GBL (750 mg/kg). Rats sacrificed 2 hr after administration of AMPT. Norepinephrine and dopamine analyses conducted on whole brain. Number of individual experiments indicated by numbers in center of bar graph. Vertical bars depict standard error of the mean.

limited extent both the disappearance of brain dopamine and brain norepinephrine. However, the effect of pentobarbital on brain dopamine was not nearly as potent as was GBL (Fig. 3).

Similar studies employing α-methyl-*p*-tyrosine methyl ester to inhibit catecholamine biosynthesis but in this case analyzing only effects in subcortical brain regions indicated that GBL completely antagonized the disappearance of subcortical dopamine but exerted no antagonism on the disappearance of subcortical norepinephrine

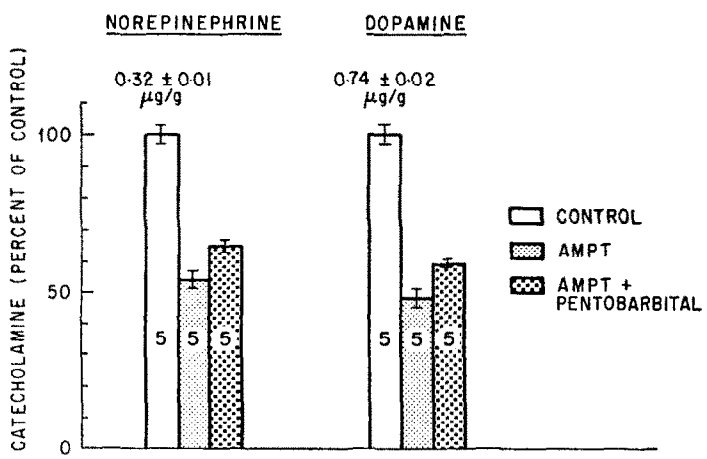

FIG. 3. The effect of pentobarbital on the utilization of whole brain catecholamines. α-Methyl-*p*-tyrosine methyl ester (AMPT, 250 mg/kg) given 10 min after pentobarbital (60 mg/kg). Rats sacrificed 2 hr after administration of AMPT. Norepinephrine and dopamine analyses conducted on whole brain. Number of individual experiments indicated by numbers in center of bar graph. Vertical bars depict standard error of the mean.

(Figs. 4 and 5). Pentobarbital did not selectively antagonize the α-methyltyrosine-induced disappearance of subcortical dopamine (Fig. 5).

Behavioral studies. Depletion of brain 5-hydroxytryptamine with *p*-chlorophenylalanine (PCPA) (320 mg/kg) did not produce any significant changes in the duration

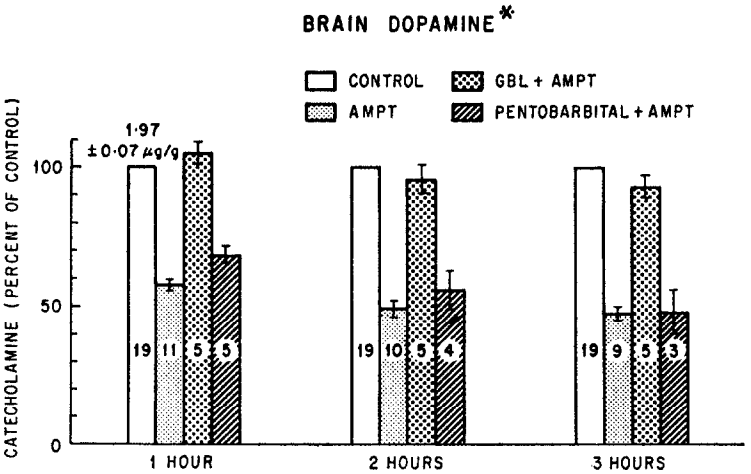

FIG. 4. The effect of γ-butyrolactone and pentobarbital on the utilization of subcortical dopamine. *Dopamine analysis conducted on subcortical regions (basal ganglia, diencephalon and midbrain) of three pooled rat brains. α-Methyl-*p*-tyrosine methyl ester (AMPT, 250 mg/kg) given 10 min after GBL (750 mg/kg) or pentobarbital (60 m/kg). Rats sacrificed 1, 2 or 3 hr after administration of AMPT. Number of individual experiments indicated by numbers in the center of the bar graphs. Vertical bars depict standard error of the mean.

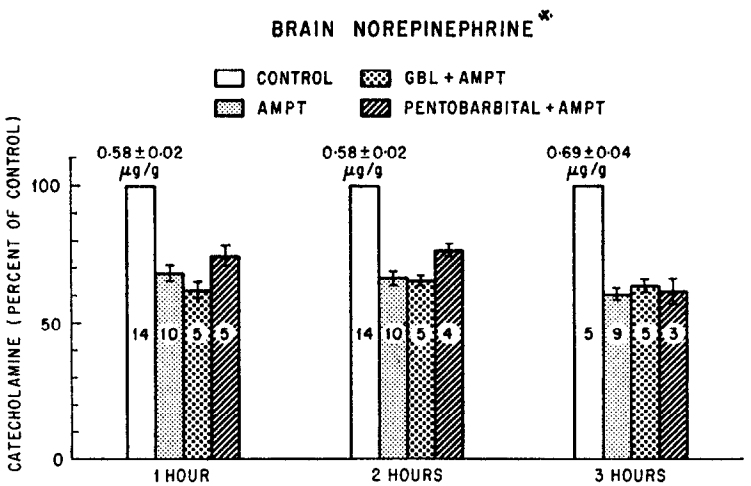

FIG. 5. The effect of γ-butyrolactone and pentobarbital on the utilization of subcortical norepinephrine. *Norepinephrine analyses conducted on subcortical regions (basal ganglia, diencephalon and midbrain) of 3 pooled rat brains. α-Methyl-*p*-tyrosine methyl ester (AMPT, 250 mg/kg) given 10 min after GBL (750 mg/kg) or pentobarbital (60 mg/kg). Rats sacrificed 1, 2 or 3 hr after administration of AMPT. Number of individual experiments indicated by numbers in the center of the bar graphs. Vertical bars depict standard error of the mean.

of GBL- or GHB-induced sleep. On the other hand, depletion of brain catecholamines with α-methyl-*p*-tyrosine caused about a 100 per cent increase in the sleep time induced either by GBL- or GHB (Table 4). This potentiation of sleep time by α-methyl-*p*-tyrosine appeared to be somewhat specific for GBL- or GHB-induced sleep since the sleep time of pentobarbital-treated rats was not significantly increased by this agent (Table 4).

TABLE 4. EFFECT OF MONOAMINE DEPLETION ON SLEEP INDUCED BY γ-HYDROXYBUTYRIC ACID, γ-BUTYROLACTONE AND PENTOBARBITAL

	No.*	Sleep time† (min)	per cent increase	P values
GBL (350 mg/kg)	9	81 ± 7		
AMPT + GBL‡	5	161 ± 13	99	<0·02
PCPA + GBL§	4	94 ± 6	16	NS‖
GHB (500 mg/kg)	10	58 ± 4		
AMPT + GHB	6	120 ± 11	107	<0·01
PCPA + GHB	6	75 ± 6	29	NS‖
Pentobarbital (30 mg/kg)	8	65 ± 5		
AMPT + Pentobarbital	8	74 ± 2	14	NS‖

*Number of experiments.
†Values expressed as mean ± S.E.M.
‡α-Methyl-*p*-tyrosine (AMPT, 200 mg/kg) given 4½ hr prior to drugs.
§*p*-Chlorophenylalanine (PCPA 320 mg/kg) given 24 hr prior to drugs.
‖Not significant at the 0·05 level.

Pretreatment with 100 mg/kg of dihydroxyphenylalanine (DOPA), the immediate precursor of dopamine, did not antagonize or potentiate GBL-induced sleep. Treatment with D-amphetamine (10 mg/kg), a drug known to release central dopamine,[20,21] did however partly antagonize GBL-induced sleep (Table 5).

TABLE 5. EFFECT OF DIHYDROXYPHENYLALANINE AND AMPHETAMINE ON γ-BUTYROLACTONE-INDUCED SLEEP

Treatment	No.*	Sleep time† (min)	Per cent change
GBL (350 mg/kg)	10	88·2 ± 2·6	
GBL (350 mg/kg) + DOPA (100 mg/kg)	5	89·4 ± 7·5	+ 1·3
GBL (350 mg/kg) + amphetamine (10 mg/kg)	5	63·2 ± 4·1	− 29·4‡

*Number of experiments.
†Values expressed as the mean ± S.E.M.
‡Significance < 0·01.

DISCUSSION

Our initial studies and those of Gessa *et al.*[2] indicate that both GBL and GHB produce a marked and rather specific increase in brain dopamine. Little change is observed in the levels of other monoamines or GABA.

A number of theoretical mechanisms to explain this GHB-induced increase in brain dopamine are apparent: 1. GHB could interfere with the catabolism of brain dopamine. 2. GHB could cause an increase in the rate of dopamine biosynthesis. 3. GHB could decrease utilization of brain dopamine by (a) interference with release and (b) increasing the binding capabilities of the neuron.

The first possibility seems unlikely since (a) norepinephrine and 5-hydroxytryptamine are both metabolized by enzymes which also degrade dopamine and yet no marked changes are observed in the level of these monoamines; (b) examination *in vitro* of MAO or COMT after pretreatment of rats with GHB or after addition of GHB directly to brain homogenates indicated that this drug had no inhibitory effect on the activity of these enzymes.[7]

The second possibility, that of the drug producing an increase in the biosynthesis of dopamine, was examined by infusing control and GBL-treated rats with ^{14}C-tyrosine and then isolating the labeled brain catecholamines and determining their specific activity. In this case GBL produced no significant increase in the specific activity of brain norepinephrine while it caused about a 90 per cent increase in the specific activity of brain dopamine. Although these experiments are suggestive that GHB might be acting to increase the biosynthesis of brain dopamine, they are not necessarily definitive and other explanations for this drug-induced increase in dopamine specific activity are plausible. For example, it could not be ruled out that the drug might alter the specific activity of tyrosine at the site of dopamine biosynthesis. However, the observation that the specific activity of norepinephrine is not significantly increased by treatment with GBL would argue against this possibility. It is also feasible that GHB might interfere with the utilization or release of newly synthesized dopamine, thus causing a marked increase in the specific activity of the whole brain pool of dopamine. The possibility that GHB might be interfering with the utilization of brain dopamine is also suggested by our observation that GBL produces a significant decrease in the amount of HVA found in subcortical areas of the brain. The action of GBL in antagonizing dopamine utilization was examined more directly by inhibiting synthesis of brain catecholamines with α-methyl-*p*-tyrosine and then following the disappearance of brain catecholamines both in the presence and absence of GBL. Experiments of a similar nature were also conducted for pentobarbital. In whole brain, GBL interfered with the utilization of brain dopamine but not with the utilization of brain norepinephrine. On the other hand, pentobarbital antagonized to some extent the disappearance of both whole brain norepinephrine and dopamine; however, its effect on brain dopamine was much less pronounced than that of GBL and it was not selective in action. The specificity of this action of GBL on the utilization of dopamine was even more apparent if certain brain regions were analyzed. Thus, GBL had no effect on the utilization of subcortical norepinephrine but completely blocked the utilization of subcortical dopamine for about 3 hr (Fig. 5). In this brain region, pentobarbital had no significant effect on the disappearance of either dopamine or norepinephrine (Figs. 4 and 5).

All the above experiments are consistent with the hypothesis that GHB increases brain dopamine levels by antagonizing its utilization. This would be the resultant effect if GHB specifically blocked the release of dopamine. A blockade of dopamine release would also be consistent with our finding that GBL causes a significant reduction in the subcortical levels of HVA, since under this condition dopamine would no longer be

exposed to metabolic enzymes. This hypothesis also fits best with recent fluorescence histochemical observations which appear to localize this GBL-induced increase in dopamine to the terminals of the dopamine-containing neurons.[5] At this time the possibility cannot be ruled out that an inhibition of dopamine release might lead to a compensatory activation of dopaminergic neurons, resulting also in a temporary increase in the rate of dopamine biosynthesis. In fact, this is the most likely explanation for the very rapid increase in brain dopamine. Previous experiments by Gessa *et al.*[7] have demonstrated that dopamine accumulates selectively not only in the brain of normal animals but also in animals treated with reserpine. This effect is most prominent after administration of DOPA. Since reserpine is believed to impair binding capacity for monoamines, causing their subsequent release from the bound form, all brain monoamines should be directly exposed to degradative enzymes, especially MAO, and thus should not accumulate unless these metabolic enzymes are inhibited. The observation of the specific reversal of reserpine-induced depletion of dopamine by GHB has been difficult to reconcile with the fact that GHB does not interfere with either COMT or MAO, and yet promotes the selective accumulation of dopamine but not that of norepinephrine after reserpine. The findings of our present experiments suggest the possibility that GHB may exert this action by specifically antagonizing the reserpine-induced release of dopamine via the same mechanism by which it appears to block the normal release of dopamine. Thus, in effect, GHB could prevent the metabolism of dopamine by keeping it in a sequestered or bound form and inaccessible to the metabolic enzymes.

However, even if GHB does exert its effect on dopamine neurons strictly by blocking the release of dopamine from the nerve terminals, it still remains to be determined if this action is linked in some way to the central nervous system depressant properties of this drug. Some indirect evidence has already been presented to indicate that the central nervous system depressant properties of GHB and dopamine accumulation might be causally related.[7]

(a) There is a good temporal correlation between the sedative action and the accumulation of brain dopamine after GHB administration especially in rabbits.[2]
(b) The striatum, an area high in dopamine, is most sensitive to the actions of GHB when the drug is given intracerebrally to unanesthetized cats.[22]
(c) Only butyric acid congeners endowed with anesthetic activity selectively increase brain dopamine.[3]* However, it should be pointed out that all the active butyric acid congeners investigated so far are converted to GHB *in vivo* which is presumably the active metabolite responsible for both the central nervous system depression and the alterations in brain dopamine.[17,18,23,24]

If GHB-induced sleep and dopamine accumulation are causally related it would be expected that agents which either further antagonize or facilitate dopamine release would also respectively either potentiate or reverse the central nervous system depression exerted by GHB. Experiment with α-methyl-*p*-tyrosine (an agent which interferes with catecholamine biosynthesis and thus leaves little catecholamine left to participate in release processes) reveals that this drug produces about 100 per cent

*R. H. Roth, unpublished data.

increase in the "sleep time" of rats treated with either GBL or GHB (Table 4). α-Methyl-*p*-tyrosine did not, however, potentiate the sleep time of pentobarbital, indicating that this action of α-methyl-*p*-tyrosine is not because of a nonspecific potentiation of the action of all central nervous system depressants. Amphetamine, on the other hand, a drug known to cause release of central catecholamines,[20,21] produces a significant reduction in the GBL-induced sleep.

These experiments provide some indirect support for the contention that the "sleep"-inducing properties of GHB might be related to its action in blocking dopamine release. Experiments are in progress to further clarify this possibility.

Acknowledgements—This work was supported in part by USPHS Grant No. MH-14092. We are grateful to Mr. Karlis Rozitis for his competent technical assistance.

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