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EFFECT OF GAMMA-HYDROXYBUTYRATE ON DOPAMINE AND DOPAMINE METABOLITES IN THE RAT STRIATUM*

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Abstract—Gamma-butyrolactone (GBL), a precursor for the naturally occurring central nervous system depressant, gamma-hydroxybutyrate (GHB), administered in anesthetic doses, produces an increase in rat corpus striatum dopamine levels without affecting norepinephrine or serotonin levels. The rise and fall of the dopamine levels coincide with the changes of brain GHB levels and the behavioral effects of the drug. The specific activity of striatal dopamine was found to be greater in rats injected with ^{3}H-tyrosine shortly before or shortly after GBL, as compared with controls, which were not treated with GBL. The specific activity of cortical norepinephrine in GBL-treated rats was not significantly different from that observed in untreated controls. No significant difference was observed in blood or striatal tyrosine specific activity of GBL-treated rats. Levels of dopamine metabolites, dihydroxyphenylacetic acid and homovanillic acid, also increased in the corpus stratium after GBL, but the increase did not occur until after brain levels of GHB began to fall. These results suggest that the drug either increases dopamine synthesis and or blocks the release of dopamine from a rapidly turning over functional compartment within the neurons, or both. Perhaps as a result of the ability of GHB to block the release of dopamine, this drug also interferes with the metabolism of dopamine for a certain period of time after administration.

GAMMA-HYDROXYBUTYRATE (GHB) is an interesting drug for several reasons. It has a very simple structure and has been shown to be a naturally occurring compound in the brains of several mammals.[1] It has also been found to produce behavioral depression or an anesthesia-like state in many species including man. Gamma-butyrolactone (GBL) and 1,4-butanediol have been shown to produce effects identical to those of GHB.[2] They are both metabolized to GHB *in vivo*, and GHB has been shown to be the active form in both cases.[3–5]

The depressive effect of GHB has led to its use as an anesthetic or anesthetic adjuvant in several countries including the United States. However, GHB has neurophysiological and biochemical effects which seem to distinguish it from other anesthetics. EEG recordings have been characterized by some central excitation properties[6,7] and the drug has been shown to have a specific effect on brain dopamine levels. It is with this last neurochemical effect that this paper is concerned.

Gessa *et al.*[8] showed that GHB can cause a marked increase in brain dopamine levels without producing a very significant effect on brain norepinephrine or serotonin

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levels. They also found that GHB does not produce this effect by inhibiting the dopamine-metabolizing enzymes, monoamine oxidase or catechol-*O*-methyl transferase.[9] By means of fluorescence microscopy, Aghajanian and Roth[10] further localized this increase in dopamine to sites presumed to represent the nerve endings or terminals of the dopamine-containing neurons. No increase in fluorescence was observed in fiber tracts or cell bodies.

Recently, Roth and Surh[11] demonstrated that when the biosynthesis of catecholamines was blocked by α-methyl-*para*-tyrosine, GHB prevented the normal disappearance of dopamine but not the disappearance of norepinephrine. It has also been demonstrated that GHB in anesthetic doses blocks the initial increase in homovanillic acid caused by chlorpromazine.[12] Taken together, these observations suggest that GHB may be inhibiting neuronal release or utilization of dopamine. An action of this sort might in part, be responsible for the observed increase in dopamine concentration found in the nerve terminals after administration of GHB. However, an acceleration of dopamine synthesis might also be occurring. The present experiments were designed to investigate the mechanism responsible for the large increase in brain dopamine observed after administration of anesthetic doses of GBL. The drug was administered as GBL, since this lactone form is quickly converted to GHB in the plasma and the liver, and is more rapidly and uniformly taken up and distributed after intraperitoneal injection than is GHB.[13]

METHODS

Time course study. Male Sprague–Dawley rats (150–250 g) obtained from Charles River, Inc., were injected with GBL (750 mg/kg) intraperitoneally (i.p.). In some cases a second dose of GBL (375 mg/kg) was given 45 min after the first. In one set of animals, chlorpromazine (10 mg/kg) was administered i.p. The rats were sacrificed after various time periods by decapitation. The brains were rapidly removed and dissected over ice. In the initial experiments, an area referred to as the subcortex was taken. This consisted of the midbrain, the diencephalon and the basal ganglia regions. The average weight of the subcortex section was 500 mg. In later experiments, only the corpus striatal area was used, average weight being 107·3 $\pm$ 0·9 mg ($n = 75$). After the tissue was dissected, it was rapidly frozen on dry ice, weighed and stored at −70° until assayed.

Dopamine determination. Tissues were homogenized in 0·4 M perchloric acid. After centrifugation, EDTA (10 mg/ml) and 0·2 ml of 1 M Tris buffer, pH 8·4, were added to the extract. The extracts were then adjusted to pH 8·4 and passed twice over 1·4 × 0·4 cm columns of aluminium oxide (British Drug House) as described previously.[14] The columns were eluted with 2 ml of 0·2 M perchloric acid plus 2 ml of distilled water. A portion of the eluate was taken for assay essentially by the method of Laverty and Taylor,[15] with two exceptions. The phosphate buffer used was 0·15 M, pH 7·2, and 0·13 ml of glacial acetic acid was used to bring the final pH to 4·4 before reading the fluorescence. A standard curve of external standards was run with each assay. All values are corrected for an average recovery of 81 $\pm$ 4 per cent ($n = 74$) based on internal standards which were carried through the extraction procedure and assayed fluorimetrically for dopamine.

GHB determination. Rats were injected i.p. with 750 mg/kg of ^{14}C-GBL (specific activity, 0·8 mc/m-mole) prepared from 1-^{14}C-GHB (sp. act., 8·0 mc/m-mole, Schwarz

Bioresearch Inc.), which had been lactonized by heating at 85° for 10 min at pH 1·0. The rats were sacrificed as described above. The subcortical sections were homogenized in 4 ml of 15% trichloroacetic acid and centrifuged. The supernatants were lactonized as above and then buffered with phosphate to a final concentration of 0·05 M, pH 7·0. The pH was brought to 6·0 with NaOH, 2 vol. benzene was added, and the mixtures were again shaken and centrifuged. The benzene layers were pooled and a sample was taken for determination of radioactivity in a scintillation fluid containing 240 g naphthalene, 15 g 2,5-diphenyloxazole, 0·3 g dimethyl-1,4-bis-2-(5-phenyloxazolyl) benzene and 1 l. each of toluene, dioxane, and absolute ethanol (DTE scintillation fluid). Reagent blanks were carried through the extraction with known amounts of ^{14}C-GHB for determinations of recovery, which was 52 per cent. The values are corrected for recovery and for counting efficiency.

Dihydroxyphenylacetic acid (DOPAC) determination. A modification of the method of Murphy *et al.*[16] was used for determination of DOPAC. Corpora striata (obtained as described above) from two rats were pooled for each determination. Cerebellar tissue of the same weight as the pooled corpora striata was used for recovery and tissue blank determinations. The samples were homogenized in 3 ml of cold 0·1 N HCl, and 0·12 ml of concentrated perchloric acid and 1·4 g KCl were added to each sample. After centrifugation, the supernatants were poured into tubes containing 7 ml of cold butyl acetate (Baker's superior grade, redistilled) and approximately 0·4 g KCl. The tubes were capped, shaken in a cold room for 10 min, and centrifuged for 2 min. Then 6·5 ml of the butyl acetate layer was added to 2·4 ml of cold ethylene diamine solution (35 ml water, 1 ml of 2 N HCl and 1·5 ml ethylene diamine; Mallinkrodt, twice redistilled), shaken 6 min in a cold room, centrifuged, and the butyl acetate layer aspired off. DOPAC was then assayed fluorimetrically.[16] Cerebellar blanks were approximately the same as reagent blanks carried through the extraction procedure. The latter however, were significantly higher than reagent blanks carried through the assay procedure only, perhaps due to some quenching material picked up during the extraction procedure. For these reasons, reagent blanks carried through the extraction procedure were used for standards and standard blanks. Recovery of varying amounts of DOPAC added to tissue blanks was 68 $\pm$ 1·7 per cent (S.E.M.) for 23 tests. Values were corrected for recovery.

Homovanillic acid (HVA) determination. HVA was determined on two pooled corpora striata and the extraction procedure was the same as that for the DOPAC, except that the shaking times for the extraction were doubled and it was not found necessary to carry out the shaking in a cold room. In the final step of the extraction, HVA was extracted from the butyl acetate into 2 ml of Tris buffer, pH 8·5, 0·05 M. HVA was assayed by a modification of the method of Andén *et al.*[17] Two 0·6-ml portions of the buffer phase were mixed with 1·0 ml of 5 N ammonium hydroxide. To one of each pair, 0·20 ml of a 0·1 mg/ml potassium ferricyanide solution was added and mixed. Four min later, 0·20 ml of a 1 mg/ml cysteine solution was added and mixed. The second of the pair served as a blank and to it the cysteine solution was added before the potassium ferricyanide. Fluorescence was determined with an activating wavelength of 315 nm and fluorescence of 420 nm. Standards were run with each assay. Recovery was determined by adding tracer amounts of ^{14}C-labeled HVA[11] to the tissue homogenate and measuring radioactivity in a sample of the final extract. Each sample was corrected for its own recovery, which averaged 54·8 $\pm$ 0·4 per cent ($n = 42$).

Synthesis in vivo *of dopamine and norepinephrine.* Sprague–Dawley male rats weighing 165–175 g were injected intravenously with 100 μc of ^{14}C-tyrosine (sp. act., 42·9 c/m-mole, New England Nuclear Corp., purified on alumina just prior to injection). One group of rats received GBL (750 mg/kg, i.p.) 10 min before receiving the tyrosine injection, and another group was injected with GBL 2 min after the tyrosine injection. Control animals received only the tyrosine injection. All rats were sacrificed either 10 or 20 min after the tyrosine injections. The corpora striata were dissected as described above and a sample of blood was collected into 200 units of heparin. Sections of neocortex were also removed and treated in the same manner as the corpora striata.

The specific activity of dopamine in the corpora striata was determined by preparing the tissues for dopamine assay as described above, with two exceptions. The samples were homogenized in 3 ml of 15% trichloroacetic acid, and 1·0 ml of 0·5 M K_2HPO_4 was added to the samples before the pH was adjusted to 8·4. The first 20 ml of the alumina column effluent and wash was saved for tyrosine assay as described below. Two ml of the alumina column eluate was taken for dopamine assay and the remainder for determination of total radioactivity in a scintillation fluid containing 5·5 g 2,5-diphenyloxazole, 300 mg dimethyl-1,4-bis-2-(5-phenyloxazolyl) benzene, 2 l. of toluene and 1 l. of Triton X-100 (Rohm & Hass) which was previously purified by passage through silica gel. Internal standards were used to correct the radioactivity to disintegrations per minute.

The specific activity of norepinephrine in the neocortex was determined in the same way as that of dopamine with some modification due to the lower levels of the transmitter present in this tissue. The procedure for the column extraction was followed as with dopamine, except that a column of 1·0 × 0·4 cm was used and the sample was passed over it three times before the wash. Norepinephrine was eluted with 1 ml of 0·2 N perchloric acid and 1 ml water. Half of the eluate was taken for assay and the other half was taken for determination of total radioactivity in DTE scintillation fluid. Internal standards of ^{3}H-toluene were used for correction to disintegrations per minute.

Norepinephrine assay. Levels of norepinephrine were determined by a modification of the fluorimetric method of von Euler and Lishajko.[18] One deviation from their procedure was 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.

Tyrosine assay. Tyrosine was assayed by a modification of the method of Weiner and Rabadjija.[19] The alumina column effluents from the corpora striata were acidified to pH 1·5 with HCl and placed on a Dowex 50-X4, 200–400 mesh column (25 × 2·9 mm) prepared as described by Häggendal.[20] The column was eluted with 5 ml of 1·0 M ammonium hydroxide. The eluate was taken to dryness and dissolved in 1 ml water. A portion was taken for fluorimetric assay of tyrosine and the remainder was counted to determine radioactivity.

For the determination of tyrosine in blood, 2 ml blood was deproteinized with 6 ml of 1·0 N perchloric acid. After centrifugation, the supernatant was brought to pH 4·0 with KCl, recentrifuged, reacidified to pH 1·5 with HCl and purified on Dowex 50 in the manner described above. For the blood samples, radioactivity and tyrosine determinations were made directly upon the ammonium hydroxide eluate. It was found necessary to use reagant blanks carried through the column procedure for preparation of standards.

RESULTS

Time course. The time course of the levels of GHB and dopamine in the subcortex of the rat after an anesthetic dose of GBL is shown in Fig. 1. The levels of GHB rose rapidly in the subcortex, peaked at about 60 min, and then fell off. The increase in dopamine followed the rise and fall of the brain levels of GHB, peaking at about 90 min. The anesthetic effects of the drug were evident shortly after the injection and within 4–6 min the animals became unresponsive and appeared "anesthetized". The period of "anesthesia" was timed from the point when the animals lost the righting reflex, and would remain on their backs when placed there, until they regained the righting reflex. This period lasted approximately 3 hr, and the rats began to right themselves at a point where GHB and dopamine levels were nearing control values. It has been shown that there is not a very significant effect on norepinephrine or serotonin over the time course of this drug's effects.[8,10]

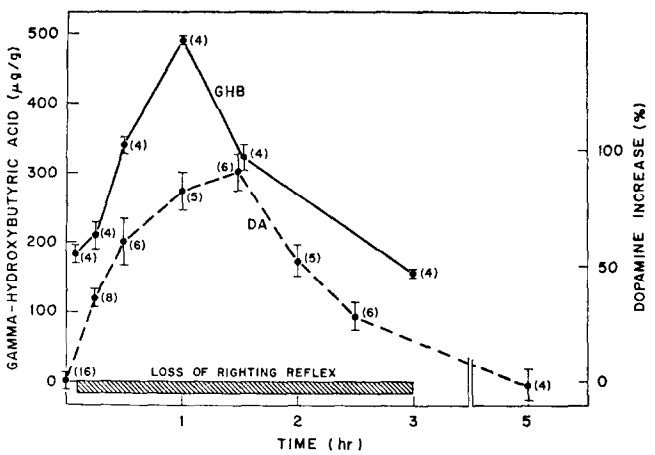

FIG. 1. ^{14}C-GHB concentration and per cent dopamine increase in subcortex after 750 mg/kg o GBL. Vertical bars represent the standard error of the mean. The number in parentheses represents the number of individual experiments. The endogenous level of dopamine in the subcortex of untreated rats is 2·52 ± 0·09 μg/g (n = 16).

Dopamine synthesis study. The initial rate of increase of dopamine after GHB was greater than one would expect from the synthesis rates of dopamine reported in the literature.[21] To investigate this, the degree of incorporation of ^{3}H-tyrosine into catecholamines was determined in the corpus striatum of control and drug-treated rats (Fig. 2). Previous experiments in our laboratory have indicated that only negligible amounts of labeled norepinephrine are formed from ^{14}C-tyrosine in the corpus striatum. Therefore, it was assumed that all the catecholamine radioactivity in this area represented dopamine. In rats treated with GBL 2 min after the infusion of ^{3}H-tyrosine, the specific activity of corpus striatum dopamine was greater than in the controls. It was increased still further in animals treated with GBL 10 min before the tyrosine infusion. As the dopamine levels are increasing over this time period, the amount of label incorporated into the dopamine increases even more dramatically in the drug-treated rats than does the specific activity. The specific activity of tyrosine

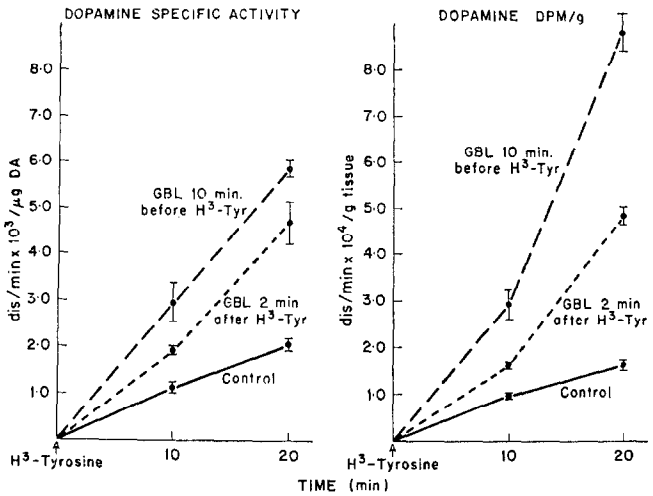

FIG. 2. Effect of GHB on the specific activity of dopamine (left panel) and the level of ^{3}H-dopamine (right panel) in the corpus striatum after rapid i.v. infusion of ^{3}H-tyrosine. Vertical bars represent the standard error of the mean. Each point is the mean of four or more determinations.

in blood and in the corpus striatum was determined, and no significant differences were seen between the drug-treated and control animals as shown in Table 1. Similar experiments attempting to determine effects of GBL on dopamine synthesis at later time points than those used here have been complicated by the fact that the distribution of the labeled tyrosine seems to be affected in the more deeply sedated animals. The increased specific activity of the tyrosine in both the brain and blood of the drug-treated animals at the longer time points makes the interpretation of the results more complicated. We have established, however, that this effect does not occur in the first 30 min after drug treatment.

TABLE 1. EFFECT OF GHB ON BLOOD AND STRIATAL TYROSINE SPECIFIC ACTIVITY

Treatment*	Blood tyrosine sp. act.† (dpm/ng × 10^4)	N‡	Corpus striatum tyrosine sp. act.† (dpm/ng)	N‡
^{3}H-tyrosine, 10 min before sacrifice				
Control§	2·28 ± 0·23	4	66·7 + 11·0	4
GBL, 2 min after ^{3}H-tyrosine	2·19 ± 0·16	4	68·1 + 5·8	4
GBL, 10 min before ^{3}H-tyrosine	2·62 ± 0·33	4	80·2 ± 5·0	4
^{3}H-tyrosine, 20 min before sacrifice				
Control§	1·14 ± 0·10	5	38·9 + 2·3	5
GBL, 2 min after ^{3}H-tyrosine	1·46 ± 0·10	3	44·4 + 1·8	3
GBL, 10 min before ^{3}H-tyrosine	1·37 ± 0·12	3	48·0 + 4·2	4

* ^{3}H-tyrosine (100 µc) was administered i.v. where indicated. GBL (750 mg/kg) was administered i.p.
† Results are expressed as mean ± standard error of the mean.
‡ N = number of experiments.
§ Drug-treated animals do not differ significantly from control animals ($P > 0·1$).

TABLE 2. EFFECT OF GHB ON THE SPECIFIC ACTIVITY OF CORTICAL NOREPINEPHRINE FORMED FROM ^{3}H-TYROSINE

Treatment*	Norepinephrine sp. act.† (dpm/ng)	N‡
^{3}H-tyrosine, 10 min before sacrifice		
Control	13·2 ± 0·9	4
GBL, 2 min after ^{3}H-tyrosine	16·0 ± 1·6	4
GBL, 10 min before ^{3}H-tyrosine	8·0 ± 0·6§	4
^{3}H-tyrosine, 20 min before sacrifice		
Control	9·1 ± 0·6	4
GBL, 2 min after ^{3}H-tyrosine	9·7 ± 2·0	3
GBL, 10 min before ^{3}H-tyrosine	7·8 ± 1·1	3

* ^{3}H-tyrosine (100 μc) was administered i.v. Where indicated, GBL (750 mg/kg) was administered i.p.
† Results are expressed as mean ± standard error of the mean.
‡ N = number of experiments.
§ Significantly different from control ($P < 0·05$). Other drug-treated animals do not differ significantly from control animals ($P > 0·1$).

The specific activity of norepinephrine in the cortex of these animals was also determined and did not show the increase seen with dopamine (Table 2). (Here again previous experiments had shown very little dopamine was formed under these conditions in the cortex and all catecholamine radioactivity was assumed to be norepinephrine.) The lower specific activity of norepinephrine 10 min after the tyrosine in the animals which had received GBL before tyrosine perhaps reflects the effect of the stress associated with the tail vein injection of labeled tyrosine on the synthesis of cortical norepinephrine. The animals already anesthetized with GBL would be spared this stress. The opposite effect on the specific activity of dopamine as compared with that on norepinephrine is further indication that the change in dopamine's specific activity is due to the actions of GBL, not to stress associated with the tyrosine injection procedure.

Time course of metabolites. To obtain further information on the effects of GBL on dopamine, the time course of two dopamine metabolites was followed after the administration of 750 mg/kg of GBL (Fig. 3). As noted above, the brain levels of both GHB and dopamine increase rapidly after an injection of this amount of GBL, with the drug levels peaking at about 60 min. It was observed that there was no corresponding increase in levels of DOPAC or HVA in the striatum until after this time period when drug levels were beginning to fall. DOPAC seemed to be a more rapid and sensitive indicator of changes in dopamine metabolism. There was a significant decrease in DOPAC levels at the 30-min time point, and DOPAC levels began to rise after 60 min, whereas HVA did not show a significant change until after 90 min. Both metabolites peaked at 3 hr when the drug and dopamine levels were close to control and the animals were regaining the righting reflex. DOPAC levels fell off fairly rapidly and were back to normal by 5 hr, but HVA levels were still elevated at the 5-hr point.

To investigate the relationship between the lag phase before the increase in dopamine metabolite levels and the rising phase of the drug curve, a second dose of the drug was

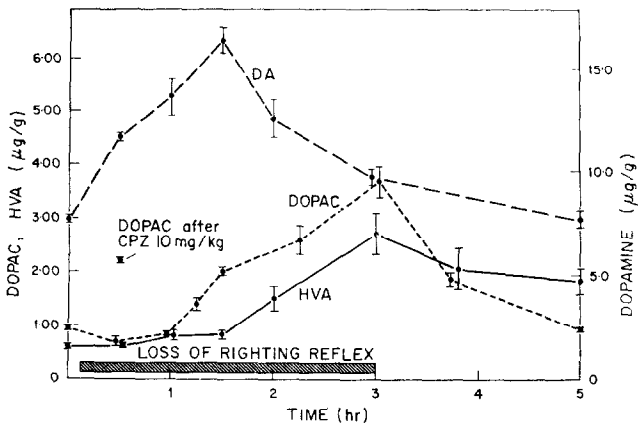

FIG. 3. Dopamine, HVA and DOPAC concentrations in rat corpus striatum after 750 mg/kg of GBL. Vertical bars represent the standard error of the mean. Each point is the mean of four or more determinations. Zero time values are control values from rats which did not receive GBL.

given 45 min after the first to prolong the rising phase of the drug curve (see Fig. 1). The results are shown in Fig. 4. In 45 min after this second dose, the DOPAC levels were significantly lower than after just one dose, suggesting that there might be some relationship between the rising drug levels and the fact that there was no increase in dopamine metabolites in spite of the increase in dopamine levels. The second dose

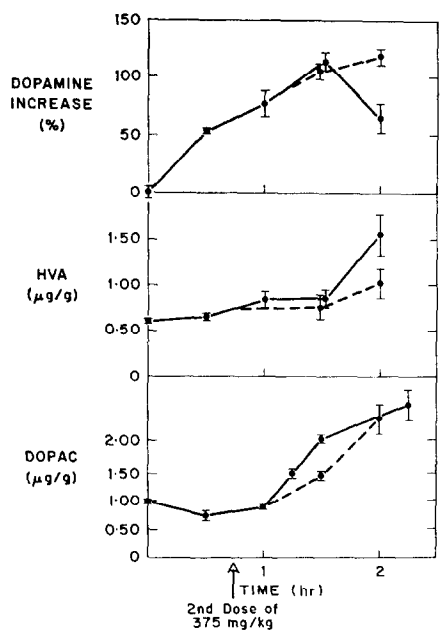

FIG. 4. Effect of multiple doses of GBL on dopamine, HVA and DOPAC in rat corpus striatum. Vertical bars represent the standard error of the mean. Each point is the mean of four or more determinations. The arrow represents the time of the second dose, 375 mg/kg of GBL. Zero time values are control values from rats which did not receive GBL.

also delayed the rise in HVA, but this effect was not statistically significant. Dopamine levels did not increase further after the second dose, suggesting that at high amine levels the storage capacity of the neuron might be exceeded and the excess dopamine may spill over to be metabolized, or alternatively, that this surplus dopamine might serve as a feedback effector, slowing dopamine synthesis.

DISCUSSION

The rise and fall in dopamine levels in rat corpus striatum were shown to follow the changes in GHB levels and to coincide with the behavioral effects of the drug. It has been shown, however, that if the increase in dopamine is prevented by the administration of α-methyl-*para*-tyrosine,[9,11] the animals still exhibit behavioral depression, suggesting that increased dopamine levels *per se* do not seem to be necessary for the behavioral effect of the drug. In fact, it has been reported that α-methyl-*para*-tyrosine prolongs the period of "anesthesia" after GBL.[11] The hypothesis that GHB prevents the release or functional utilization of dopamine is not inconsistent with the idea that a drug which decreases dopamine synthesis might prolong the effects of GHB. It remains to be proven, however, that there is a causal relationship between the effect of GHB on behavior and its effect on dopamine in the caudate or on the dopamine-containing limbic areas, which have also been shown to have increased dopamine fluorescence after GHB.[10] It should perhaps also be noted that changes in levels of acetylcholine have been reported after GHB.[22]

Most of the techniques used to obtain synthesis rates for the brain catecholamines which have involved labeled precursors have assumed that newly synthesized dopamine is mixed evenly with pre-existing dopamine and that there is no preferential release.[23,24] Evidence has been growing, however, for the concept that newly synthesized dopamine may be formed in a small pool which does not mix evenly with the total amount of intraneuronal dopamine.[25–27] It would seem possible that this smaller pool might be released and refilled at a faster rate than the larger pool. Besson *et al.*[28] and Javoy and Glowinski[29] have shown some evidence that the synthesis and turnover rates of dopamine may be much higher than most techniques have indicated. They suggest that the synthesis rate for dopamine in the caudate is in the order of 24 μg/g/hr, while other techniques have reported lower values such as 2·8 μg/g/hr.[23]

The increase of dopamine after the administration of GBL corresponds to approximately 5–6 μg/g of tissue in the first hour. Similar results have also been obtained recently by Spano *et al.*[30] after administration of the sodium salt of GHB. If one assumes that the lower synthesis rates are accurate, this would suggest that the drug was causing an increase in synthesis of dopamine and, indeed, the increase in specific activity of the corpus striatum dopamine after administration of GBL and ^{3}H-tyrosine would also suggest an increase in dopamine synthesis. However, if one considers the synthesis rates reported by Javoy and Glowinski,[29] one could account for the apparent rate of increase in dopamine levels after GBL solely by postulating an effect on release or metabolism, without an effect on synthesis. If the newly synthesized dopamine is preferentially released, an effect of the drug on release or metabolism of newly formed dopamine might account for the rapid increase in specific activity of dopamine observed after the drug. At present, it does not seem possible to distinguish between these two possibilities in regard to dopamine synthesis. However, recent *in vitro*

studies in our laboratory have demonstrated that GHB can antagonize the release of newly synthesized dopamine.[31]

The hypothesis that GBL may be preventing the release or functional utilization of dopamine is supported by the metabolite data. It is evident that, during the initial period of the drug effect, the increased levels of dopamine seem to some extent protected from metabolism. The rapid rise in DOPAC after the administration of chlorpromazine indicated that the levels of DOPAC can respond rapidly after a change in dopamine metabolism.

Monoamine oxidase, which catalyzes the conversion of dopamine to DOPAC, is considered to be located largely, but not exclusively, inside the nerve ending,[28,32] so that changes in DOPAC levels may not indicate whether the transmitter is being metabolized intraneuronally or extraneuronally. As DOPAC is not confined to an intraneuronal site,[33] HVA, the *O*-methylated product of DOPAC (and the deaminated product of 3-methoxytyramine), does not seem a good indicator of the site of metabolism of dopamine either. While one cannot rule out the possibility of compensatory or corresponding changes in levels of 3-methoxytyramine or of neutral metabolites of dopamine,[34] if it is assumed that HVA and DOPAC do reflect changes in dopamine metabolism, it is interesting to note that the protection of dopamine from metabolism seems related to high or rising levels of GHB. This protection might also be involved in the mechanism whereby GBL blocks the increase in HVA seen normally after chlorpromazine.[12]

In summary, the above results suggest that GHB is affecting the release and—depending upon the drug levels in the CNS—metabolism of central dopamine. Also, an increase in dopamine synthesis cannot be ruled out. If one assumes some kind of specific interaction of the drug with the dopaminergic neurons, one possible explanation for this protection from metabolism might be an effect of the drug directly on the nerve endings. This could result in a change in the binding or localization of the transmitter within the neuron in such a way that the access of dopamine to monoamine oxidase is altered. Another possibility might be a competition between the high levels of GHB and dopamine for some factor used in the metabolism of both, such as NAD.

However, preliminary results from extracellular recording experiments conducted in the substantia nigra and midbrain reticular formation suggest that GHB may be depressing neuronal firing in general, and that the increase in dopamine levels reflects a uniqueness in the response of dopaminergic neurons to a decrease in firing. The possibility that the suppression of neuronal firing may be associated with and perhaps even be the cause of the increase in dopamine levels observed in GBL-treated animals has been suggested by experiments in which lesions in the substantia nigra seem to cause rapid short-term increases in dopamine in the corpus striatum comparable to those seen after GBL.[35–37] Experiments are in progress to investigate this possibility further.

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