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SHORT COMMUNICATIONS

Effect of gamma-hydroxybutyrate and gamma-butyrolactone on dopamine synthesis and uptake by rat striatum

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GAMMA-Hydroxybutyrate (GHB), a naturally occurring central nervous system depressant,[1] and its lactone precursor, γ-butyrolactone (GBL), cause a marked and rapid increase in brain dopamine (DA).[2,3] This effect seems to be somewhat specific in that the levels of other putative neurotransmitters such as norepinephrine, 5-hydroxytryptamine and γ-aminobutyric acid are not markedly altered by anesthetic doses of GHB,[2–4] although acetylcholine is significantly increased.[4,5] The increase in brain DA induced by GHB is localized mainly in the striatum,[2] which is known to contain most of the dopaminergic nerve terminals arising from cell bodies located in the substantia nigra.[6] Recent findings *in vivo* by Spano *et al.*[7] suggest that GHB might increase striatal DA through a stimulation of DA synthesis. However, these investigators were not able to discriminate whether this increase in DA synthesis was mediated through a direct effect of the drug on the DA-containing nerve terminals or an indirect effect on the cell bodies of the DA-containing neurons. Furthermore, they did not rule out the possibility that GHB might produce this apparent increase in synthesis through an impairment in the utilization or release of striatal DA. Recent reports by our laboratory as well as by others indicate that it is possible to study DA synthesis in resting and K^+ depolarized slices from rat striatum.[8,9] The purpose of this communication therefore is two-fold: (1) to study the effect of GHB and GBL pretreatment on DA synthesis in resting and K^+ depolarized slices from rat striatum; and (2) to study the effect of GHB and GBL on DA uptake by "synaptosomal" preparations from rat striatum. Theoretically, an increase in DA re-uptake by nerve terminals after GHB treatment might also account in part for the increase in brain DA observed after administration of this drug.

Studies on DA synthesis. Striatal tissue slices (0·18 mm in thickness) were prepared with a Sorvall tissue chopper from the striatum of adult, male, Sprague–Dawley rats. Tissue slices weighing about 30–40 mg were incubated at 37° in beakers containing 5 ml of either Krebs–Ringer phosphate (KRP), pH 7·4, or KRP-high K^+ (55 mM), pH 7·4, saturated with 95% O_2 + 5% CO_2. After a 10-min preincubation, labeled L-tyrosine-^{14}C (U) with a specific activity of 5 mc/m-mole was added to the media, producing a final tyrosine concentration of 5×10^{-5} M. Thereafter the slices were incubated an additional 30 min, the beakers were chilled on ice, and slices were separated from the media by centrifugation at 10,000 rev/min for 10 min in a Sorvall refrigerated centrifuge. The slices were then homogenized in 15% trichloroacetic acid (TCA); each incubation medium was acidified with 0·5 ml of 50% TCA. Unlabeled DA, norepinephrine and tyrosine (50 μg each) were added to both tissue homogenates and media and the precipitated protein was then removed by centrifugation at 10,000 rev/min for 20 min. Separation and analysis of the tissue and the media for labeled DA and its metabolites were carried out by absorption chromatography through Alumina columns and ion-exchange chromatography through Amberlite CG-120 columns as described previously.[10] Eluates from the columns containing labeled DA were analyzed for ^{14}C in a Packard liquid scintillation spectrometer. Results were calculated as described previously[8] and are expressed in terms of nanomoles of ^{14}C-DA per gram, wet weight, per hour. Tissue blanks were run by incubating striatal slices as described above, but in the presence of α-methyl-*p*-tyrosine (2×10^{-4} M), an inhibitor of tyrosine hydroxylase.[11]

Tyrosine assay. After a 30-min incubation of striatal slices in normal KRP with ^{14}C-tyrosine, as described above, the specific activity of the tyrosine isolated from the tissue was determined. Tyrosine was separated from catechols and other labeled tyrosine metabolites by passage through Alumina columns and columns of Dowex 50W-X8 (H^+), 100–200 mesh.[8] Eluates from the Dowex 50 columns were evaporated to dryness, redissolved in distilled water, the radioactivity was determined by liquid scintillation counting, and the quantity of tyrosine estimated by the nitrosonaphthol fluorescent method.[12,13]

Studies on DA uptake. Synaptosomal uptake activity was measured essentially by the method of Coyle and Snyder,[14] with some minor modifications as previously described.[15] Nialamide, a monoamine oxidase inhibitor, was added to the incubation media to a final concentration of $7{\cdot}5 \times 10^{-5}$ M, and ^{3}H-DA (specific activity, 10 c/m-mole) was added to a final concentration of 8×10^{-7} M.

A 3-min incubation period at 37° was utilized for routine assay. In experiments employing GHB, a 3-min preincubation was performed before addition of ^{3}H-DA. All assays were performed in duplicate. Diffusion blanks were measured by maintaining some samples in an ice-water bath. Under the experimental conditions used, the uptake activity was linear for 8 min. Evidence that the pellet radioactivity is mostly unmetabolized ^{3}H-DA and that the observed uptake activity is predominantly synaptosomal has been presented elsewhere.[14]

The conversion of ^{14}C-tyrosine to ^{14}C-dopamine in striatal slices was followed through a wide range of tyrosine concentrations. The apparent K_m for ^{14}C-tyrosine was $2{\cdot}4 \times 10^{-6}$ and 1×10^{-5} M, respectively, when determined in a normal K^+ and high-K^+ media. Synthesis of ^{14}C-DA was linear for up to 40 min when using a ^{14}C-tyrosine concentration of 5×10^{-5} M. Table 1 shows that the addition of GHB directly to the incubation media produced no significant effect on the conversion of ^{14}C-tyrosine to ^{14}C-DA. As reported previously,[8] the presence of K^+ (55 mM) increased about 2-fold the formation of ^{14}C-DA. Most of the increased ^{14}C-DA synthesized after high K^+ was present in the media (Table 1). This, of course, is due both to the stimulatory effect of high K^+ on the release[16] of newly synthesized DA and to its inhibitory effect on the re-uptake of the monoamine (J. E. Harris, personal communication). The presence of GHB in the media was not able either to block or to potentiate the K^+-induced formation of newly synthesized ^{14}C-DA (Table 1).

TABLE 1. EFFECT OF GHB ON SYNTHESIS OF DOPAMINE BY STRIATAL SLICES*

Incubation media	^{14}C-dopamine synthesis (nmoles/g wet wt/hr)		
	Tissue	Media	Total
KRP	20·1 ± 2·9	1·9 ± 0·4	22·0 ± 2·6
KRP + GHB (1×10^{-3} M)	23·7 ± 2·0†	1·9 ± 0·5†	25·6 ± 1·7†
KRP − high K^+ (55 mM)	30·8 ± 2·3	34·2 ± 1·9	65·0 ± 3·1‡
KRP − high K^+ (55 mM) + GHB (1×10^{-3} M)	32·0 ± 2·1†	31·6 ± 2·7†	63·6 ± 4·6†‡

* Striatal slices were prepared by means of a Sorvall tissue chopper and incubated, as indicated above, in media containing saturating concentrations of ^{14}C-tyrosine (5×10^{-5} M, sp. act., 5·0 mc/m-mole and 1·25 μc/flask) for 30 min at 37°. ^{14}C-dopamine was separated by column chromatography and its radioactivity determined in a Packard scintillation spectrometer. Results represent the mean ±S.E.M. of six different experiments.

† Not significantly different when compared to respective control without GHB.

‡ $P < 0{\cdot}02$ when compared to respective normal KRP control.

TABLE 2. EFFECT OF GBL PRETREATMENT ON DOPAMINE SYNTHESIS IN STRIATAL SLICES*

Incubation media	Treatment of rats	^{14}C-dopamine synthesis (nmoles/g wet wt/hr)		
		Tissue	Media	Total
KRP	Saline injection	27·9 ± 1·7	2·9 ± 0·6	30·9 ± 2·0
KRP	GBL injection	26·8 ± 1·9†	1·2 ± 0·2‡	27·9 ± 2·1†
KRP − high K^+	Saline injection	35·8 ± 2·7	47·7 ± 4·9	83·5 ± 7·3
KRP − high K^+	GBL injection	31·8 ± 1·4†	45·2 ± 5·8†	77·0 ± 3·0†

* Rats were killed 30 min after GBL (750 mg/kg, i.p.). The striatum was dissected out and slices were prepared by means of a Sorvall tissue chopper. Incubation conditions and separation of ^{14}C-dopamine were as described under Table 1. Results represent the mean ±S.E.M. of six different experiments.

† Not significantly different from saline-treated rats.

‡ $P < 0{\cdot}05$ when compared to saline-treated rats.

Pretreatment of rats with anesthetic doses of GBL was without significant effect on the formation of ^{14}C-DA in striatal slices, either in a normal or high K^+ media (Table 2). The only significant effect observed in these experiments was the ability of the drug to antagonize the spontaneous release of newly synthesized DA (Table 2).

It is possible that a stimulatory effect on GHB on ^{14}C-DA synthesis could be masked if the drug significantly inhibited the uptake of ^{14}C-tyrosine. However, this seems unlikely, since the specific activity of ^{14}C-tyrosine found in the tissue at the end of the incubation was not significantly altered in the presence of GHB or after GBL pretreatment. The values obtained (mean ±S.E.M.; n = 4) at the end of the incubation period were, respectively, 51,688 ± 8951, 50,997 ± 2,229 and 43.232 ± 3992 dis./min/μg of tyrosine for the control slices, slices incubated with GHB, and slices from GBL-treated animals.

Table 3 shows that pretreatment of rats with anesthetic doses of GBL produced a significant inhibitory effect on ^{3}H-DA uptake by crude "synaptosomal" preparations from rat striatum. This apparent inhibition of uptake might be explainable in part due to a small dilution in the specific activity of the added labeled DA by the increased levels of endogenous DA found in the homogenates prepared from the GBL-treated rats. Addition of GHB directly to the incubation media did not affect the uptake of ^{3}H-DA (Table 3).

TABLE 3. EFFECT OF GHB AND GBL PRETREATMENT ON ^{3}H-DA UPTAKE BY CRUDE "SYNAPTOSOMAL" PREPARATIONS FROM RAT STRIATUM*

Incubation media	Treatment†	^{3}H-DA uptake (pmoles/3 min/0·9 mg protein)
KRP		116·7 ± 2·3
KRP	GBL	94·0 ± 4·2‡
KRP + 3-min preincubation		112·2 ± 5·5
KRP + 3-min preincubation + GHB (1×10^{-3} M)		113·1 ± 5·9

* Synaptosomal suspension containing 0·9 mg protein was incubated in media containing ^{3}H-DA (8×10^{-7} M; sp. act., 10 c/m-mole and 0·41 μc/tube) for 3 min at 37°. Results represent the mean ±S.E.M. of four different experiments.

† GBL (750 mg/kg, i.p.) was injected 30 min before sacrifice.

‡ $P < 0·005$ when compared to control rats.

In conclusion, the results shown here do not support the idea that GHB increases brain DA by a direct action on dopaminergic nerve terminals resulting in an increase in DA synthesis or a potentiation of DA re-uptake. However, the possibility still remains that GHB exerted the above effects at the dopaminergic terminals through some change arising only after administration of the drug *in vivo*. So far the results obtained in brain slices from rats pretreated with GBL seemed to argue against this possibility (Tables 2 and 3). In fact, GBL pretreatment was shown to have an apparent inhibitory rather than stimulatory effect on ^{3}H-DA uptake by striatal synaptosomes.

While GHB administered *in vivo* undoubtedly possesses the ability to cause a rapid increase in endogenous DA[2,3,7] as well as an apparent increase in the formation of ^{3}H-DA from ^{3}H-tyrosine,[17] this action is not necessarily due to an accelerated rate of synthesis. In fact, recent experiments in our laboratory suggest that a different mechanism is responsible.[5] For example, when DA synthesis is inhibited by administration of α-methyl-*p*-tyrosine, GHB causes a striking decrease in the rate of disappearance of DA from the subcortex.[3] In addition, administration of GHB and GBL causes a significant reduction in the levels of dihydroxyphenylacetic acid (DOPAC)[17] and homovanillic acid (HVA)[3,18] found in the striatum at a time when the level of striatal DA is still rising. Furthermore, anesthetic doses of GHB and related agents antagonize the initial increase in HVA and DOPAC normally produced by neuroleptic drugs such as chlorpromazine and haloperidol.[5,18]

The above observations are consistent with the suggestion that GHB blocks the release or utilization of DA. The actual mechanism responsible for this action of GHB appears to be the ability of this compound to block impulse flow in the nigro-neostriatal pathway, thus leading to a marked reduction in dopamine release.[5,19] It is most likely that this effective block in dopamine release is responsible

for the rapid accumulation of DA within the nerve terminals of the striatum observed when the drug is administered *in vivo*.

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Effect of puromycin on binding and on metabolism *in vitro* of substrates by rat liver microsomes

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THE ADMINISTRATION of puromycin, an inhibitor of protein synthesis, prevents drug-induced increases in microsomal enzyme activity.[1–3] The mechanism of this effect is thought to be due to puromycin interaction with the ribosome-messenger RNA complex leading to the release of incomplete protein chains from the ribosomes. Since puromycin is demethylated by rat liver microsomes,[4] it also could be expected to act as an alternate substrate affecting the metabolism of other drugs.[5] Gelboin and Blackburn[2] reported that treatment of rats with puromycin did not alter the basal level of benzypyrene hydroxylase in several tissues. However, data presented by Alvares *et al.*[6] indicate that puromycin administered hourly by i.p. injections in a dose of 20 mg/kg, caused a slight decrease in V_{max} of benzypyrene hydroxylase of rat liver microsomes.

The purpose of the research presented in this communication was to characterize the reaction of puromycin with rat liver microsomes, to investigate the effects of puromycin on the binding of sub-