

γ -Hydroxybutyrate Modulates Synthesis and Extracellular Concentration of γ -Aminobutyric Acid in Discrete Rat Brain Regions In Vivo¹

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ABSTRACT

γ -Hydroxybutyrate possesses most of the properties of a neurotransmitter/neuromodulator that acts via specific pathways and receptors in brain. Beside its regulatory effects on dopaminergic transmission, γ -hydroxybutyrate was thought for many years to interfere with γ -aminobutyric acid (GABA)ergic processes in the brain. The present study demonstrates that in the rat frontal cortex in vivo, γ -hydroxybutyrate or its agonist NCS-356 administered systemically at a high dose (500 mg/kg) increases GABA contents in dialysates via a mechanism blocked by the peripheral administration of the γ -hydroxybutyrate antagonist NCS-382. Under the same conditions, the extracellular concentration of this amino acid was not modified in the hippocampus. However, when administered at a low dose (250 mg/kg), γ -hydroxybutyrate decreases GABA content of the dialysates of the frontal cortex by an NCS-382-sensitive mechanism. Spontaneous [³H]GABA release was observed in

the frontal cortex of rats at 160 min after i.p. [³H]- γ -hydroxybutyrate administration. This result indicates that γ -hydroxybutyrate in vivo could be the precursor of an extracellular GABA pool in the frontal cortex. After i.p. [³H]- γ -hydroxybutyrate administration in the rat, the amino acid contents of several brain regions were quantified 160 min later, and the radioactivity in each region was measured. [³H]GABA, [³H]glutamate, and [³H]glycine were detected in most, but not all, of the brain regions studied. In particular, radioactive GABA was not detected in the hippocampus. The other amino acids were not labeled. These results show that γ -hydroxybutyrate modulates the synthesis and the extracellular concentrations of GABA in specific regions of the rat brain. Identification of these GABA pools and determination of their functional role remain to be defined.

About 1% to 2% of the metabolic flux through the cerebral γ -aminobutyric acid (GABA) shunt pathway leads to the production of γ -hydroxybutyrate (GHB) (Gold and Roth, 1977). This substance is present at concentrations ranging from 2 to 5 μ M in all brain regions investigated, but this amount could be increased by several orders of magnitude after the peripheral administration of GHB to various animals or humans (Shumate and Snead, 1979; Vayer et al., 1988). This pharmacological increase is used for therapeutic benefits in anesthesia, for the treatment of narcoleptic patients, and for addiction and withdrawal therapy (Laborit, 1973; Mamelak et al., 1986; Gallimberti et al., 1993).

In recent years, evidence has accumulated that support a role for endogenous GHB as a neuromodulator in the central nervous system (Maitre, 1997). GABA is the main precursor of GHB in brain after its conversion by GABA-T into succinic semialdehyde. Then, succinic semialdehyde reductase (EC

1.1.1.1.2), a neuronal enzyme, reduces succinic semialdehyde to GHB, which is accumulated in synaptic nerve endings (Weissmann-Nanopoulos et al., 1982; Snead, 1987). Cortical or hippocampal slices, preloaded with [³H]GHB, release this substance after neuronal depolarization via a Ca²⁺-sensitive mechanism (Maitre et al., 1983; Vayer and Maitre, 1988). Specific high-affinity receptors for GHB are distributed mainly in the cortex, hippocampus, olfactory tracts, striatum, and thalamus, but the hypothalamus, cerebellum, and pons medulla are devoid of binding sites (Hechler et al., 1992). In addition to their distribution, these receptors are specific as judged by kinetic, pharmacological, and developmental points of view. Their stimulation enhances Ca²⁺, inositol phosphates, and cGMP levels in brain slices or in pure cultures of neurons (Vayer and Maitre, 1989). GHB exerts a regulatory effect on dopaminergic neurons of the brain via a mechanism that is blocked by the GHB receptor antagonist NCS-382 (6,7,8,9-tetrahydro-5-[H]benzocycloheptene-5-ol-4-ylidene acetic acid) (Roth et al., 1980; Maitre et al., 1990).

However, several reports argue for a GABAergic effect of

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ABBREVIATIONS: GHB, γ -hydroxybutyrate; GABA, γ -aminobutyric acid.

GHB because GHB-induced electrophysiological processes are sometimes blocked by the GABA_B antagonist CGP 35348 (*P*-[3-aminopropyl]*P*-diethoxymethylphosphinic acid) *in vivo* or in brain slices (Xie and Smart, 1992; Williams et al., 1995). Behavioral studies also seem to indicate a GABA-like effect of GHB, especially because the sedative, anesthetic, or anxiolytic properties of GHB are usually described to be induced or potentiated via GABAergic phenomena (Schmidt-Mutter et al., 1998). Apart from a direct hypothetical effect of GHB on GABA_B receptors, a GHB-induced inhibition of GABA release has been described in the thalamus of the rat (Banerjee and Snead, 1995). In addition, GHB can be converted into GABA, both *in vivo* and *in vitro* (Della Pietra et al., 1966; DeFeudis and Collier, 1970; Vayer et al., 1985). Thus, it seems that GHB can induce GABA mechanisms by modulating GABA release and/or synthesis.

The present study demonstrates that the peripheral administration of GHB to rats decreases at a low dose and increases at a high dose the GABA extracellular concentration in the frontal cortex but not in the hippocampus of freely moving rats. In addition, [³H]GHB has been shown to be the precursor of [³H]GABA, [³H]glutamate, and [³H]glycine in some regions of the rat brain, but only the first two radioactive amino acids were released after neuronal depolarization. Thus, it appears that pharmacological doses of GHB regulate several GABA pools, at least in the frontal cortex, and thus a similar modulation at physiological GHB levels cannot be excluded and most probably consists of a tonic inhibitory control of the activity of some GABAergic synapses in the brain.

Materials and Methods

In Vivo Microdialysis Experiments

Surgical Procedure. Male Wistar rats weighing 350 to 400 g at the beginning of the experiments (bred in the Center de Neurochimie, Strasbourg) were housed in individual cages on a standard 7 AM to 7 PM light/dark cycle with free access to food and water. All experiments were carried out in accordance with the European Community Council directive of November 24, 1986 (86/609/EEC). Rats were anesthetized with ketamine (100 mg/kg *i.p.*) and placed in a stereotaxic frame (Narishige). Two holes were drilled in the skull, and two continually perfused (2.0 μ l/min) dialysis probes (polycarbonate-polyether, 20-kDa molecular mass cutoff, 2 or 4 mm long and 0.52-mm diameter (CMA 12; CMA Microdialysis) were slowly lowered into the brain after piercing of the meninges. Coordinates used for the tip of the probes from bregma were rostral 3.0 mm, lateral 1.8 mm, and ventral 5.5 mm with an angle of 15 degrees for frontal cortex and caudal 3.5 mm, lateral 2.5 mm, and ventral 3.5 mm for dorsal hippocampus, according to Paxinos and Watson (1986). The two dialysis probes were permanently fixed to the skull with three stainless steel screws and methacrylic cement and protected with a tube. Rats were used between 24 and 48 h after implantation.

In Vivo Microdialysis. During the experiments, the animals were placed in hemispheric bowls of 40-cm diameter, with free access to water, and the microdialysis probes were connected to a microinjection pump (CMA 100; CMA Microdialysis). The perfusion medium contained 147 mM NaCl, 4.0 mM KCl, and 2.4 mM CaCl₂, pH 6.5 (Guevara-Guzman et al., 1994), filtered through 0.22- μ m Millex Millipore filters before each experiment. The perfusion rate was 1.0 μ l/min (20 min collection). Under these conditions, the *in vitro* recovery was 16.8% for GABA. Ca²⁺ dependence of GHB-induced GABA release was tested by perfusing the same medium but with 12 mM MgCl₂ and no Ca²⁺ (Banerjee and Snead, 1995).

Verification of Probe Placement. At the end of each experiment, the rats were sacrificed by an anesthetic overdose, and their brains were removed and stored in buffered formalin for at least 1 week. Tissue sections were made, and the correct placement of the microdialysis probe was verified by examination of the sections (Fig. 1).

Measurements of Free Amino Acid Levels in Dialysates. The amino acid content of the dialysates was determined by fluorimetric detection of the *o*-phthalaldehyde derivatives after their separation by HPLC, using a modification of the method of Allison et al. (1984). Briefly, all chromatography was performed with a Nucleosil C 18 column (5 μ m, 25 \times 0.4 cm) with two Waters pumps (model 590) and a Waters Baseline 810 integrator. Detection was carried out with a Waters fluorimeter 470 (excitation, 345 nm; emission, 455 nm). The mobile phase was a binary gradient between solution A (0.1 M sodium phosphate, pH 6.0, containing 2% methanol, pH 6.0) and solution B (40% 0.1 M sodium phosphate, pH 6.0, 30% methanol, and 30% acetonitrile). Precolumn autoderivatization (2 min) and injection were achieved with a CMA 200 refrigerated microsampler (CMA Microdialysis) by the addition to 20 μ l of dialysate of a volume of 20 μ l of the following derivatization mixture: 5 ml of 0.1 M sodium tetraborate, pH 9.5, containing 10 μ l of 3-mercaptopyruvic acid (Sigma Chemical Co., St. Louis, MO) and 15 mg of *o*-phthalaldehyde (Sigma) in 500 μ l of methanol. Elution was carried out at a rate of 0.8 ml/min and at a temperature of 35°C with the following steps: 0 min, 90% A/10% B; 15 min, 40% A/60% B (linear gradient); 16 min, 40% A/60% B (isocratic); 19 min, 100% B (isocratic); and 24 min, 90% A/10% B (isocratic) until 29 min. β -Aminoisobutyric acid was used as internal standard.

Incorporation of Radioactivity into Amino Acids of Some Brain Tissue Regions

In Vivo Experiments. Animals were injected *i.p.* with [³H]GHB (500 μ Ci, 100 Ci/mmol; CEA/Saclay, Gif-sur-Yvette, France) and 4.0 mmol/kg nonradioactive GHB, Na⁺ salt (Sigma). After 160 min (the delay that was necessary to observe a spontaneous [³H]GABA release), the rats were sacrificed by microwave irradiation (2.0-s irradiation time for rats weighing 450 g; Püschner, Strasbourg, Germany). Their brains were removed and dissected on a cold glass plate. Twenty-eight brain structures were dissected according to the protocol described by Vayer et al. (1988), and nuclei were isolated using the Palkovits and Brownstein microdissection procedure (1988). Care was taken to not contaminate the different brain slices. After tissue collection, samples were immediately immersed in liquid nitrogen until the day of analysis.

Amino Acid Tissue Level Determination. After being weighed, brain structures were homogenized in 10 volumes of 0.1 N

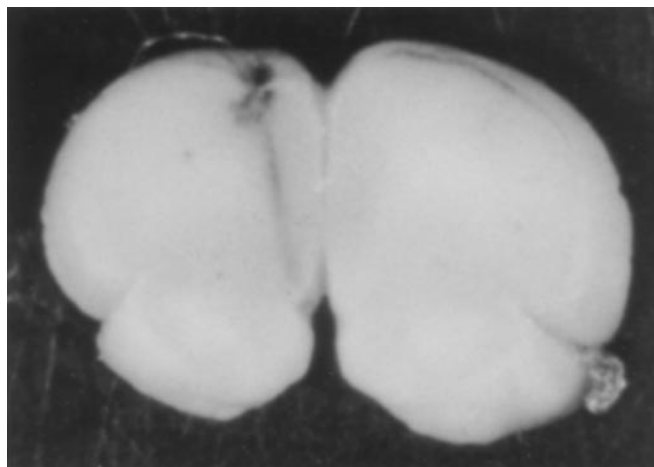


Fig. 1. Histological control showing the position of the 4-mm CMA 12 probe during dialysis.

HCl (w/v) and centrifuged. The internal standard (β -aminoisobutyric acid) was added to the supernatants, and the free amino acid levels were determined by the same method as that used for the dialysis samples. The eluates of each chromatography were collected in 1 ml/min fractions and counted for radioactivity in a liquid scintillation spectrometer. This protocol allowed the quantification of each individual amino acid.

Statistical Analysis

Microdialysis experiments were evaluated statistically using a one-way ANOVA followed by the nonparametric test of Friedman for repeated measurements on absolute levels of released amino acids (pmol/20-min fraction).

Results

GHB-Induced Modifications in GABA Extracellular Concentration in Frontal Cortex. Simultaneous microdialysis experiments of the rat frontal cortex and hippocampus were carried out because these regions are among the richest in GHB receptors. The baseline GABA level was followed during 7×20 min before rats received 4.0 mmol/kg i.p. GHB at zero time, and dialysates (20-min fractions) were analyzed for their GABA content. The increase in extracellular GABA release was significant 80 min after GHB, and the maximum was reached at 100 to 120 min ($+120 \pm 10\%$ of the basal level, $p < .01$). This increase remained significant up to 220 min (Fig. 2). In the absence of Ca^{2+} in the perfusion medium, the GHB-induced increase in extracellular GABA was not significantly modified (not shown). NCS-356 (γ -*p*-chlorophenyl-*trans*-4-hydroxycrotonate) (2.0 mmol/kg i.p.), another agonist of the GHB receptor, produced a similar GABA increase ($+100 \pm 7\%$ with a maximum at 40–80 min, $p < .01$; Fig. 3). Pretreatment of the rats with the GHB receptor antagonist NCS-382 completely abolished the GABA increase due to GHB or to NCS-356.

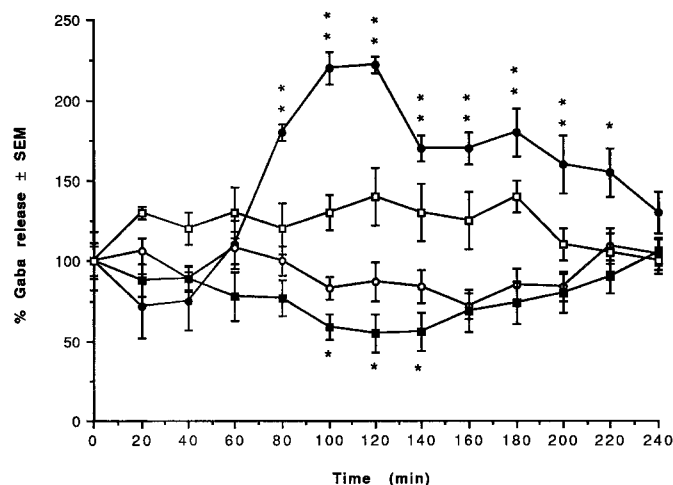


Fig. 2. ●, GHB (4.0 mmol/kg i.p.) injected at time zero (mean basal release \pm S.E.M. = 2.54 ± 0.12 pmol/20 min, $n = 3$ rats). ○, inhibition of the GHB induced GABA increase by NCS-382 (2.0 mmol/kg i.p.) injected 20 min before GHB (mean basal release \pm S.E.M. = 2.31 ± 0.27 pmol/20 min, $n = 3$ rats). ■, decrease in extracellular GABA content after i.p. administration of 2.0 mmol/kg GHB (mean basal release \pm S.E.M. = 1.50 ± 0.11 pmol/20 min, $n = 3$ rats). □, this decrease is blocked by coadministration of NCS-382 (1.0 mmol/kg i.p.) with GHB (2.0 mmol/kg i.p.; mean basal release \pm S.E.M. = 1.53 ± 0.25 pmol/20 min, $n = 3$ rats). Results are expressed as percentage of the mean basal release of GABA (mean of 4×20 min dialysate fractions before the first drug injection). * $p < .05$; ** $p < .01$.

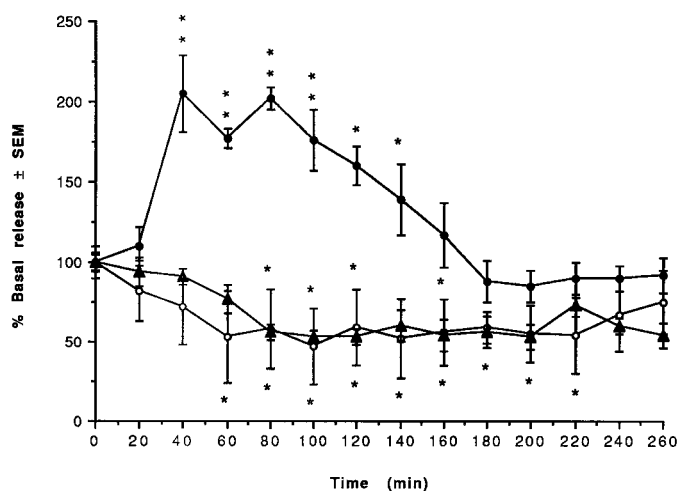


Fig. 3. ●, NCS-356 (2.3 mmol/kg i.p.) injected at time zero (mean basal release \pm S.E.M. = 1.40 ± 0.14 pmol/20 min, $n = 3$ rats). ▲, inhibition of NCS-356 induced GABA increase by NCS-382 (2.0 mmol/kg i.p.) injected 20 min before NCS-356 (mean basal release \pm S.E.M. = 2.3 ± 0.27 pmol/20 min, $n = 3$ rats). ○, NCS-382 alone. Results are expressed as percentage of the mean basal release of GABA (mean of 4×20 min dialysate fractions before the first drug injection). * $p < .05$; ** $p < .01$.

In the hippocampus, no modification of GABA release was seen after GHB administration (data not shown).

To investigate the effect of GHB concentrations closer to endogenous brain levels, the modification of GABA release in the frontal cortex was monitored after the administration of 2.0 mmol mg/kg i.p. GHB. Under these conditions, the extracellular concentration of GABA rapidly declines, reaching about -50% compared with control levels after 120 min ($p < .05$, Fig. 2). Treatments of the rats with GHB (2.0 mmol/kg i.p.) plus NCS-382 (1.0 mmol/kg i.p.) completely blocks the GHB-induced decrease in GABA extracellular levels.

[^3H]GHB as Precursor of GABA Present in Extracellular Space. In subsequent experiments, after stabilization of the baseline level of GABA, labeled GHB was administered to rats at time zero (4.0 mmol/1.25 mCi/g), and the release of radioactive GABA was monitored. About 7000 cpm of tritiated GABA was present in the total peak of GABA that was spontaneously released. The amount of radioactive GABA represented about 2% of the total GABA released at 160 min after GHB administration (Fig. 4).

At 260 min after the first peak, a second peak was initiated by a high potassium shock. A 100 mM KCl pulse was included in the perfusion medium during 20 min, and NaCl was reduced to 51 mM in the same medium to maintain normal osmolarity. This K^+ -induced depolarization provoked a second peak of spontaneously released GABA. However, no radioactive GABA was detected in this second peak (Fig. 4).

Distribution of Radioactive Amino Acids in Brain Tissues after Administration of Radioactive GHB. Twenty-eight regions of the rat brains were analyzed for their contents of various free radioactive amino acids at 160 min after the administration of radioactive GHB (4.0 mmol/1.25 mCi/kg i.p.). It was observed in previous experiments that such a delay was necessary for a spontaneous release of radioactive GABA.

Among the brain regions investigated, 12 had no detectable radioactive amino acids (caudate nucleus anterior part, caudate-putamen, ventral pallidum, hypothalamus, amyg-

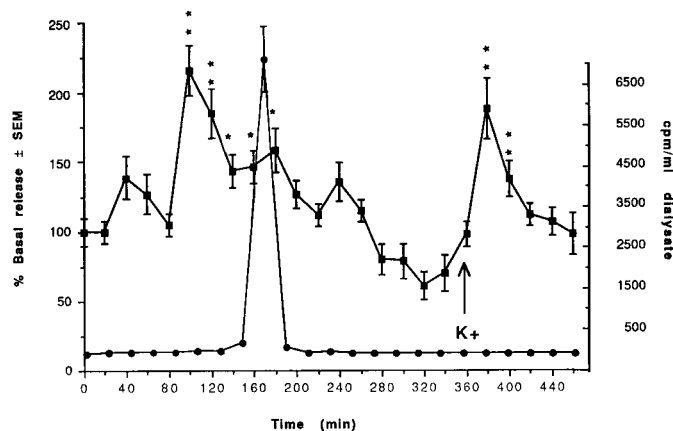


Fig. 4. Simultaneous determination of total GABA (radioactive plus nonradioactive) released in the frontal cortex after peripheral administration of 2,3-³H-GHB (4.0 mmol/kg, 1.5 mCi/kg; ■) and of tritiated GABA alone (●) (mean basal release ± S.E.M. = 3.6 ± 0.27 pmol/20 min, *n* = 3 rats). After the spontaneous release of radioactive GABA, a potassium shock (100 mM KCl in the dialysis probe during 20 min) induced a supplementary release of GABA. However, this second peak was not radioactive. The radioactive GABA represented about 2% of the total GABA released in the extracellular space. Results are expressed as the percentage of the mean basal release of GABA (mean of 4 × 20 min dialysate fractions before the first drug injection). **p* < .05; ***p* < .01.

dala, occipital cortex, substantia nigra, ventral tegmental area, ventral hippocampus, temporal and retrosplenial cortices, cerebellum, and inferior colliculi). For the other brain areas, only glutamate, GABA, and glycine were found to be radioactive.

Table 1 and Fig. 5 show the distribution of radioactive amino acids in picomoles per milligram of tissue. Radioactive glutamate was highest in the prefrontal and parietal cortices, in the medial thalamus, but the lateral thalamus showed no glutamate labeling. Radioactive GABA was present especially in the globus pallidus, prefrontal and frontal cortices, and septum but absent from the parietal and entorhinal cortices, dorsal hippocampus, lateral thalamus, superior colliculi, and medulla oblongata. Finally, radioactive glycine was particularly abundant in the prefrontal cortex, globus pallidus, lateral and medial thalamus, and raphe nuclei, but the septum, parietal and entorhinal cortices, and medulla oblongata were devoid of radioactive glycine.

The specific activities (percentage of the different radiola-

TABLE 1
Distribution of radioactive glutamate, GABA, and glycine after peripheral administration of 2,3[³H]GHB (4.0 mmol/kg; 1.5 mCi/kg)
Results are expressed in pmol/mg of tissue (mean ± S.E.M., *n* = 3 rats).

Lateral thalamus	N.D.	N.D.	106 ± 12
Medulla oblongata	13 ± 1	N.D.	N.D.
Raphe nuclei	13 ± 1	14 ± 0.1	166 ± 77
Superior colliculi	13 ± 4	N.D.	12 ± 1
Dorsal hippocampus	14 ± 0.7	N.D.	14.1
Frontal cortex	14 ± 1	35 ± 1	33 ± 2
Entorhinal cortex	15 ± 1	N.D.	N.D.
Globus pallidus	16 ± 2	114 ± 30	129 ± 45
Parietal cortex (posterior)	17 ± 3	10 ± 2	N.D.
Olfactory tubercles	23 ± 4	N.D.	43 ± 5
Nucleus accumbens	28 ± 1.1	16 ± 4	21 ± 11
Septum	28 ± 8	36 ± 1	N.D.
Olfactory bulbs	31 ± 9	13 ± 1	76 ± 40
Parietal cortex (anterior)	39 ± 9	N.D.	N.D.
Medial thalamus	56 ± 23	30 ± 2	157 ± 117
Prefrontal cortex	62 ± 11	49 ± 4	125 ± 40

N.D., not detected.

beled amino acids in the corresponding tissular compartment) of the glutamate, GABA, and glycine pools of the different brain regions investigated are indicated in Table 2 and Fig. 6. The nucleus accumbens, globus pallidus, septum, and medial thalamus were heavily labeled in the glutamate compartment. GABA specific activities were highest in the frontal and prefrontal cortices, globus pallidus, septum, and medial thalamus. For glycine, the highest specific activities were found in the lateral and medial thalamus, raphe nuclei, globus pallidus, prefrontal cortex, and olfactory bulbs.

Discussion

Several results argue in favor of GHB receptors belonging to the metabotropic class, coupled via G protein to Ca²⁺ and K⁺ conductances and working mainly as presynaptic heteroreceptors (Harris et al., 1989; Diana et al., 1991; Ratomponirina et al., 1995). Thus, these receptors appear to be closely related to GABA_B receptors, at least from a functional point of view. In addition, several electrophysiological effects of GHB, in vivo or in vitro, have been blocked by CGP 35 348, a GABA_B antagonist (Xie and Smart, 1992; Engberg and Nissbrandt, 1993; Williams et al., 1995). These results have led some authors to claim a GABAergic effect for GHB, possibly via a direct interaction of GHB with GABA_B receptors. Although the IC₅₀ value of GHB for GABA_B binding is weak (Bernasconi et al., 1992) or very weak (Ito et al., 1995; Ishige et al., 1996), the possibility remains that large doses of GHB administered peripherally could interfere with GABA_B-mediated effects in the brain. However, GABA_B binding experiments carried out with [³H]GABA in the presence of isoguvacine suggest that GHB effects on this binding is mainly due to rapid conversion of GHB into GABA under the conditions used (40-min incubation at 20°C and pH 7.4) (Hechler et al., 1997).

Also in vivo, several reports suggest that GHB is the precursor of GABA, even though the total brain concentration of GABA remained unchanged after GHB administration (Della Pietra et al., 1966; DeFeudis and Collier, 1970). This finding could explain why GHB possesses some GABA-like effects on behavior, namely sedative and anxiolytic effects, and could

TABLE 2
Distribution of the specific activities (percentage of labeled amino acid molecules in the corresponding amino acid pool) of the different radioactive pools of tritiated glutamate, GABA, and glycine after administration of 2,3[³H]GHB (4.0 mmol/kg; 1.5 mCi/kg)
Results are expressed in percentage of each total amino acid (mean ± S.E.M., *n* = 3 rats).

Lateral thalamus	N.D.	N.D.	1.85 ± 0.20
Frontal cortex	0.09 ± 0.01	1.92 ± 0.02	0.67 ± 0.04
Parietal cortex (posterior)	0.10 ± 0.01	0.41 ± 0.03	N.D.
Dorsal hippocampus	0.10 ± 0.03	N.D.	0.37 ± 0.05
Entorhinal cortex	0.13 ± 0.03	N.D.	N.D.
Superior colliculi	0.15 ± 0.07	N.D.	0.19 ± 0.04
Medulla oblongata	0.16 ± 0.04	N.D.	N.D.
Raphe nuclei	0.17 ± 0.05	0.40 ± 0.08	2.47 ± 1.18
Parietal cortex (anterior)	0.24 ± 0.01	N.D.	N.D.
Olfactory bulbs	0.25 ± 0.01	0.22 ± 0.03	1.79 ± 1.07
Olfactory tubercles	0.25 ± 0.09	N.D.	0.84 ± 0.03
Prefrontal cortex	0.37 ± 0.10	0.77 ± 0.06	1.67 ± 0.17
Septum	0.63 ± 0.19	2.19 ± 0.45	N.D.
Nucleus accumbens	0.68 ± 0.09	0.39 ± 0.04	0.40 ± 0.26
Medial thalamus	0.73 ± 0.13	1.26 ± 0.13	3.94 ± 1.94
Globus pallidus	1.52 ± 0.53	2.28 ± 0.01	1.90 ± 0.41

N.D., not detected.

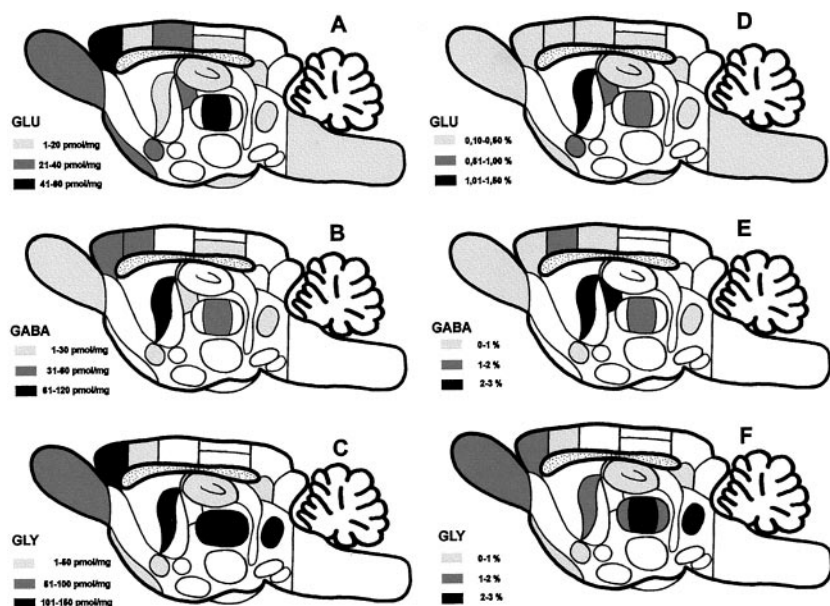


Fig. 5. Schematic drawings of the brain distribution of [³H]glutamate (GLU), [³H]GABA, and [³H]glycine (GLY) resulting from the conversion of [³H]GHB. Intensity of gray-shaded areas according to the distribution of picomoles of radioactive amino acids per milligram of tissue (images A–C) or to the distribution of specific activities of each labeled amino acid compartment (images D–F).

induce petit mal epilepsy in rodents, which is thought to be mediated by a GABA_B effect (Snead, 1992, 1994). However, these phenomena are probably the results of a specific activation of the GHB system, associated with a selective potentiation of some GABAergic mechanisms. In this regard, GHB is also a possible modulator of GABA release. A GHB-induced inhibition of GABA release has been described in the thalamus of the rat (Banerjee and Snead, 1995).

The present study confirms and describes two mechanisms for a GHB-mediated control of GABAergic mechanisms with a specific brain distribution. The experiments were carried out in the frontal cortex and the hippocampus, which possess a high density of GHB receptors and GABAergic innervation. First, GHB receptors are implicated in the control of GABA release, but the nature of this control depends on the brain region. In the frontal cortex, a strong increase in GABA release was induced about 60 min after 4.0 mmol/kg GHB administration, but this increase was not abolished by the absence of Ca²⁺ in the perfusion medium. This release was blocked by pretreatment with the GHB receptor antagonist NCS-382 and reproduced by the GHB receptor agonist NCS-356. However, perhaps due to its higher affinity for the GHB receptor (Hechler et al., 1993) or for pharmacokinetic reasons, the latency for GABA increase in the case of NCS-356 was much lower. In the hippocampus, GHB has no effect on GABA release.

If the dose of administered GHB is much lower and closer to endogenous physiological levels of this substance in brain, the effect of GHB on the GABA release in the frontal cortex is radically different. According to the observation of Banerjee and Snead (1995) in the thalamus, at low doses GHB induces a decrease in GABA release. In this last study, the actual concentrations of GHB applied locally were 30 to 250 μM if a 15% recovery for GHB dialysis through the probes is considered. In our present study, for a GHB dose of 4.0 mmol/kg administered i.p., we can assume a concentration of GHB in the rat frontal cortex of about 400 to 500 μM after 120 min, when the increase in GABA release is maximum (Lettieri and Fung, 1979; Shumate and Snead, 1979). In contrast, for a GHB dose of 2.0 mmol/kg i.p., the concentra-

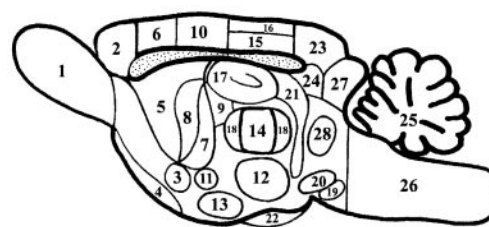


Fig. 6. Identification of the dissected brain areas. 1, olfactory bulbs; 2, prefrontal cortex; 3, nucleus accumbens; 4, olfactory tubercles; 5, nucleus caudatus (anterior part); 6, frontal cortex; 7, nucleus caudatus (posterior part); 8, globus pallidus; 9, septum; 10, parietal cortex (anterior part); 11, ventral pallidum; 12, hypothalamus; 13, amygdala; 14, medial thalamus; 15, parietal cortex (posterior part); 16, cingulate cortex (posterior part); 17, dorsal hippocampus; 18, lateral thalamus; 19, substantia nigra; 20, ventral tegmental area; 21, ventral hippocampus; 22, entorhinal cortex; 23, occipital cortex; 24, superior colliculi; 25, cerebellum; 26, medulla oblongata; 27, inferior colliculi; 28, raphe nuclei.

tion of GHB in the frontal cortex is only about 200 to 250 μM after 120 min (when the maximal GABA decrease is observed). This dose corresponds to the maximal dose used by Banerjee and Snead (1995).

To reconcile these findings, the following hypothesis can be drawn. For doses of administered GHB of ≤2.0 mmol/kg (which induce a maximal concentration of GHB in brain below 400–500 μM), an inhibition of GABA release is observed in the thalamus (Banerjee and Snead, 1995) or in the frontal cortex (the present study). This effect, which begins rapidly after GHB administration, could be due to the direct stimulation of GHB receptors antagonized by NCS-382. This GHB-induced inhibitory control of GABA release possibly represents the physiological effect of GHB and its endogenous role, via presynaptic GHB receptors, for the control of the activity of some specific GABAergic pathways. At higher doses of GHB (4.0 mmol/kg in the present study, which is about 800–1000 μM GHB in brain, maximal concentration), a rapid and short decrease in GABA release is observed and is followed by a large increase, starting with a delay of 60 to 80 min. Thus, GHB exerts a biphasic effect on GABA release in the rat frontal cortex. For the lower dose studied, an inhibitory effect of GHB on GABA release is observed,

whereas at the higher dose of GHB, an increase in GABA release is induced. It could be postulated that a high dose of GHB desensitizes GHB receptors, and this is installed 60 to 80 min after GHB administration. This phenomenon (which could be blocked by large dose of the antagonist NCS-382) inactivates the inhibitory control of GHB on GABA release, which is seen at lower GHB doses. However, a direct or an indirect effect of large concentrations of GHB on other receptors cannot be ruled out, and some evidence has been reported in favor of a direct GABA_B receptor effects (Bernasconi et al., 1992).

On the other hand, the present study demonstrates that in vivo, radioactive GHB is converted into radioactive GABA, which is released into the extracellular space of the frontal cortex. This release takes place 160 min after GHB administration, and a subsequent neuronal depolarization 200 min later shows no further release of radioactive GABA. This indicates that GHB is converted in vivo into GABA after a rather long delay, and thus could explain why the formation of GABA has not been detected in some previous studies (Möhler et al., 1976; Doherty and Roth, 1978). It is interesting to note that GHB gives rise to a GABA pool possibly implicated in GABAergic neurotransmission and that this property exists in the frontal cortex but not in the hippocampus. However, it remains to be demonstrated that the GABA pool that could be released or synthesized under GHB influence is of neuronal origin and is due to a exocytotic release (Timmerman and Westerink, 1997). A modulation of some GABAergic terminals via presynaptic heteroreceptors can be proposed, but these receptors do not exist in hippocampus. In this region, [³H]GHB does not give rise to [³H]GABA; thus, it appears that this conversion does not take place in all fields of GABAergic innervation.

Moreover, of the 28 brain regions investigated, 19 regions showed the amount of radioactive GABA to be undetectable. In the other regions, levels of radioactive GABA were very heterogeneous, showing that GHB conversion into GABA is determined by a regionally selective mechanism. However, the precise functional role of this GABA pool is presently unknown. Radioactive glutamate was found in the majority of the brain regions investigated; only 13 of 28 regions were devoid of radioactive glutamate. Despite the fact that glutamate is considered to be the major precursor of GABA in brain, the specific activities of tritiated glutamate pools in the different regions investigated were very different from the specific activities of the corresponding GABA pools. However, several glutamate pools exist in brain, and the [³H]GABA synthesized from [³H]GHB might come from a specific brain glutamate compartment whose labeling probably originates in Krebs' cycle intermediates. In addition, the possibility exists that the GABA derived from GHB comes from the conversion of GHB into GABA via GHB dehydrogenase and GABA-T.

Taken together, these experiments favor a GABAergic influence of GHB when administered at a high dose, mostly in the extracellular space of the frontal cortex, via an increased GABA release and via a conversion of GHB into GABA. However, these phenomena occur with a time lapse of about 40 min (increase in GABA release) or 160 min (spontaneous [³H]GABA release after [³H]GHB). At least for the frontal cortex, these modifications of extracellular GABA concentration are too late to explain GHB-induced sedation, anxiolysis,

or absence epilepsy (Laborit, 1973; Bernasconi et al., 1992; Snead, 1992; Maitre, 1997; Schmidt-Mutter et al., 1998) through a modification of GABAergic transmission. However, the results described here have been obtained for a single dose of GHB or NCS-356. A higher dose of GHB might induce a more precocious increase of GABA in the frontal cortex. The kinetics of this effect might also be different in other brain regions. The present study show, for example, an absence of GHB-induced GABA effects in the hippocampus. Other brain regions, like globus pallidus, accumulates more [³H]GABA and could be able to release GABA more rapidly after GHB administration.

Finally, the role of GHB-derived glutamate in the glutamatergic neurotransmission remains doubtful because no radioactive glutamate was detected in the extracellular space, even after potassium shocks. Similarly, radioactive glycine, present in several brain structures after the radioactive GHB pulse, was absent from dialysates. Thus, it is difficult to attribute a specific functional role to this pool of glycine synthesized from GHB. However, its high concentration in the thalamus and in the frontal/prefrontal cortices might explain some aspects of the GHB role in glycinergic inhibitory mechanisms or in *N*-methyl-D-aspartate receptor stimulation involved in the regulation of epileptic activity (Banerjee and Snead, 1992).

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