Naunyn-Schmiedeberg's Arch Pharmacol (1984) 328:62–68

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Evidence for down-regulation of GABA receptors following long-term gamma-butyrolactone

G. Gianutsos and P. D. Suzdak

Section of Pharmacology and Toxicology, University of Connecticut, Box U-92, Storrs, CT 06268, USA

Summary. Long-term oral administration (12 weeks) of γ-butyrolactone (GBL) to mice resulted in pharmacological and neurochemical changes which may be interpreted as a decrease in GABA-mediated synaptic activity. The depression in motor activity produced by the GABA-mimetic muscimol was reduced following long-term GBL. The binding of GABA to its putative receptor was reduced in the GBL group as evidenced by a decrease in the Bmax in the cerebral cortex, cerebellum, and striatum, but not in the hippocampus. No difference in the concentration of GABA was observed between the two groups. However, the reduction in GABA accumulation which normally results after an acute injection of GBL (560 mg/kg) was markedly attenuated in the mice receiving 12 weeks of GBL. Similarly, the muscimol-induced alteration in the concentration of DOPAC, a dopamine metabolite, was also reduced in the GBL group. These results, illustrating tolerance to the pharmacological effects of GBL and muscimol and in vitro evidence of a reduction in GABA binding sites, suggest that long-term exposure to GBL is accompanied by an alteration in GABA receptors and provides a possible mechanism for the tolerance to GBL-induced changes in dopamine.

Key words: GABA – Gamma butyrolactone – Dopamine – Receptors

Introduction

Gamma-butyrolactone (GBL) and its active metabolite γ-hydroxy butyrate GHB) (Giarman and Roth 1964) exert a profound CNS depressant effect on animals (Giarman and Schmidt 1963) and in man, which may be clinically significant (Laborit et al. 1961). At anesthetic doses, these drugs also produce characteristic changes on dopaminergic parameters in the CNS which resemble the short-term effects produced by surgical or chemical destruction of the dopamine neuron. These include inhibition of neuronal activity (Walters et al. 1973) and increases in dopamine content (Gessa et al. 1966) and synthesis rate (Walters et al. 1973). With long-term treatment, a tolerance develops to both the behavioral depression and to the dopaminergic actions of the drug (Gianutsos and Moore 1978; Nowycky and Roth 1979).

The mechanism underlying these effects remains obscure. The structural similarity between GHB and GABA suggest that a GABA-mediated mechanism may play a role in its acute pharmacological effects and the involvement of GABA has previously been proposed (Roth and Nowycky 1977; Anden and Stock 1973) but also disputed (Osorio and Davidoff 1978).

Because of the possibility that GABA may play a role in the pharmacological activity of GBL, we chose to investigate whether changes in GABAergic parameters may constitute a potential mechanism for the tolerance resulting from long-term GBL administration. We have examined the effects of the GABA agonist, muscimol (Naik et al. 1976; Beaumont et al. 1978) in mice treated with GBL and report a reduction in the behavioral and neurochemical effects produced by muscimol, as well as an apparent decrease in the binding of GABA to its receptor. These results are consistent with a possible down-regulation of central GABA receptors as a result of long-term GBL.

Materials and methods

Drug administration. Male CD-1 mice (Charles River Farms, Wilmington, MA) were used in all experiments. GBL was diluted in a fruitpunch flavored solution (Hawaiian Punch) and served as the animal's only source of fluid; controls received unadulterated punch. After 2 days on plain punch, the mice began receiving GBL at a concentration of 0.25% V/V. After 3 weeks, the concentration was raised to 0.5% and then to 0,75% after 3 additional weeks. The mice remained at this concentration for 6 weeks. The GBL was removed from the fluid for 24 h prior to performing the experiments described. While some decrease in fluid consumption was noted in some mice during the course of treatment, most mice adapted to the presence of the drug.

For acute experiments, GBL was diluted in saline and injected i.p. Muscimol (Research Organics, Cleveland, OH) and THIP (4,5,6,7, tetrahydroisoxazolo pyridin-3-ol) were dissolved in distilled water and injected s.c.

Motor activity. Activity was measured in a Stoelting Activity Monitor during the morning hours. A mouse was placed in one the 6 sensors and acclimated to the device for 20 min. They were then removed, injected and returned to

Send offprint requests to G. Gianutsos at above address

the box and activity was recorded for one hour. Activity is expressed as total "counts" recorded by the machine for the observation period.

Dopac. The concentration of dopamine and its metabolite, DOPAC, was estimated using an HPLC method with electrochemical detection based on the assay described by Magnusson and coworkers (1980). The mice were sacrified and the amount of DOPAC in the striatum and the mesencephalic region containing the substantia nigra was determined in the supernatant derived after centrifugation of the tissue homogenized in 0.1 N perchloric acid. In brief, the supernatant was filtered through a 0.5 micron cellosic membrane and a 20 µl aliquot was injected into the HPLC system. The mobile phase consisted of 0.15 M citric acid/Na acetate buffer with 12.5% methanol (final pH of 3.95), and included 0.5 mM octyl sodium sulfate. The sample was separated on a Waters Boundapack (C18) column. Typical retention times were 9.5 min for DOPAC, 11 min for DA and 7.9 min for the internal standard, dihydroxybenzylamine. A Bioanalytical LC-4 amperometric detector fitted with a glassy carboe electrode maintained at a potential of +0.8 V vs a Ag/AgCl reference electrode was used. The amount of material in the sample was calculated by integrating the area under the curve (by use of a microprocessor), and was based upon values (sample to internal standard ratio) from standards assayed simultaneously. The pellet from the original centrifugation was saved for the determination of proteine by the method of Bradford (1976).

Gaba. The concentration of GABA was measured by the enzymatic- fluorometric method of Graham and Aprison (1966), which depends on the fluorometric determination of NADPH derived from NADP during the enzymatic degradation of GABA by GABAse in vitro. In brief, frozen brain tissue was homogenized in 50 mM sodium acetate, boiled to inactivate residual glutamic acid decarboxylase and centrifuged. A 20 µl aliquot the clear supernatant was incubated in a mixture containing α-keto glutarate, GABAse (Sigma), NADP and pyrophosphate buffer (pH = 8.4). The NADPH generated by the enzymatic reaction was quantified after conversion to highly fluorescent NADP by alkaline peroxide.

In experiments designed to assess GABA turnover, aminooxyacetic acid (AOAA) was injected (20 mg/kg, i.p.) 15, 30 or 60 min prior to sacrifice. This regimen leads to a time-dependent accumulation of GABA which can be used to determine relative turnover (see Pericic et al. 1978). GABA was quantified as above. GBL or saline was injected s.c. 5 min before AOAA and the GABA turnover in different brain regions was analyzed. Differences between the groups were analyzed by analysis of variance. Data is expressed as percent of control (i.e., values obtained from mice receiving two injections of saline) for clarity.

Gaba binding. The binding of [^{3}H]-GABA to its putative receptor was performed essentially as described by Enna and Snyder (1977). Briefly, mouse brain tissue was pooled and homogenized in 15 volumes of ice-cold 0.32 M sucrose. After a series of centrifugations to separate the synaptosomal fraction, the pellet was finally resuspended in 100 volumes of 50 mM Tris-citrate buffer (ph = 7.1) containing 0.05% Triton X-100 and was incubated for 30 min at 37° C. The mixture was centrifuged at 40,000 × g for 10 min and the resulting pellet was suspended in the tris-citrate buffer and used in the binding assay. The detergent-treated membrane was added to a solution containing different concentration of [^{3}H]-GABA (New England Nuclear, 29.3 Ci/mmole) in tris-citrate buffer. The tubes were incubated on ice for 20 min and then centrifuged at 4° C for 10 min at 48,000 × g. The supernatant was gently poured off and the pellet was washed with 1 ml of ice-cold buffer. After decanting, the tube was wiped dry and the pellet is dissolved with 0.25 ml Protosol (New England Nuclear) After addition of Enconoflour, the radioactivity in the sample was quantified by liquid scintillation spectrometry. Non-specific binding was defined as label which was not displaced by 100 µM GABA. Binding constants (B_{max}, Kd) were calculated by the method of Rosenthal (1967) and are based on the assumption of two binding sites. A minimum of 9 concentrations of GABA (10^{-9} to 10^{-5} M) was used to calculate the kinetic values. All points were run in triplicate and the values for Km and B_{max} reported in the results represent means of three separate determinations on independent groups of animals assayed at different times.

Statistics. Unless otherwise indicated, comparisons were made between controls and chronic GBL treated animals under the same experimental condition (e.g., receiving identical acute injection). Student's t-test was used to determine statistical differences with $p<0.05$ chosen as the level of significance.

Results

Activity

In normal mice, the GABA agonist muscimol exerts a depressant effect on motor activity as illustrated in Fig. 1 (see also Biggio et al. 1977). This effect is of brief duration (approximately 45 min) and dose-dependent, with doses above 0.5 mg/kg producing significant depression. There was no difference in activity between control and chronic-GBL mice when injected with saline. However, after long-term GBL, the mice were less sensitive to the depressant effects of muscimol. Doses of 0.5 or 1 mg/kg of muscimol were significantly less effective in reducing motor activity in the chronic GBL group than in controls. At high doses (above 1.5 mg/kg), the tolerance was no longer significant. These mice are also tolerant to the depressant effects of an acute injection of GBL (Gianutsos and Moore 1978 and data not shown).

In order to further test the possible tolerance to GABA agonists, a limited experiment was also conducted with THIP. The group receiving long-term GBL was also tolerant to the motor depression produced by this drug as well (1,044 + 156 counts/hr in control vs. 1,544 + 241 in GBL group treated with 6 mg/kg THIP, $n = 8$; $p < 0.05$).

Binding. The results from the activity experiment could be interpreted as evidence for decreased sensitivity of GABA receptors as a result of the long-term GBL treatment. In order to test this hypothesis, the binding of [^{3}H]-GABA was compared in control and GBL-treated animals. As summarized in Table 1, the specific binding of [^{3}H]-GABA was reduced following long-term GBL.

The binding was resolved into both a high affinity and low affinity component in each brain structure examined. In the striatum, cortex and cerebellum obtained from GBL-exposed mice,, there was a decrease in the number of both the high and low affinity binding sites (decreased B_{max}). For example, in the cerebral cortex, there was a 53% decrease in the number of high affinity sites and a 58% decrease in the low affinity site. There was no significant change in affinity of either site in any brain region, with the exception of the low affinity site in the striatum. This decrease in binding suggests that long-term GBL produces a decrease in the available number of GABA receptors. In the hippocampus, however, no change in GABA binding was detected. Incubation of GHB (the active metabolite of GBL) did not displace GABA when added to membrane preparations in vitro at concentrations up to 0.1 mM, suggesting that residual GHB could not account for the reduction in GABA binding.

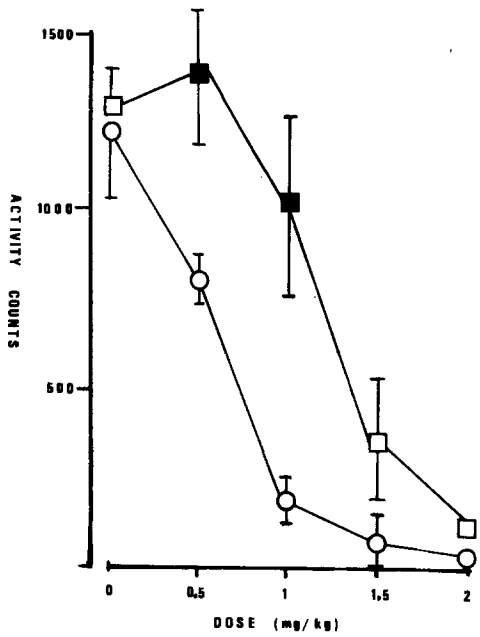

Fig. 1. Effect of muscimol on motor activity after chronic GBL. Muscimol or saline was injected sc in control mice (*cicles*) or mice receiving oral GBL (squares) for 12 weeks as described in text. Motor activity was accumulated for the 50 min period beginning 10 min after injection. Activity is expressed as total "counts" during the measurement period. *Filled symbols* represent values significantly different ($p<0.05$) from mice in the control group receiving the identical injection

Gaba

In an effort to determine a possible explanation for the effect of long-term GBL on GABA receptors, the concentration of GABA and relative GABA turnover was measured in the four brain regions and also in the mesencephalic region containing the substantia nigra. The content of GABA in all five brain regions was unchanged by either acute or chronic administration of GBL as summarized in Table 2. In the acute experiment, GBL was injected at a dose of 560 mg/kg; this dose produces a marked increase in brain DA content and is approximately 1/3 the daily dose consumed by the mice on the chronic regimen. In the chronic study, the paradigm was identical to that used in the binding (i.e., they received oral GBL for 12 weeks and were then withdrawn from the GBL for 24 h before testing). No change in GABA levels was detected in any brain region under these conditions.

Similarly, data summarized in Fig. 2 illustrate that the rate of accumulation of GABA, after inhibition of degradation, was the same in the control and long-term GBL groups. However, acute injections of GBL markedly slowed the accumulation of GABA in all brain regions, in agreement with previous studies reported by Pericic and

Table 1. Effect of chronic GBL on the binding of GABA[a]

Brain region	^{3}H-GABA binding			
	High affinity site		Low affinity site	
	B_{max}	K_d	B_{max}	K_d
A. Cerebral cortex				
Control	240±35	22+7	925± 50	217+25
Chronic GBL	113±10*	38+5	388± 15*	176+18
B. Striatum				
Control	305±27	14+4	1,250± 97	223+15
Chronic GBL	165±15*	13+3	590± 20*	150+28*
C. Cerebellum				
Control	414±23	20+9	1,500±105	208+19
Chronic GBL	265±15*	33+8	920± 30*	185+15
D. Hippocampus				
Control	290±20	10+3	990± 35	165+19
Chronic GBL	290±16	25+9	875± 20	150+25

[a] Mice received oral GBL for 12 weeks or control solution as described in text. The specific binding of ^{3}H-GABA was measured 24 h after drug withdrawal in the brain regions noted. B_{max} is expressed as fmoles/mg protein while K_d is nM concentration. Values are means±SEM for determinations made in three different experiments (see text)

* Represents values significantly different from corresponding control group

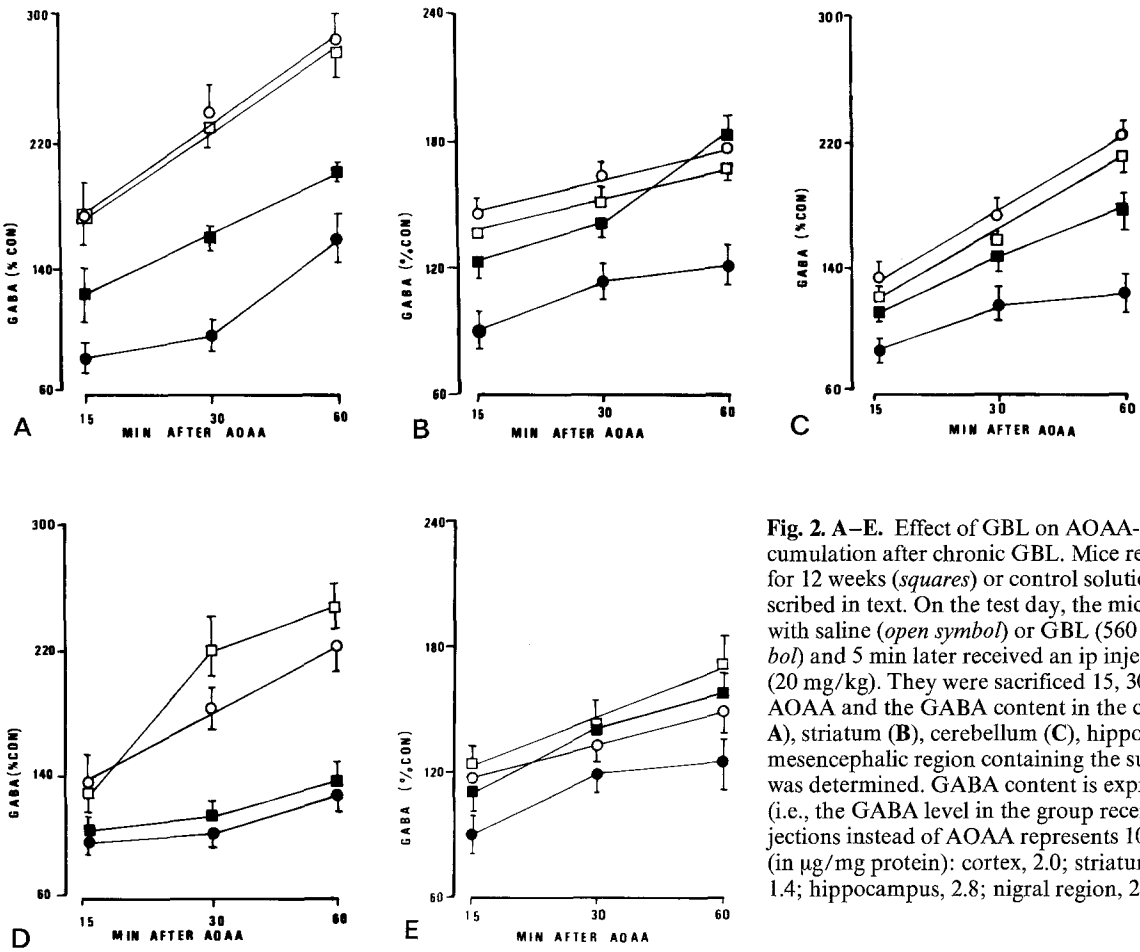

Fig. 2. A–E. Effect of GBL on AOAA-induced GABA accumulation after chronic GBL. Mice received GBL orally for 12 weeks (*squares*) or control solution (*circles*) as described in text. On the test day, the mice were pretreated with saline (*open symbol*) or GBL (560 mg/kg, *closed symbol*) and 5 min later received an ip injection of AOAA (20 mg/kg). They were sacrificed 15, 30 or 60 min after the AOAA and the GABA content in the cerebral cortex (panel **A**), striatum (**B**), cerebellum (**C**), hippocampus (**D**) or the mesencephalic region containing the substantia nigra (**E**) was determined. GABA content is expressed as % control (i.e., the GABA level in the group receiving saline injections instead of AOAA represents 100%. These values are (in μg/mg protein): cortex, 2.0; striatum, 2.2; cerebellum 1.4; hippocampus, 2.8; nigral region, 2.5)

Table 2. Effect of acute or chronic GBL on brain GABA concentrations

Brain Area	GABA concentration (μg/mg protein; mean±S.E.)			
	Acute group[a]		Chronic group[b]	
	Control	GBL	Control	GBL
Striatum	2.6±0.2	2.6±0.4	2.8±0.6	2.7±0.4
Cerebellum	1.2±0.2	1.2±0.1	1.6±0.2	1.3±0.3
F. cortex	1.5±0.3	2.1±0.4	1.5±0.3	1.4±0.3
Hippocampus	2.5±0.7	2.7±0.4	3.0±0.4	2.7±0.7
S. nigra area	2.0±0.6	2.1±0.4	2.1±0.4	2.7±0.5

[a] Mice were injected ip with GBL (560 mg/kg) or saline 30 min before sacrifice for the determination of GABA. $N=8$

[b] Mice received GBL orally for 12 weeks as described in text. All mice were removed from drug for 24 h before GABA measurements

coworkers (1978). Evidence for tolerance to the GBL-induced reduction of GABA turnover was found in the mice receiving long-term GBL in the cerebral cortex, striatum, cerebellum and nigral region. For example, in the striatum (Fig 2b), an acute injection of GBL reduced the normal linear accumulation of GABA occurring after inhibition of degradation. However, when this normally effective dose of GBL (560 mg/kg) was tested in mice which had previously been receiving GBL, the rate of GABA accumulation was similar to that seen in control animals injected with saline plus AOAA. In contrast, no tolerance to the GBL-induced reduction in GABA accumulation was noted in the hippocampus.

Dopac

In order to test whether the previously observed tolerance to the effects of GBL on DA neurons (Gianutsos and Moore 1978) might be due to these postulated changes in GABA-mediated mechanisms, we investigated the effect of muscimol on the accumulation of the DA metabolite DOPAC as a marker of DA turnover (Roth et al. 1976). In normal mice, muscimol produced a biphasic effect on the concentration of DOPAC in the striatum and in the area containing the DA cell bodies in the substantia nigra, as summarized in Table 3. A low dose (1 mg/kg) of muscimol reduced DOPAC concentrations, while a higher dose (3 mg/kg) increased DOPAC. The content of DA was unaffected by any dose tested (data not shown). In the mice maintained on GBL, a tolerance to the effect of muscimol was noted in both brain regions and this occurrend at a dose which either increased or decreased DOPAC. In other words, the dose of 1 mg/kg muscimol produced less of a decrease in DOPAC in the chronic-GBL group than in controls, while the 3 mg/kg dose produced less of an increase in DOPAC in the GBL-group.

Table 3. Effect of muscimol on DOPAC after chronic GBL

Group[a]	DOPAC concentration (ng/mg protein)		
	Treatment[b]	Striatum	S. nigra area
Control	Saline	22.9±1.7	2.4±0.2
Chronic GBL	Saline	25.2±2.7	2.7±0.6
Control	Muscimol (1 mg/kg)	12.4±2.7	1.3±0.2
Chronic GBL	Muscimol (1 mg/kg)	19.1±3.0*	1.8±0.2*
Control	Muscimol (3 mg/kg)	49.9±3.9	7.5±0.2
Chronic GBL	Muscimol (3 mg/kg)	36.3±1.3*	6.2±0.4*

[a] Mice were maintained on oral GBL or control solution for 12 weeks as described in text. All animals received control solution for 24 h before test. Values are mean±S.E. from 6–8 mice

[b] Mice were injected sc with saline or muscimol (1 or 3 mg/kg) and were sacrificed 30 min later for the determination of DOPAC

* Represents values significantly different ($p<0.05$) from corresponding treatment in control group

Discussion

Long-term oral administration (12 weeks) of GBL resulted in changes in in vivo and in vitro GABAergic parameters which are consistent with a decrease in GABA-mediated receptor activity. The in vivo effect of the GABA agonist muscimol (Naik et al. 1976; Beaumont et al. 1978) on motor activity and on DA turnover was reduced after long-term GBL as were the number of GABA binding sites (presumably receptors) in several brain regions measured in vitro. These results suggest that a GABAergic system in the CNS is capable of undergoing plasticity changes by down-regulating in response to GBL. The exact mechanism by which GBL exerts this effect is at present unclear.

An alternate explanation of these effects could be provided if GBL altered drug metabolism. In a previous study, there was no evidence for alterations in drug metabolism after chronic GBL (Gianutsos and Moore 1978). Furthermore, changes in binding in vitro were detected in this study. Therefore, it appears likely that GBL affects a GABAergic mechanism to produce the observed changes. A simple interpretation could be developed if GBL acted as a GABA agonist such that long-term receptor occupation would lead to a compensatory receptor subsensitivity. The DA elevation effect of GBL is reportedly antagonized by the GABA antagonist picrotoxin (Roth and Nowycky 1977) which would be constistent with this interpretation. However, neither GBL nor its active metabolite GHB displaced GABA from its binding sites (see also Beaumont et al. 1978), suggesting that direct receptor interaction is unlikely.

An alternative interpretation could be based upon changes in GABA synthesis or metabolism. However, neither acute (see also Giarman and Schmidt 1963) nor chronic GBL altered the content of GABA in the brain, suggesting that direct interference with GABA synthesis or metabolism is equally unlikely. However, GBL did reduce the accumulation of GABA after inhibition of enzymatic degradation (see also Pericic et al. 1978), suggesting that GBL alters GABA release or, more likely, its regulation by feed-back mechanisms. The observation that tolerance developed to the GBL-induced change in GABA turnover lends some validity to its importance in explaining the results.

Recently, endogenous GHB has been detected in the brains of normal animals (Roth and Giarman 1970) along with a specific synthesis mechanism (Rumigny et al. 1981) and selective binding sites (Benavides et al. 1982). This information suggests that endogenous GHB may play a role in brain function and, in fact, it has been suggested as a possible mediator in epilepsy (Snead et al. 1980). It is possible that the GHB system (and, by inference, exogenously administered GBL) may be functionally coupled to the GABA system whereby stimulation of a GHB-sensitive site results in a physiological response which is either identical to or equivalent to stimulation of GABA receptors. It is interesting that in cultured neurons, GHB and GABA induce similar changes in chloride ion conductance (Hoesli et al. 1983). If, therefore, GHB and GABA induce equivalent physiological actions, repeated administration of GBL could be expected to lead to a down-regulation of GABA-mediated synaptic events (e.g., receptors) as a means of compensation for the excess "GABA-like" stimulation produced by GBL via GHB. In all likelihood, GHB "binding sites" are also altered under these conditions, although there is presently no experimental verification of this.

Although evidence supporting a possible change in GABA receptors is presented, it has been proposed that two different receptor sites, termed GABA-A and GABA-B may exist (Hill and Bowery 1981). Our results suggest the participation of the GABA-A site because of the characteristics of the binding assay and the observed tolerance to muscimol. However, our previous studies in which there was tolerance to baclofen (Gianutsos and Moore 1978), the prototype GABA-B agonist (Hill and Bowery 1981), following long-term GBL, suggest that the B site may also be affected.

One puzzling aspect is the lack of tolerance development in the hippocampus, which cannot be resolved at this time. GBL clearly produced an effect on GABA utilization in this structure upon acute administration and a high concentration of GHB binding sites have been reported in this structure (Benavides et al. 1982), suggesting that the hippocampus responds pharmacologically to GBL. It is possible that the interaction between GBL and GABA in the hippocampus is different from other brain regions or that GABA receptors in the hippocampus are less susceptible to plasticity changes.

The brain region where GBL exerts its influence on DA utilization, on the other hand, are sensitive to long-term GBL. Previously, it was demonstrated that tolerance develops to the effect of GBL on DA neurons (Gianutsos and Moore 1978; Nowycky and Roth 1979), and the results of this investigation suggest that this is due to a decrease in the responsiveness of GABA receptors which may regulate DAergic activity (Gale et al. 1978). Although there was insufficient tissue to perform in depth binding studies in the substantia nigra area which contains the DA cell bodies, tolerance was demonstrated to the GBL-induced decrease in GABA accumulation (i.e., relative GABA turnover) in this area. This results would be consistent with a decrease in GABAergic influence in this area.

Furthermore, the effect of the GABA agonist muscimol an DA metabolism was reduced in the chronic GBL group. It must be pointed out however, that relatively little intact muscimol penetrates into the CNS, and this may (Baraldi et al. 1979) or may not (Maggi and Enna 1979) be sufficient to stimulate GABA receptors in vivo. The metabolites generated from in vivo muscimol administration also may or may not have GABAergic activity, and may even be related to GHB itself (Unnerstall and Pizzi 1981). Muscimol does, however, produce effects in vivo which would be expected of a GABA agonist (Naik et al. 1976). In our study, pharmacological activity was produced by the muscimol injection and tolerance was noted to both its motor depressant activity and its effects on DA utilization. Furthermore, tolerance was also demonstrated to the depressant effects to THIP, a muscimol analog with better pharmacokinetic properties (Moroni et al. 1982).

Muscimol produced a biphasic effect on DA metabolism, reducing DOPAC at low doses and elevating DOPAC at higher doses. Biphasic effects of muscimol have previously been reported on the content of DOPAC (Biggio et al. 1977), DA (Gundlach and Beart 1981) and on the behavioral effects of DA antagonists (Worms and Lloyd 1980) depending on factors such as dose, pretreatment time and route of administration. One possible explanation for these effects is based on the observation of Grace and Bunney (1979) who reported that microiontophoretic application of muscimol into the substantia nigra produces biphasic effects on DA neuronal activity depending on the site of application and proposed that GABA interneurons in the mesencephalon may mediate these effects. If so, our results suggest that the GABA receptors on these GABAergic interneurons as well as GABA receptors on the DA neurons in the substantia nigra may be sensitive to long-term GBL administration, since tolerance was noted to both components of muscimol's action.

In summary, long-term administration of GBL may produce a decrease in the number of GABA receptors in different regions of the CNS and may represent a model system to investigate changes in the pharmacology of GABAergic neurons.

Acknowlegements. This research was supported in part by a starter grant from the Pharmaceutical Manufacturers Association Foundation. The effort of Patricia McGinley in the development of the HPLC methodology and the technical assistance of Laura Elliott and Carol Brown is gratefully acknowledged. THIP was generously donated by H. Lundbeck & Co. (Copenhagen).

References

Anden NE, Stock G (1973) Inhibitory effect of gammahydroxybutyric acid and gammaaminobutyric acid on the dopamine cells in the substantia nigra. Naunyn-Schmiedeberg's Arch Pharmacol 279:89–92

Baraldi M, Grandison L. Guidotti A (1979) Distribution and metabolism of muscimol in the brain and other tissues of the rat. Neuropharmacology 18:57–62

Beaumont K, Chilton WS, Yamamura HI, Enna SJ (1978) Muscimol binding in rat brain: association with synaptic GABA receptors. Brain Res 148:153–162

Benavides J, Rumigny JF, Bourguignon JJ, Cash C, Wermuth CG, Mandel P, Vincendon G, Maitre M (1982) High affinity binding sites for γ-hydroxybutyric acid in rat brain. Life Sci 30:953–961

Biggio G, Casu M, Corda MG, Vernaleone F, Gessa GL (1977) Effect of muscimol, a GABA-mimetic agent, on dopamine metabolism in the mouse brain. Life Sci 21:525–32

Bradford, MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal Biochem 72:248–254

Enna SJ, Snyder SH (1977) Properties of γ-aminobutyric (GABA) binding in rat brain. Brain Res 124:185–190

Gale K, Costa E, Toffano G, Hong JS, Guidotti A (1978) Evidence for a role of nigral γ-aminobutyric acid and substance P in the haloperidol- induced activation of striatal tyrosine hydroxylase. J Pharmacol Exp Ther 206:29–37

Gessa GL, Vargiu L, Crabai F, Bocro GC, Caboni F, Camba R (1966) Selective increase of brain dopamine induced by γ-hydroxybutyrate. Life Sci 5:1921–1930

Gianutsos G, Moore KE (1978) Tolerance to the effects of baclofen and γ-butyrolactone on locomotor activity and dopaminergic neurons in the mouse. J Pharmacol Exp Ther 207:859–869

Giarman NJ, Roth RH (1964) Differential estimation of gamma-butyrolactone and gamma-hydroxybutyric acid in rat blood and brain. Science 145:583–584

Giarman NJ, Schmidt KF (1963) Some neurochemical aspects of the depressant action of γ-butyrolactone on the central nervous system. Br J Pharmacol 20:63–568

Grace AA, Bunney BS (1979) Paradoxical GABA excitation of nigral dopaminergic cells: Indirect mediation through reticulata inhibitory neurons. Eur J Pharmacol 59:211–218

Graham LT, Aprison MH (1966) Fluorometric determination of aspartate, glutamate and aminobutyrate in nerve tissue using enzymatic methods. Anal Biochem 15:487–497

Gundlach AL, Beart PM (1981) Differential effects of IV and IP muscimol on central dopamine metabolism. Psychopharmacology 74:71–73

Hill DR, Bowery NG (1981) ^{3}H-baclofen and ^{3}H-GABA bind to bicuculline-intensive GABA sites in rat brain. Nature 290:149–152

Hoesli L, Hoesli E, Lehmann R, Schneider J, Borner M (1983) Action of γ-hydroxybutyrate and GABA on neurons of cultured rat central nervous system. Neurosci Lett 37:257–260

Laborit H, Kind A, Regil CDL (1961) 220 cas d'anesthesie en neurochirurgie avec le 4-hydroxybutyrate de sodium. Presse Med 69:1216–1217

Maggi A, Enna SJ (1979) Characteristics of muscimol accumulation in mouse brain after systemic administration. Neuropharmacology 18:361–366

Magnusson O, Nilsson LB, Westerlund D (1980) Simultaneous determination of dopamine, DOPAC and homovanillic acid. Direct injection of supernatants from brain tissue homogenates in a liquid chromatography-electrochemical detection system. J Chromatography 221:237–247

Moroni F, Forchetti MC, Krogsgaard-Larsen P, Guidotti A (1982) Relative disposition of the GABA agonists THIP and muscimol in the brain of the rat. J Pharm Pharmacol 34:676–678

Naik SR, Guidotti A, Costa E (1976) Central GABA receptor agonists: Comparison of muscimol and baclofen. Neuropharmacology 15:479–484

Nowycky MC, Roth RH (1979) Chronic gamma-butyrolactone (GBL) treatment: a potential model of dopamine hypoactivity. Naunyn Schmiedeberg's Arch Pharmacol 309:247–254

Osorio I, Davidoff RA (1979) γ-hydroxybutyric acid is not a GABA-mimetic agent in the spinal cord. Ann Neurol 6:111–116

Pericic D, Eng N, Walters JR (1978) Post-mortem and aminooxyacetic acid-induced accumulation of GABA: Effect of gamma-butyrolactone and picrotoxin. J Neurochem 30:767–773

Rosenthal HE (1967) A graphical method for the determination and presentation of binding parameters in a complex system. Anal Biochem 20:525–532

Roth RH, Giarman NJ (1970) Natural occurence of gamma-hydroxybutyrate in mammalian brain. Biochem Pharmacol 19:1087–1093

Roth RH, Murrin LC, Walters JR (1976) Central dopaminergic neurons: Effects of alterations in impulse flow on the accumulation of dihydroxyphenylacetic acid. Eur J Pharmacol 36:163–172

Roth RH, Nowycky MC (1977) Dopaminergic neurons: Effects elicited by γ-hydroxybutyrate are reversed by picrotoxin. Biochem Pharmacol 26:2079–2082

Rumigny JF, Maitre M, Cash C, Mandel P (1981) Regional and subcellular localization in rat brain of the enzymes that can synthesize γ-hydroxybutyrate. J Neurochem 36:1433–1438

Snead OC, Bearden JL, Pegram V (1980) Effect of acute and chronic anticonvulsant administration on endogenous γ-hydroxybutyrate in rat brain. Neuropharmacology 19:47–52

Unnerstall JR, Pizzi WJ (1981) Muscimol and γ-hydroxybutyrate: similar interactions with convulsant agents. Life Sci 29:337–344

Walters JR, Roth RH, Aghajanian GK (1973) Dopaminergic neurons: similar biochemical and histochemical effects of gamma-hydroxybutyrate and acute lesions of the nigroneostriatal pathway. J Pharmacol Exp Ther 186:630–639

Worms P, Lloyd KG (1980) Biphasic effects of direct, but not indirect, GABA mimetics and antagonists on haloperidol-induced catalepsy. Naunyn-Schmiedeberg's Arch Pharmacol 311:179–184

Received October 7, 1983/Accepted July 4, 1984