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Comparison of Epileptogenic Properties of Unsubstituted and β-Alkyl-Substituted γ-Butyrolactones

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SUMMARY

β-Ethyl-β-methyl- and β,β-dimethyl-γ-butyrolactones (β-EMGBL, β-DMGBL), their corresponding hydroxy acids (β-EMGHB, β-DMGHB), and rigid *cis* and *trans* analogues of γ-hydroxybutyrate were synthesized. These compounds were tested in mice and guinea pigs *in vivo* and in incubated guinea pig hippocampal slices, and their effects were compared with those of unsubstituted γ-butyrolactone (GBL) and γ-hydroxybutyrate (GHB). β-EMGBL produced convulsive seizures and epileptiform electroencephalographic (EEG) discharges consisting of spike and polyspike activity. β-DMGBL, β-EMGHB, and the rigid *cis* analogue of GHB produced behavioral and EEG effects similar to that of β-EMGBL, but all were less potent. In contrast, GBL, GHB, and the rigid *trans* analogue of GHB caused nonconvulsive seizures and epileptiform EEG discharges consisting mainly of irregular slow-wave activity. Neuronal activity in hippocampal slices was markedly activated by β-EMGBL, but β-EMGHB, GBL, and GHB had little or no effect. Seizures produced by both the substituted and unsubstituted GBLs were suppressed by ethosuximide but not by phenytoin. The results of this study demonstrate that β-alkyl-substituted GBLs are convulsant agents and the effects of these compounds are different from those of GHB or GBL. The results also suggest that the β-substituted compounds are active in a lactone form whereas the unsubstituted compounds are active as hydroxy acids. Thus the β-substituted and the unsubstituted compounds probably act at different sites and have different mechanisms of action.

INTRODUCTION

GBL[2] was synthesized over 100 years ago (1) and has been known to be a neuropharmacologically active agent for the past 35 years (2). Recent studies have also demonstrated that GHB, the hydrolysis product of GBL, is a naturally occurring substance in brain tissue of several mammalian species (3), including man (4). The molecular and cellular actions of GBL and GHB in nerve tissue and their roles in normal and abnormal neurological function have recently been reviewed (5). Although their mechanisms of action are poorly understood at present, available information does suggest that they may be involved in the pathophysiological mechanisms of some seizure types.

It has been reported (6–8) that the systemic administration of GBL or GHB to experimental animals produces seizures, and their behavioral and EEG characteristics have been suggested to resemble petit mal absence seizures. Experimental seizures produced by GBL or GHB are blocked by drugs such as ESM and trimethadione, which are effective in the treatment of petit mal absence seizures in humans. Of interest, there are structural similarities between the heterocyclic rings of GBL, ESM, and trimethadione. However, GBL lacks the small alkyl group substitutions known to be essential for the anticonvulsant activity of ESM and trimethadione (9). Possibly, alkyl-substituted GBL may have properties very different from those of the unsubstituted compound. To test this idea, we have synthesized several alkyl-substituted GBLs. The behavioral and electrophysiological effects of these compounds were tested in mice and guinea pigs *in vivo* and in incubated hippocampal slices, and several were found to have convulsant or anticonvulsant properties. In the present report, the epileptogenic effects of β-substituted GBLs and rigid *cis* and

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[2] The abbreviations used are: GBL, γ-butyrolactone; GHB, γ-hydroxybutyrate; EEG, electroencephalograph(ic); ESM, ethosuximide; β-EMGBL, β-ethyl-β-methyl-γ-butyrolactone (β-EMGHB, the corresponding hydroxy acid); β-DMGBL, β,β-dimethyl-γ-butyrolactone (β-DMGHB, the corresponding hydroxy acid); NHCL, *endo*-bicyclo[2.2.1]hept-5-ene-*cis*-2-hydroxymethyl-3-carboxylic acid lactone; NHCE, bicyclo[2.2.1]hept-5-ene-*trans*-2-hydroxymethyl-3-carboxylic acid ethyl ester; *cis*-NHCA, *endo*-bicyclo[2.2.1]hept-5-ene-*cis*-2-hydroxymethyl-3-carboxylic acid; *trans*-NHCA, bicyclo[2.2.1]hept-5-ene-*trans*-2-hydroxymethyl-3-carboxylic acid; PHT, phenytoin; CD_{50}, convulsant dose 50%.

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trans analogues of GHB are described and compared with unsubstituted GBL and GHB. Companion reports describe the anticonvulsant actions of α- and γ-substituted GBLs (10) and discuss the structure-activity relationships of alkyl-substituted GBLs (11).

MATERIALS AND METHODS

Chemical Syntheses

Melting points were determined on a Kofler micro hot stage and are uncorrected. Boiling points were recorded during distillation at reduced pressure and also are uncorrected. Proton magnetic resonance spectra were recorded in chloroform-*d* on a Varian Associates Model T-60 spectrometer. Chemical shifts are given in δ units (parts per million) downfield from tetramethylsilane internal standard. The multiplicity is identified by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets), and dq (doublet of quartets). Relative peak heights are noted as integers after the multiplicity. IR spectra were recorded neat or in KBr on a Beckman Model IR-18 instrument. Characteristic bands are listed in units of reciprocal centimeters.

Synthesis of β-EMGBL, β-DMGBL, and NHCL. β-EMGBL, β-DMGBL, and NHCL were synthesized by regiospecific sodium borohydride reduction of the corresponding cyclic anhydride (12) by the method of Bailey and Johnson (13). All compounds had satisfactory IR and proton magnetic resonance spectra.

Synthesis of NHCE. The Diels-Alder adduct formed by the reaction of cyclopentadiene and fumaric acid monoethyl ester [Aldrich Chemical Company (Milwaukee, Wisc.)] was converted from the monoethyl ester-acid to the monoethyl ester-alcohol by the procedure of Paquette and Nelson (14). Briefly stated, the monoethyl ester acid was converted to the monoethyl ester-acid chloride with $SOCl_2$ [Fisher Scientific Company (Pittsburgh, Pa.)] and then reduced to the monoethyl ester-alcohol with $NaBH_4$. The ester alcohol thus produced was a mixture of two pairs of stereoisomers and was not further purified. It was a fragrant, colorless liquid, b.p. 118–127° (0.8 mm Hg). The IR spectra had a single strong carbonyl absorption at 1735 cm^{-1} and a hydroxyl absorption at 3500 cm^{-1}. The proton NMR in $CDCl_3$ was complex and showed the following: δ 1.25 (*dt*, 3, $-COOCH_2CH_3$); 1.54 (*m*, 2, $-CHCH_2CH-$); 1.88 (*m*, 1, $-CHCH_2OH$); 2.65 (*m*, 1, $-CHCOOCH_2CH_3$); 2.9–3.9 (*m*, 4, $-CHCH_2CH-$ and $-CH_2OH$); 4.14 (*dq*, 2, $-COOCH_2CH_3$); 6.18 (*m*, 2, $-CH=CH-$); the —OH proton appeared anywhere from 5.0 to 7.0 depending on concentration.

Hydrolysis of lactones and esters. An ethanolic solution of excess NaOH was added to a solution of the lactone or ester in ethanol, and the mixture was refluxed for 60 min. The ethanol was removed under vacuum, the residue was dissolved in an appropriate volume of water, and the pH was adjusted to 7.5–8.0 with dilute HCl before the solution was brought to the final volume.

Testing of Behavioral and Electrophysiological Effects

Behavioral effects in mice. Female Swiss-Webster mice (20–30 g, Lab Supply) received i.p. injections or i.v. injections (in the lateral tail vein) of drugs in a volume of 10 μl/g. ESM (Parke-Davis, Morris Plains, N. J.) was given as an aqueous solution 30 min before convulsant challenges, and PHT (Parke-Davis) was given as a suspension in 30% propylene glycol 60 min before convulsant challenges. All other drugs were given as solutions in 30% propylene glycol in the doses indicated. Seizures were monitored by three markers: myoclonic twitches, generalized clonic seizures, and tonic seizures. The CD_{50} was determined graphically. The propylene glycol solvent alone, in the amounts used in these experiments, had no apparent effects.

EEG recording in paralyzed-ventilated guinea pigs. Female albino guinea pigs (200–400 g, Issacs) were lightly anesthetized with ether, tracheotomized, and paralyzed with Flaxedil (50 mg/kg) (gallamine triethiodide, Davis & Geck). A 1% solution of procaine HCl (Novocain; Breon Laboratories, New York, N. Y.) was immediately applied to all wound margins and the animals were ventilated with an N_2O/O_2 mixture, the ratio of which was adjusted around 65/35 to keep the arterial pO_2 above 100 mm Hg. The arterial pCO_2 was kept at approximately 35 mm Hg by adjusting the ventilation rate. The arterial pH was thus kept approximately 7.40 ± 0.05. The body temperature was maintained at 37 ± 0.5° by a heating apparatus. A jugular vein and a femoral artery were cannulated for drug injections and blood pressure/blood gas monitoring, respectively. The skull was then exposed and four stainless steel electrodes were placed in the parietal bones, two on each side of the sagittal suture, approximately 2 mm lateral to it and 6 mm apart from the other electrode on the same side.

The blood pressure was continuously monitored and the animal was rejected if the pressure fell significantly below normal (76/48 mm Hg) except transiently after drug injections. The EEG was recorded bilaterally on a Grass Model 7D polygraph at a band-pass frequency (half-amplitude) of 1–75 Hz. Sensitivity was 500 μV/cm or 1 mV/cm.

Electrophysiological studies of hippocampal slices.

Preparation of slices. White, female guinea pigs (200–250 g, Issacs) were decapitated and their brains were rapidly removed. The hippocampus was quickly dissected free and sliced transversely in 350- to 400-μm slices on a McIlwain tissue chopper. The slices were floated onto a nylon stage constantly perfused with medium similar to the extracellular fluid in brain, containing 127 mM NaCl, 2.0 mM KCl, 1.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 25.7 mM $NaHCO_3$, 1.10 mM KH_2PO_4, and 10 mM glucose. The medium was constantly bubbled with 95% O_2/5% CO_2 to maintain a pH of 7.4.

Recording and stimulation. Glass microelectrodes filled with 2 M NaCl with tip impedences of 1–2 Mohm were positioned with a micromanipulator in the pyramidal layer of CA_3 and led through a high-impedence amplifier in parallel to an oscilloscope and a Grass Model 7D polygraph. Evoked activity was elicited by brief stimulation (0.2-msec duration at 15 Hz) of the mossy fibers via bipolar metal electrodes. The stimulus intensity was adjusted to give a stable, submaximal response and varied from 5 to 15 V but remained constant within each experiment. The slices were allowed to equilibrate for approximately 1 hr before drug applications. The stability of the

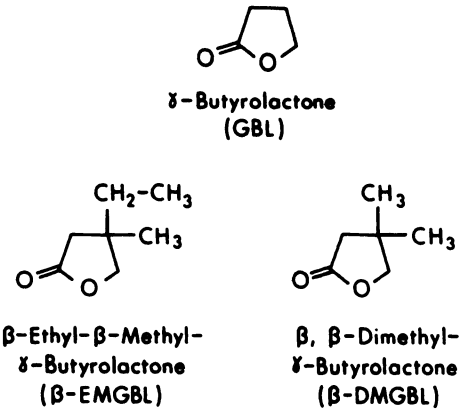

FIG. 1. *Chemical structures of GBL and two β-substituted GBLs*

equilibrated slices was determined by monitoring evoked potentials.

Drug application. Compounds to be tested were dissolved in the perfusion medium at the indicated concentration. At all concentrations of drugs used there was no significant effect on molarity or pH of the medium.

RESULTS

Effects on behavior in mice. Mice receiving i.v. or i.p. injections of GHB (125–1000 mg/kg) showed a dose-dependent change in behavior characterized by cessation of spontaneous exploratory activity, generalized tremor, ataxia, and exophthalmia. However, even at the highest dose (1000 mg/kg; 7.9 mmole/kg), no generalized clonic or tonic seizures were observed, and all animals recovered.

Mice receiving injections of β-EMGBL (Fig. 1) also had dose-dependent changes in behavior (Table 1), but these were much different from the effects of GHB. These seizures were characterized by myoclonic twitches, clonic seizures, and, at sufficiently high doses, tonic seizures. The CD_{50} for tonic seizures of β-EMGBL given i.p. was 30 mg/kg (230 μmoles/kg) (Table 1). The dimethyl derivative, β-DMGBL (Fig. 1) showed exactly the same effects as β-EMGBL except that it was 7-fold less potent, having a CD_{50} for tonic seizures of 185 mg/kg (1.6 mmoles/kg).

When given i.v., the effects of β-EMGBL were almost instantaneous and occurred at 9-fold lower doses (Table 1). The CD_{50} for tonic seizures when β-EMGBL was given i.v. was reduced to 27 μmoles/kg. The β-substituted derivatives were also more potent when given i.v. as lactones than as the hydroxy acids. When β-EMGBL was given i.v. in the hydrolyzed form (β-EMGHB), the CD_{50} for tonic seizures was increased 6 times, to 165 μmoles/kg (Table 1). Also, tonic seizures were delayed and required approximately 60 sec to occur.

Table 2 shows the actions of two anticonvulsant drugs on the effects of β-substituted lactones in mice. At a 50 mg/kg i.p. dose of β-EMGBL, mice typically had a first twitch at 30 sec after injection, a clonic seizure at 40 sec, and all had a fatal tonic seizure at approximately 2 min. Pretreatment with ESM (250 mg/kg) had little effect; with 500 mg/kg the first twitch occurred at approximately 60 sec and the clonic seizure at 90 sec; however, no animal had a tonic seizure, and all survived. With a dose of PHT of 50 mg/kg, twice its ED_{50} for maximal electroshock seizures, the twitch occurred at 25 sec and the clonic seizure at 30 sec. The animals then had extremely vigorous continuous clonic seizures for the next 5–15 min, ending in exhaustion and death. Although no

TABLE 1

Time course and dose response of β-EMGBL and β-DMGBL in mice

Compound	Dose		Time[a] to	
	mg/kg	mmoles/kg	First clonic seizure	Tonic seizure
			sec	
β-EMGBL (i.p.)	12.5	0.10	— (0/4)	— (0/4)
	18.0	0.14	98 ± 14 (3/4)	— (0/4)
	30.0	0.23	42 ± 4 (3/4)	122 ± 4 (2/4)
	50.0	0.39	37 ± 10 (4/4)	121 ± 61 (4/4)
β-DMGBL (i.p.)	100	0.88	105 (1/4)	— (0/4)
	150	1.30	52 ± 6 (4/4)	— (0/4)
	225	2.00	32 ± 1 (4/4)	82 ± 17 (4/4)
	500	4.40	28 ± 3 (4/4)	37 ± 8 (4/4)
β-EMGBL (i.v.)	2.5	0.02	8 ± 1 (2/4)	— (0/4)
	3.5	0.03	Immediate	8 ± 2 (2/4)
	4.5	0.04	Immediate	7 ± 1 (3/4)
	6.0	0.05	Immediate	6 ± 1 (4/4)
	9.0	0.07	Immediate	11 ± 3 (4/4)
β-EMGHB (i.v.)	13	0.08	— (0/4)	— (0/4)
	20	0.12	13 ± 8 (3/4)	14 (1/4)
	40	0.23	18 ± 3 (4/4)	59 ± 19 (3/4)
	65	0.39	4 ± 1 (4/4)	6 ± 1 (4/4)

[a] Times indicate the period elapsed between injection of the compound and the occurrence of the specified event. The number of animals exhibiting seizure activity versus the number receiving injections is indicated in parentheses. Dose of β-EMGHB refers to the sodium salt of the hydroxy acid. Values are means ± standard error of the mean.

TABLE 2

Effect of anticonvulsant drugs on seizures induced by β-EMGBL and β-DMGBL

Pretreatment	Fraction protected	
	β-EMGBL[a]	β-DMGBL[a]
None	0/4	0/4
ESM, 250 mg/kg	1/4	3/4
ESM, 500 mg/kg	4/4	4/4
PHT, 50 mg/kg	0/4[b]	0/4[b]

[a] β-EMGBL, 50 mg/kg i.p.; β-DMGBL, 250 mg/kg i.p.
[b] Continuous clonic seizure resulting in death.

tonic seizure occurred, the clonic seizures were exacerbated and no animal was protected from death. The effects of these anticonvulsants were similar on seizures induced by β-DMGBL (250 mg/kg i.p.), except that ESM (250 mg/kg) provided 75% protection.

Effects on the EEG in guinea pigs. The GHB-induced changes in guinea pig EEGs (Fig. 2) were characterized by hypersynchronous slow waves with high-frequency oscillations; continuous, irregular, high-voltage hypersynchrony; and then polyspike bursts followed by increasingly long periods of electrical silence. These effects were dose-related and reversible over the range of 50–1000 mg/kg.

The EEG changes induced by β-EMGBL, β-EMGHB, and β-DMGBL (Figs. 2 and 4) were characterized by intermittent hypersynchronous bursts of spikes and slow waves which proceeded to a generalized high-frequency discharge. This was followed by polyspike bursts gradually decreasing in frequency until a period of electrical silence was observed. These effects were also dose-dependent but not reversible with doses of β-EMGHB over 70 mg/kg. The threshold dose for generalized high-frequency discharges with β-EMGHB was 50 mg/kg and the latency was approximately 60 sec. When injected as the lactone (β-EMGBL) discharges began within 10 sec at doses as small as 10 mg/kg. The dimethyl derivative, β-DMGBL, produced exactly the same effects except that a dose of approximately 100 mg/kg was required. At no dose did these β-substituted compounds produce changes similar to those of GHB.

Norbornene analogues with *cis*- and *trans*-GHB moieties (Fig. 3) were synthesized and tested in an attempt to determine the active conformational state of GHB. The *cis* analogue produced discharges identical with those of β-EMGHB, although with a slower onset of 15–20 min and a reduced potency, requiring 500–1000 mg/kg to produce the generalized high-frequency discharge (Fig. 4B). Both the lactone and hydroxy acid of the *cis* analogue were active, but the lactone was 10 times more potent. The *trans* analogue produced EEG changes similar to those of GHB (Fig. 4C), but again the potency was less, requiring 500–1000 mg/kg to produce brief (5- to 10-min) changes.

The effects of anticonvulsant drugs on GHB were similar to those observed in mice. As has been previously reported (7), ESM prevented the GHB-induced EEG changes whereas PHT had no beneficial effect. A 250 mg/kg i.v. dose of ESM 30 min before a 200 mg/kg i.v.

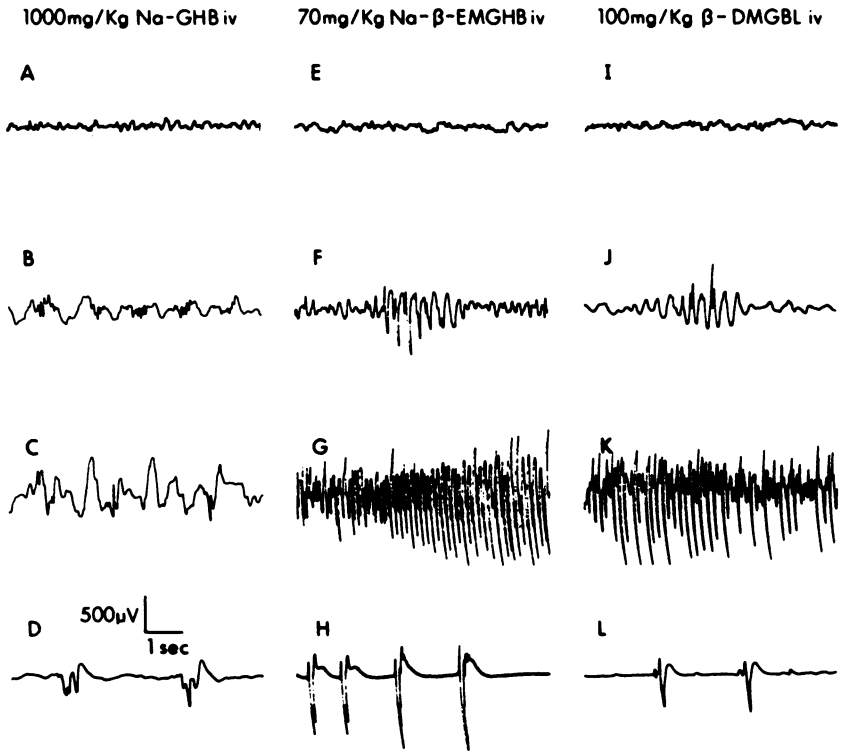

FIG. 2. *Progressive stages of seizure activity induced by GHB, β-EMGHB, and β-DMGBL*

A–D, Na-GHB, 1000 mg/kg i.v.; E–H, Na-β-EMGHB, 70 mg/kg i.v.; I–L, β-DMGBL, 100 mg/kg i.v. EEGs were recorded from paralyzed-ventilated guinea pigs as described under Materials and Methods. A, E, and I are control traces obtained before administration of the convulsant drugs.

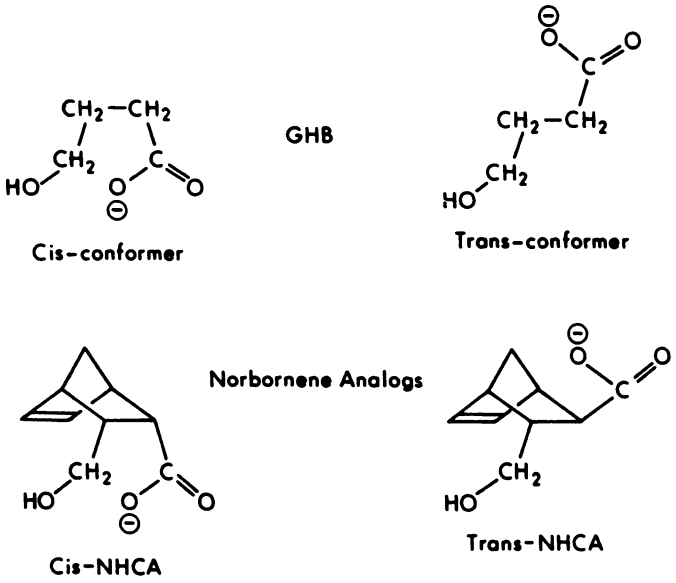

FIG. 3. *Chemical structure of GHB showing cis and trans conformers and the rigid norbornene analogues, cis-NHCA and trans-NHCA*

dose of GHB prevented all abnormal EEG discharges. A 50 mg/kg dose of PHT 60 min before the same dose of GHB had no effect. A 250 mg/kg dose of ESM also completely prevented all epileptiform activity induced by β-EMGHB (50 mg/kg). In contrast, PHT (50 mg/kg) did not prevent the generalized high-frequency discharge produced by β-EMGHB, but changed its frequency from approximately 7 Hz to 2–3 Hz and extended the duration of subsequent phases of hypersynchrony from under 5 to over 15 min.

Doses of GHB of 200–800 mg/kg had no effect on the discharges produced by β-EMGHB (50 mg/kg). When GHB was given first and the typical hypersynchrony was established, treatment with β-EMGHB interrupted this activity with a progression through the stages typical of this convulsant; the GHB pattern then returned and proceeded as usual to completion.

Effects on blood pressure were also noted with GHB and β-EMGHB. A 1000 mg/kg dose of GHB caused an increase from 75/40 mm Hg before injection to an average value of 109 ± 10/65 ± 11 during the first 15 min after injection. However, a 250 mg/kg dose of GHB, which still produced epileptiform activity, did not cause an increase in blood pressure. The effects of β-EMGHB were more striking. A 50 mg/kg dose raised the blood pressure from 75/40 to 129 ± 8/80 ± 7 during the first 15 min after injection. However, this increase in blood pressure could still be observed without epileptiform activity while infusing small amounts of the drug. When given to an animal with a high transection of the spinal cord, β-EMGHB had no pressor effect. The blood pressure before injection was 95/40 and averaged 96 ± 8/36 ± 5 during the first 15 min after injection. However, epileptiform discharges occurred as usual. This would suggest that the effect on blood pressure is centrally mediated and that the convulsant and pressor effects can be separated. ESM (250 mg/kg) prevented this rise in blood pressure as well as the epileptiform discharges in normal animals.

Effects on the electrical activity of incubated hippocampal slices. Spontaneous electrical activity recorded with an extracellular electrode placed in the pyramidal cell layer of CA_3 consisted of random single-unit activity in slices in control buffer. Stimulation of dentate mossy fibers produced a complex evoked potential of approxi-

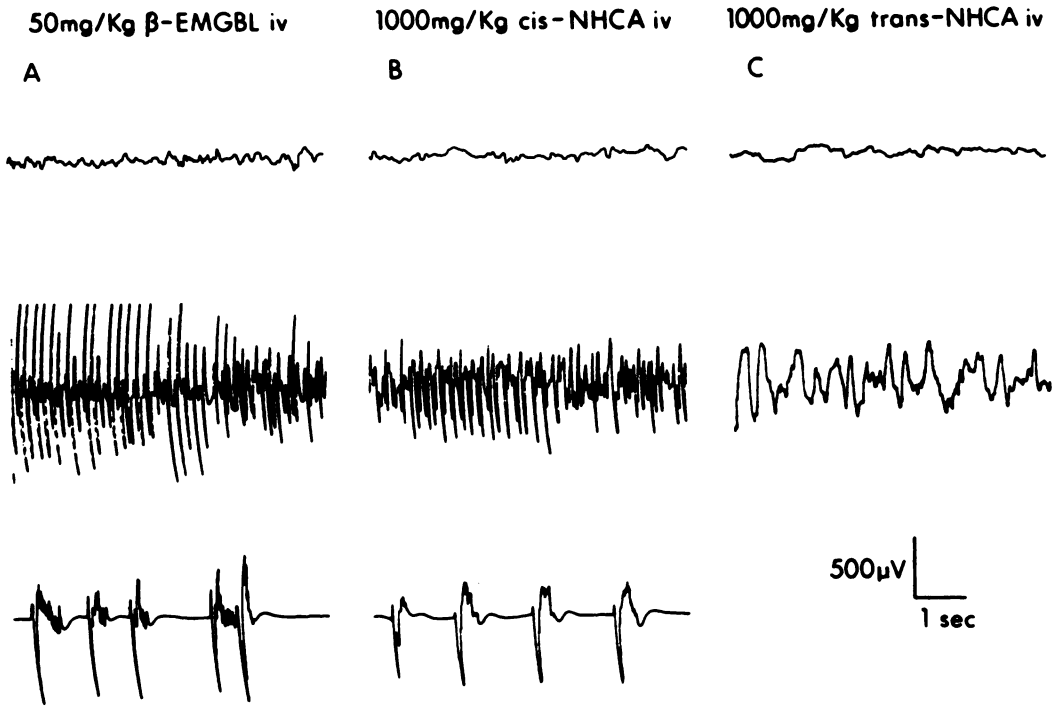

FIG. 4. *Seizure activity induced by convulsants*

A, β-EMGBL, 50 mg/kg i.v., control, 12 sec, and 100 sec after injection; B, Na-*cis*-NHCA, 1000 mg/kg i.v., control, 26 min, and 27 min after injection; C, Na-*trans*-NHCA, 1000 mg/kg i.v., control and 2 min after injection. EEGs were recorded as in Fig. 3.

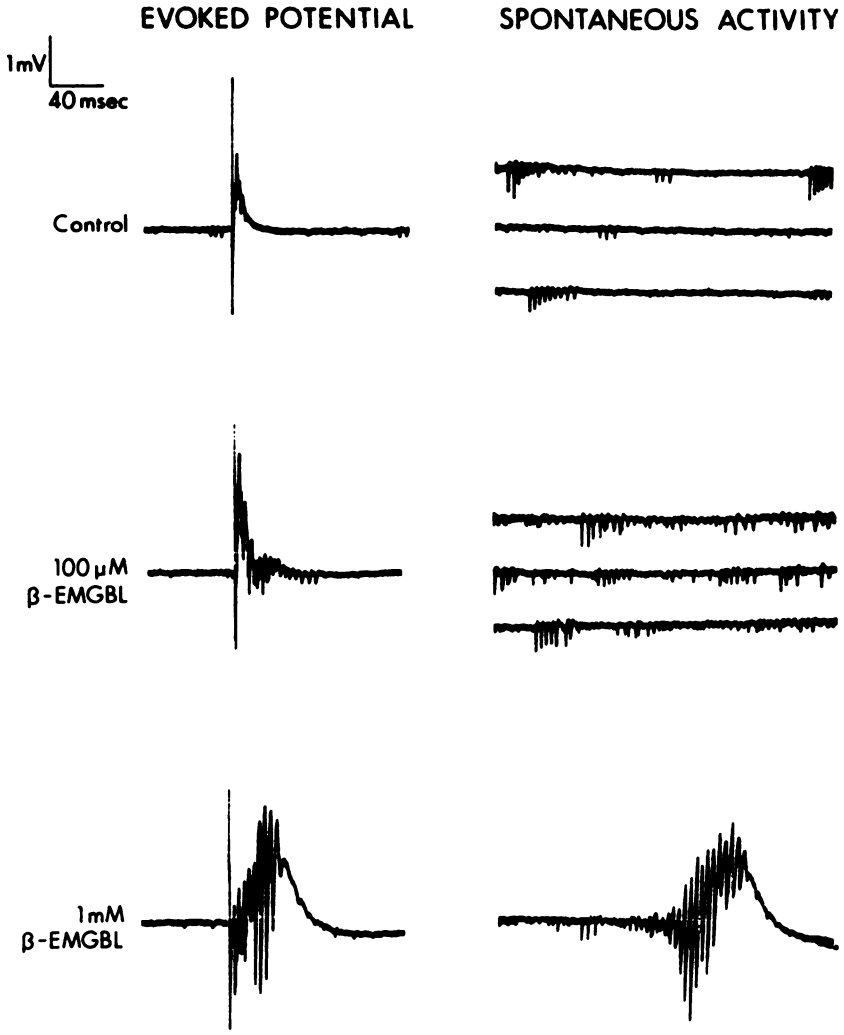

FIG. 5. *Effects of β-EMGBL on evoked and spontaneous electrical activity of incubated hippocampal slices*
The tissue was prepared as described under Materials and Methods and incubated in control buffer alone or control buffer plus 100 µM or 1 mM β-EMGBL. Time and voltage calibrations are shown at *upper left.*

mately 20- to 30-msec duration (Fig. 5). Concentrations of GBL of 10 µM–1 mM had no effect on spontaneous or evoked activity. GHB also had no effect (data not shown). In sharp contrast, β-EMGBL caused a dose-dependent excitation at concentrations of 10 µM or higher. Concentrations of 10–100 µM increased the duration of evoked potentials to 60–80 msec but had no effect on spontaneous activity (Fig. 5). At a concentration of 1 mM, evoked potentials were again increased, and spontaneous paroxysmal discharges of several millivolts lasting 60–100 msec appeared in six of nine experiments (Fig. 5). This complex activity, commonly referred to as epileptiform discharges, occurred at a rate of 5–20/min. At a concentration of 10 mM, β-EMGBL increased both evoked potentials, and spontaneous epileptiform discharges at a rate of 15–20/min were noted in all experiments.

In sharp contrast to the lactone, 1 mM β-EMGHB, the hydroxy acid derivative, caused no excitation of the evoked potential and little or no change in the random, spontaneous, single-unit activity. No epileptiform discharges were seen even at higher concentrations of 10 or 30 mM.

DISCUSSION

Several previous studies (6–8) have indicated that the systemic administration of GHB and GBL to experimental animals produces behavioral and EEG changes which can be best characterized as nonconvulsive seizures, and the present results confirm these effects. Structural similarities between GBL and anticonvulsant drugs such as ESM and trimethadione prompted us to synthesize and test the actions of alkyl-substituted GBLs. The only previous studies of these compounds were reported by Enders *et al.* (15) several years ago. These authors found that several β-alkyl-substituted GBLs, including β-EMGBL and β-DMGBL, produced seizures in rats, but the characteristics of the seizures were not described in any detail. We confirm the finding of Enders *et al.* (15) and further demonstrate that β-EMGBL and β-DMGBL produce convulsive seizures which have behavioral and EEG characteristics which are markedly different from those of GHB. We also found that GHB has little or no effect on neuronal activity in incubated hippocampal slices, whereas β-EMGBL consistently increases the du-

ration and amplitude of evoked potentials and frequently produces spontaneous epileptiform discharges. Although the epileptogenic effects of GHB and β-alkyl-substituted GBLs are clearly different, their responses to PHT and ESM are similar. The seizures produced by both are suppressed by ESM but not by PHT.

Systemic administration of either GHB or GBL produce identical behavioral and EEG effects. Previous studies have indicated that only GHB is active and that GBL exerts an effect only after it is hydrolyzed by blood lactonase (16, 17). In contrast to the situation with GBL and GHB, our data suggest that β-EMGBL and not its corresponding hydroxy acid is active. In support of this suggestion are the observations that β-EMGBL is much more potent than β-EMGHB and also has a more rapid onset of action. The fact that systemic administration of β-EMGHB produces an effect is probably due to its being nonenzymatically lactonized to β-EMGBL. It is unlikely that β-EMGBL is being converted to β-EMGHB prior to acting in brain, since substituted GBLs are not substrates for plasma or liver lactonases and brain apparently has no lactonase activity (16). Second, the *cis*-norbornene GHB derivative, which can relactonize, causes epileptiform discharges like the β-substituted lactones; the *trans* analogue, which cannot lactonize, never causes this type of discharge. Finally, the most convincing evidence indicating that the lactone, and not the hydroxy acid, is active is the finding that direct application of the former to incubated hippocampal slices produces increased neuronal activity and epileptiform discharges whereas the latter has no effect.

The fact that both unsubstituted and β-alkyl-substituted GBLs are epileptogenic raises the possibility that they are acting at the same site in brain. Several lines of evidence suggest that this is not the case. First, the unsubstituted and substituted compounds produce seizures and EEG changes which are markedly different. Second, β-EMGBL is stimulatory in hippocampal slices *in vitro*, whereas GHB and GBL are inactive. Third, it appears that the unsubstituted compounds are active as hydroxy acids and the substituted compounds are active as lactones (see above). Finally, the observation that the *cis*-norbornene GBL analogue causes seizures similar to those caused by β-EMGBL and the *trans*-norbornene GBL analogue causes seizures like those of GHB suggests that the configurations of the sites of action of GHB and β-EMGBL are different. Thus we conclude that the sites of action of GHB and β-EMGBL are distinct and separate. It has been proposed that GHB may act at γ-aminobutyric acid receptors (18–20). Although the site of action of β-EMGBL is still unknown, we are impressed with the fact that its effects are very similar to those of picrotoxin. The possible relationships between alkyl-substituted GBL and picrotoxin are considered in a companion paper (11).

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