our experiments $10^{-5}M$ GABA failed to produce significant changes in the contractility of isolated cat colon but did enhance the contraction induced by transmural stimulation (1-msec pulses, 30 V, 0.1 Hz) and abolished by tetrodotoxin. Thus, GABA may affect the motility of the mammalian intestine by stimulating nerves rather than smooth muscle. These findings suggest that GABA plays a physiological role in the mammalian intestine.

KOHTARO TANIYAMA
MASATO KUSUNOKI
NAOAKI SAITO
CHIKAKO TANAKA*

Department of Pharmacology, Kobe University School of Medicine, Kobe 650, Japan

References and Notes

1. K. R. Jessen *et al.*, *Nature (London)* **281**, 71 (1979); A. Krantis and D. I. B. Kerr, *Neurosci. Lett.* **23**, 263 (1981).
2. K. Taniyama, Y. Miki, C. Tanaka, *Neurosci. Lett. Suppl.* **6**, S72 (1981).
3. J. M. Walsh, N. G. Bowery, D. A. Brown, J. B. Clark, *J. Neurochem.* **22**, 1145 (1974).
4. M. C. W. Minchin and L. L. Iversen, *ibid.* **23**, 533 (1974).
5. N. G. Bowery, D. A. Brown, S. Marsh, *J. Physiol. (London)* **293**, 75 (1979).
6. A. Sellström and A. Hamberger, *Brain Res.* **119**, 189 (1977).
7. B. Katz and R. Miledi, *J. Physiol. (London)* **192**, 407 (1967); J. I. Hubbard, S. F. Jones, E. M. Landau, *ibid.* **196**, 75 (1968); C. D. Richards and R. Sercombe, *ibid.* **211**, 571 (1970); R. P. Rubin, *Pharmacol. Rev.* **22**, 389 (1970).
8. W. D. M. Paton and M. A. Zar, *J. Physiol. (London)* **194**, 13 (1968).
9. E. Bülbring and M. D. Gershon, *ibid.* **192**, 823 (1967).
10. R. Franco, M. Costa, J. B. Furness, *Naunyn-Schmiedebergs Arch. Pharmakol.* **306**, 195 (1979).
11. T. Cocks and G. Burnstock, *Eur. J. Pharmacol.* **54**, 251 (1979).
12. F. Hobbiger, *J. Physiol. (London)* **142**, 147 (1958); E. Florey and H. McLennan, *ibid.* **145**, 66 (1959); A. Inouye, M. Fukuya, K. Tsuchiya, T. Tsujioka, *Jpn. J. Physiol.* **10**, 167 (1960); G. P. Lewis, C. McMartin, S. R. Rosenthal, C. Yates, *Br. J. Pharmacol.* **45**, 104 (1972).
13. A. Krantis, M. Costa, J. B. Furness, J. Orbach, *Eur. J. Pharmacol.* **67**, 461 (1980).
14. Supported by grants from the Ministry of Education, Science, and Culture and the Ministry of Health and Welfare, Japan. We thank Y. Okada for pertinent discussions and M. Ohara for critical reading of the manuscript.
* Correspondence should be addressed to C.T.

22 February 1982; revised 26 May 1982

Alpha-Substituted γ-Butyrolactones: New Class of Anticonvulsant Drugs

Abstract. *Alkyl-Substituted γ-butyrolactones were synthesized and tested for their convulsant and anticonvulsant actions in mice and guinea pigs. The alpha-substituted compounds, α, α-dimethyl-, and α-ethyl-α-methyl-γ-butyrolactone were anticonvulsant compounds with a spectrum of activity similar to that of ethosuximide. In contrast, beta-substituted compounds were convulsant agents similar to picrotoxinin. The alpha-substituted γ-butyrolactones represent a new class of anticonvulsant drugs with experimental and clinical potential.*

γ-Butyrolactone (GBL) and its corresponding hydroxy acid, γ-hydroxybutyrate (GHB) (Fig. 1) produce nonconvulsive seizures in experimental animals that resemble petit mal absences in humans (*1*). Like petit mal absences, seizures produced by GBL or GHB are selectively blocked by ethosuximide and trimethadione. These two antiepileptic drugs are chemically and structurally similar to GBL in that all are five-membered heterocyclic rings; however, ethosuximide and trimethadione have small alkyl side chains. This observation led us to hypothesize that alkyl substitution of GBL may produce a compound with anticonvulsant properties. We synthesized several alkyl-substituted GBL's (Fig. 1) and examined their convulsant and anticonvulsant properties.

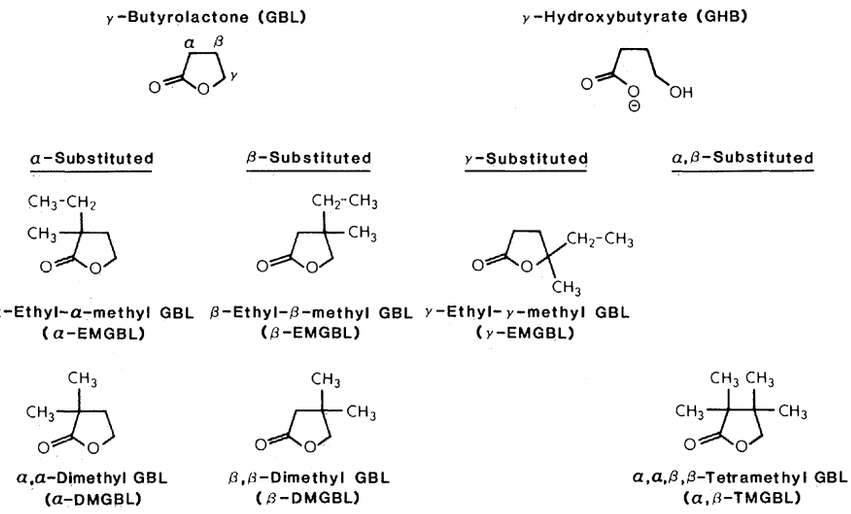

Fig. 1. Chemical structures of some alkyl-substituted GBL's.

After the drugs were synthesized (*2*), we tested each drug in mice for behavioral and toxic effects (*3*) and in paralyzed and ventilated guinea pigs for effects on the electroencephalogram (EEG) (*4*). The effects of some agents on electrical activity were also examined on incubated slices of guinea pig hippocampus (*5*). Several drugs with anticonvulsant potential were further tested for their ability to block seizures produced by maximal electroshock, by pentylenetetrazole, and by picrotoxin (*6*).

Addition of small alkyl groups to the beta position of GBL produced agents with convulsant activity. All of these beta-substituted compounds produced generalized convulsive seizures and changes in EEG activity very much like that produced by pentylenetetrazole, but much different from the nonconvulsive seizures produced by unsubstituted GBL. This suggests that beta-substituted GBL's have a different site or mechanism of action (or both) from that of GBL itself.

γ-Butyrolactones with small alkyl groups substituted in the alpha position were active anticonvulsant agents. Both the α,α-dimethyl-GBL (α-DMGBL) and the α-ethyl-α-methyl-GBL (α-EMGBL) prevented seizures in mice induced by pentylenetetrazole, picrotoxin, and the convulsant beta-substituted GBL's. These seizures are all characterized by myoclonic twitches, generalized clonic seizures, and tonic extensor seizures that usually result in death. The alpha-substituted GBL's prolonged the time to the occurrence of the twitches and clonic seizures and, at sufficiently high doses, completely prevented the clonic seizures. The tonic seizures were totally prevented at doses lower than those required to protect against clonic seizures. For example, the median effective dose (ED_{50}) (*6*) of α-EMGBL for the prevention of pentylenetetrazole induced clonic seizures was 1.8 mmole/kg (1.5 to 2.0), and the ED_{50} for tonic seizures was 1.2 mmole/kg (1.0 to 1.4). The ED_{50} of α-DMGBL for pentylenetetrazole-induced tonic seizures was 3.1 mmole/kg (2.4 to 4.1). Neither α-EMGBL nor α-DMGBL had any protective effect against maximal electroshock seizures. Similar ef-

0036-8075/82/0910-1040$01.00/0

fects were observed in paralyzed and ventilated guinea pigs. Epileptiform discharges in the EEG of these animals were produced by the convulsant β-ethyl-β-methyl-GBL (β-EMGBL). These discharges were prevented by prior treatment with α-EMGBL or α-DMGBL. If given during the epileptiform discharge, which normally lasted over 30 minutes, the alpha-substituted GBL's immediately halted the irregular activity.

Since the hydroxy acid, GHB, and not the lactone, GBL, is the active form of these two drugs—which are interconvertible in vivo (*7*)—we compared the hydroxy acid (α-EMGHB) and lactone (α-EMGBL) forms of the alpha-substituted compound. Direct comparison in intact animals is complicated by the interconversion of the two forms. We therefore examined the action of these two compounds on incubated hippocampal slices. Epileptiform discharges were induced by continuous perfusion with the convulsant β-EMGBL. The lactone, α-EMGBL, completely reversed the epileptiform activity, whereas the hydroxy acid, α-EMGHB, had no effect. Given alone, neither of the alpha-substituted forms had a significant effect on the electrical activity of the slice. In addition, the hydroxy acid form of β-EMGBL was inactive in this system. Thus, the lactone appears to be the active form of the alkyl-substituted GBL's.

The toxic effects of α-EMGBL were determined in mice by behavioral observation and by the rotorod test (*3*). The α-EMGBL produced no observable behavioral effect in doses up to 1.6 mmole/kg. Doses of 1.6 to 2.7 mmole/kg produced slight ataxia and drowsiness that were apparent soon after injection and lasted for 10 to 15 minutes. With doses greater than 3 mmole/kg, marked sedation was evident. This also was the point at which about half the animals showed neurotoxicity by the rotorod test, the median toxic dose (TD_{50}) (*3*) being 3.1 mmole/kg (2.6 to 3.7). The anticonvulsant effects of α-EMGBL against pentylenetetrazole-induced seizures were present at one-half to one-third the toxic dose (Table 1). However the ratio of the TD_{50} to the ED_{50} of α-DMGBL was very close to 1.0.

Antiepileptic drugs encompass a wide range of chemical types, but in general can be grouped into two major classes. One group, which is best represented by ethosuximide, is effective in the treatment of petit mal epilepsy, but not in the treatment of generalized tonic-clonic convulsions. Experimentally, ethosuximide is capable of preventing pentylenetetrazole-induced seizures, but not maximal electroshock seizures. The other group, represented by phenytoin, prevents generalized tonic-clonic convulsions and maximal electroshock seizures, but not petit mal absences or pentylenetetrazole-induced seizures. A comparison of the effects of α-EMGBL, ethosuximide, and pentylenetetrazole shows that α-EMGBL is much more similar to ethosuximide, which it resembles structurally, than to pentylenetetrazole.

Table 1. Comparison of the anticonvulsant effects of ethosuximide, phenytoin, and α-EMGBL. Data were obtained as described in (*3*) and (*6*) unless otherwise noted. Numbers in parentheses show the 95 percent confidence intervals. Phenytoin prevents the tonic seizure induced by pentylenetetrazole, but exacerbates the clonic seizures and protects no animals from pentylenetetrazole-induced toxicity.

Anticonvulsant	ED_{50} (mmole/kg)		TD_{50} (mmole/kg)
	Pentylenetetrazole	Maximal electroshock	
α-EMGBL	1.2 (1.0 to 1.4)	> 3.2	3.1 (2.6 to 3.7)
Ethosuximide	0.9 (0.8 to 1.1)	> 3.2	3.1* (2.7 to 3.4)
Phenytoin		0.038* (0.032 to 0.041)	0.26* (0.21 to 0.29)

*Data from (*10*), obtained by the methods described in (*3*) and (*6*).

There are, however, finer points within the structure-activity relations of the alkyl-substituted GBL's than a simple comparison between ethosuximide and α-EMGBL would reveal. For example, the beta-substituted GBL's are convulsant, but are antagonized by GBL's with identical substituents at the alpha position. We also studied compounds substituted at both the alpha and the beta positions [see Fig. 1 and (*2*)]. These compounds are not only convulsant, but are more potent that the corresponding GBL's substituted only at the beta position. Thus, it is not simply the presence of alkyl groups at the alpha position that confers anticonvulsant activity, but also the concurrent absence of alkyl groups at the beta position.

Structure-activity studies also suggest that the β-alkyl GBL convulsants work at the same site as picrotoxinin, the active component of picrotoxin. Picrotoxinin is another convulsant compound with a β-alkyl-substituted GBL moiety that is essential for its convulsant activity (*8*). The site of action of this convulsant is generally accepted to be the chloride channel associated with the γ-aminobutyric acid (GABA) receptor since picrotoxinin selectively blocks GABA-induced increases in chloride flux (*9*). The alpha-substituted GBL's could be acting at the site normally occupied by picrotoxinin and possibly the other convulsant beta-substituted GBL's. This would prevent the convulsant agent from acting and would therefore be manifested as anticonvulsant activity.

γ-Butyrolactones with either dimethyl or ethyl, methyl substitution on the alpha position represent a new class of anticonvulsant agents. These compounds should be useful for studying mechanisms of certain types of seizures and their therapy. Alpha-substituted GBL's may also have potential as clinically effective antiepileptic drugs.

WILLIAM E. KLUNK
ANN C. MCKEON
DOUGLAS F. COVEY
JAMES A. FERRENDELLI
Division of Clinical Neuropharmacology, Departments of Pharmacology and *Neurology and Neurological Surgery, Washington University Medical School, St. Louis, Missouri 63110*

References and Notes

1. M. Godschalk *et al.*, *Neurosci. Lett.* **3**, 145 (1976); O. C. Snead, *Neurology* **28**, 643 (1978); W. D. Winters and C. E. Spooner, *Electroencephal. Clin. Neurophysiol.* **18**, 287 (1965).
2. W. E. Klunk, D. F. Covey, J. A. Ferrendelli, in preparation.
3. Female Swiss-Webster mice were injected intraperitoneally with drugs in a volume of 10 μl/g. Behavior was monitored for 30 minutes before testing for neurotoxicity by the rotorod test. In this test, a mouse must remain on a rod (2.5 cm in diameter), rotating at 6 rev/min for at least 1 minute in each of three trials. The TD_{50} is that dose at which 50 percent of the animals failed the rotorod test. This value was determined by the method of Litchfield and Wilcoxon (*11*) and is given with the 95 percent confidence interval in parentheses.
4. Female albino guinea pigs were lightly anesthetized with ether, tracheotomized, and paralyzed with Flaxedil. They were ventilated with a mixture of N_2O and O_2, and the blood gases and blood pressure were monitored through a femoral arterial cannula. Drugs were injected through a cannula in a jugular vein and bilateral EEG's were recorded from the parietal bones.
5. Slices of guinea pig hippocampus, 350 to 400 μm thick, were floated on a nylon stage and constantly perfused with medium similar to extracellular fluid in the brain (*2*). Evoked activity was elicited by stimulation of the dentate mossy fibers and was recorded by a glass electrode in the pyramidal layer of CA_3. Compounds to be tested were dissolved in the perfusion medium.
6. Drugs to be tested as anticonvulsants in mice were given 30 minutes before pentylenetetrazole (100 mg/kg, intraperitoneally), β-EMGBL, hydrolyzed (50 mg/kg, intraperitoneally), picrotoxin (5 mg/kg, intravenously) or a 2-second train of square-wave pulses with a duration of 2 msec, frequency of 50 Hz, and an amplitude of 70 V through moistened ear clips for maximal electro-

shock seizures. Anticonvulsant activity was defined as prevention of the tonic extensor seizure and toxicity induced by any or all of the convulsant stimuli, unless otherwise noted. Four animals were tested at each of four to six different doses of each anticonvulsant drug. Dose ranges were 0.7 to 3.2 mmole/kg for ethosuximide, 1.1 to 4.4 mmole/kg for α-DMGBL, and 0.5 to 3.9 mmole/kg for α-EMGBL. The ED_{50} is that dose at which anticonvulsant activity was present in 50 percent of the animals. This value was determined by the method of Litchfield and Wilcoxon (*11*) and is given with the 95 percent confidence interval in parentheses.

7. N. J. Giarman and R. H. Roth, *Science* **145**, 583 (1964).
8. C. H. Jarboe, L. A. Porter, R. T. Buckler, *J. Med. Chem.* **11**, 729 (1968).
9. D. M. Woodbury, in *Antiepileptic Drugs: Mechanisms of Action*, G. H. Glaser, J. K. Penry, D. M. Woodbury, Eds. (Raven, New York, 1980), pp. 249–304.
10. R. L. Krall, J. K. Penry, B. G. White, H. J. Kupferberg, E. A. Swinyard, *Epilepsia* **19**, 409 (1978).
11. J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exp. Ther.* **96**, 99 (1949).

12. Supported in part by NIH grants NS-14834, GM-07200, and GM-24483 and by NIH research career development award CA-00829 (D.F.C.).

26 March 1982; revised 12 May 1982

Functional Restoration of the Traumatically Injured Spinal Cord in Cats by Clonidine

Abstract. *The long-term, chronic, paralysis resulting from spinal cord injury in the cat has been reversed by the use of an α_2-adrenergic receptor agonist, clonidine. Administration of this drug resulted in "normalization" of sensory-motor and autonomic dysfunctions. Preliminary studies of the use of clonidine in humans with traumatically injured spinal cord indicate that autonomic dysreflexia can be controlled and spasticity minimized. The data suggest that biochemical and pharmacologic manipulation of receptors may ameliorate paralysis following traumatic injury to the spinal cord as well as to the brain and brainstem.*

In the mammalian spinal cord, most monoaminergic neuronal pathways are descending (*1, 2*). A traumatic injury, which causes the cessation of action potentials, eventual hemorrhagic necrosis of the central gray matter, and extensive structural degeneration of long tracts of the white matter within 24 to 36 hours after injury, disrupts the neuroendocrine negative feedback (*3*), receptor activation (*1*), and end-product feedback inhibition on the molecular level (*4*).

After the transection of the spinal cord, axoplasmic transport continues to the lesion site, resulting in the accumulation of serotonin (5-hydroxytryptamine), and norepinephrine above the lesion in the spinal cord (*1, 2, 5*) and brainstem (*5*) and a loss of the biogenic amines from the distal portion of the spinal cord (*2, 5*). In contrast to monoaminergic descending pathways, substance P (SP)–containing fibers in the spinal cord represent ascending spinal tracts; SP accumulates below the lesion level (*6*). Therefore, after chordotomy, the axons of descending and ascending neurons from the cells of origin to the site of the lesion remain biologically and functionally viable. Spasticity and episodic hypertensive crises (autonomic dysreflexia) are caused by noxious stimuli arising from afferent somatic and visceral inputs. These stimuli are conducted by the spinal cord below the level of the lesion (*3*).

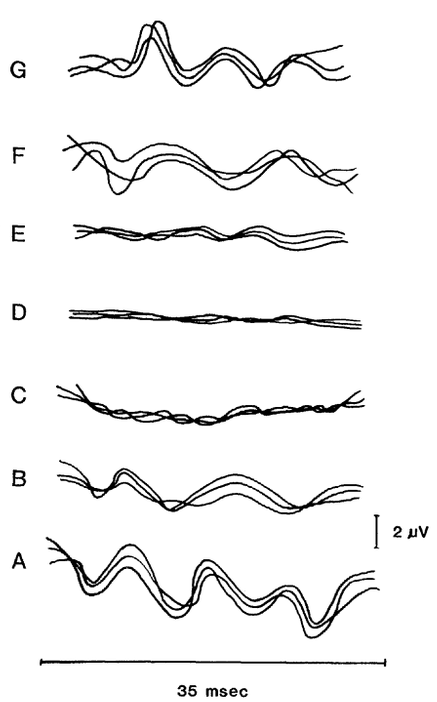

Fig. 1. In this cat the exposed dura was impacted by a 20-g weight dropped from a height of 25 cm (a 500 g-cm force). Tracings of three consecutive SEP's are shown superimposed at (A) just before, (B) 10 minutes after, (C) 20 minutes after, and (D) 30 days after impaction of the spinal cord and just before treatment. Note that SEP's are essentially absent 20 minutes after impaction and remain so for about 1 month. (E) After the first infusion of clonidine (5 μg/ml in 15 ml of saline) and administration of angiotensin II (0.1 mg/kg), SEP's were still absent. On days 2 and 3 of treatment, the animal received a 0.1-mg tablet of clonidine twice daily. On day 4 of treatment, the cat received a 0.1-mg clonidine tablet in the morning and then an infusion of clonidine and angiotensin II as on day 1. (F) Although SEP's had returned on day 4 during and after infusion, the consecutive determinations did not become constant until (G) 2 hours later. On day 5 of treatment the cat moved its tail and felt pinching by withdrawal of the hind legs. After the return of SEP's, the animals received 0.05-mg clonidine tablets four times daily. Within 4 to 8 weeks this and other cats paralyzed and treated in the same way started to walk; all tactile, thermal, pressure, and pain sensations, as well as motor coordinations, seem to have returned.

After a traumatic injury to the spinal cord, alterations of the membrane phospholipids will affect the configuration and hence the activity of the major phospholipid-dependent enzymes (*7*), as well as transmitter-receptor coupling (*8*) and the bindings of endogenous opiate receptors (*9*).

At my laboratory we have investigated membrane pathology in the traumatically injured spinal cords of cats, using physiological, biochemical (*3, 5*), immunocytochemical (*6*), and light microscopic, scanning, and transmission electron microscopic (*7*) techniques. We have also examined the pathology from the standpoint of lipid-free radical damage to neuronal membranes (*7*). It was reasoned that if functional restoration were to occur in the central nervous system (CNS), it was essential to reestablish the fluid-dynamic state of the cell membranes. As already mentioned, a significant part of the architecture of descending (*1, 2, 5*) and ascending (*6*) pathways remains intact. We postulated that a specific agonist might provide the appropriate signal or stimulus to its own membrane receptors on the descending or ascending tracts and serve as a guide for nerve tracts to grow along their genetically regulated paths.

The adrenergic system is a major descending pathway. The bulbospinal projections of the noradrenergic system, and those of the serotonergic and dopaminergic systems, project to the same or different regions of the spinal cord (*2, 10*). Therefore, the α_2-adrenergic agonist, clonidine, was chosen for our studies because it might also affect serotonin- and dopamine-containing neurons through the intimate interconnections of their circuitry in the spinal cord (*10*) and because clonidine has a high degree of selectivity for the α_2-adrenergic receptors in the CNS (*11*).

We used Allen's model of spinal cord trauma. This model is widely accepted as being the closest representative of the traumatic injury incurred by humans (*12*). Intramuscular ketamine or intravenous pentobarbital (30 mg/kg) was used to anesthetize 47 mongrel cats. Arterial blood pressure was continuously monitored in animals for at least 2 hours. After recording the stable initial arterial

0036-8075/82/0910-1042$01.00/0