would have been obliterated rapidly by weathering.

The appearance of the solar marker slabs strongly suggests natural emplacement; their position does not require moving and setting by humans. Although it is not possible to reconstruct a detailed history of the slabs, we conclude that a rockfall probably occurred, that light patterns were observed, and petroglyphs were added to develop the solar marker.

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12. We thank Superintendent W. Herriman and the staff at Chaco Culture National Historical Park for their cooperation and D. McCulloch, D. Rubin, R. Webb, and H. Wilshire for their assistance in the field.

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Release of γ-Aminobutyric Acid from Cat Colon

Abstract. *The release of γ-aminobutyric acid was confirmed in isolated cat colon loaded with tritiated γ-aminobutyric acid. Thirty to 180 minutes after loading the spontaneous efflux of tritium appeared to fit a single exponential curve with an efflux rate coefficient of 0.002 per minute. Electrical stimulation produced frequency-dependent increases in the tritium efflux and in the contractions. Even 120 minutes later over 91 percent of the total radioactivity in the superfusates was attributable to tritiated γ-aminobutyric acid. The acid release and the contractions induced by electrical transmural stimulation were inhibited by tetrodotoxin and by a calcium-free medium. Release of the acid was not significant during contractions elicited by nicotine and acetylcholine. These findings indicate that γ-aminobutyric acid is released from the terminals of neurons in the myenteric plexus of the colon.*

γ-Aminobutyric acid (GABA) may act as a neurotransmitter in the mammalian gut. Tritiated GABA accumulates in a small number of ganglion cells in the guinea pig myenteric plexus (*1*), and high concentrations of GABA and highly active glutamate decarboxylase are present in the myenteric plexus (*2*). In identifying a neurotransmitter, it is essential to show that it is released from nerve terminals as a result of presynaptic stimulation. There is no documentation of the release of GABA from neuronal elements in mammalian peripheral nervous tissue. We now report that electrical stimulation releases GABA from isolated cat colon loaded with [^{3}H]GABA.

The colon was excised from anesthetized adult cats of either sex. The mucosa was removed and strips of the colon were incubated at 37°C for 40 minutes with $5 \times 10^{-8}M$ [2,3-^{3}H]GABA (1 mCi/ml, 57 Ci/mmole) (Amersham) in Krebs solution containing $10^{-5}M$ aminooxyacetic acid and saturated with 95 percent O_2 and 5 percent CO_2. After being washed in fresh medium for 15 minutes the strips were superfused at 37°C with oxygenated Krebs solution in the presence of aminooxyacetic acid. The superfusate was collected every 2 minutes and the radioactivity of the sample was determined with a liquid scintillation spectrometer. Mechanical activity of the strips was recorded isotonically during measurement of [^{3}H]GABA release. At the end of the experiment the tissue was dissolved in Soluene and the radioactivity was measured in a scintillation counter.

The spontaneous efflux of tritium from the strips approached an exponential rate 20 to 30 minutes after superfusion. The efflux rate coefficient was a steady value of 0.0022 ± 0.0003 per minute (mean ± standard error for ten determinations).

Electrical transmural stimulation (pulse duration, 1 msec; intensity, 30 V; frequency, 1 to 20 Hz) for 1 minute resulted in a significant increase in ^{3}H efflux above the spontaneous release occurring during the period before stimulation. The efflux was accompanied by contraction of the preparation (Fig. 1a). The responses to electrical stimulation were frequency-dependent, and both the increase in ^{3}H efflux and the contraction were maximal at a frequency of 10 Hz. The frequency-response curve for electrically stimulated ^{3}H efflux was in good agreement with that for contraction (Fig. 1, b and c).

To confirm that the radioactivity in the superfusate was indeed GABA or its metabolites, superfusates from electrically stimulated and nonstimulated tissues were collected and 100-μl portions with added unlabeled GABA were subjected to high-voltage electrophoresis on Whatman 3-mm chromatography paper (*3*). GABA was identified with ninhydrin and the radioactivity of GABA was determined in a scintillation counter. Over 91 percent of the total radioactivity in superfusates from both electrically stimulated and nonstimulated samples proved to be [^{3}H]GABA, even after 120 minutes. Accordingly, the estimates of total radioactivity reported here appear to provide a satisfactory index of the level of unchanged GABA.

The effects of tetrodotoxin on the contractile response to 1 minute of electrical stimulation (1-msec pulses, 30 V, 1 to 20 Hz) were then investigated. Tetrodotoxin at the concentration of $10^{-6}M$ abolished the contractions induced by electrical stimulation at frequencies of 1, 3, and 5 Hz, indicating that the contractions were neurogenic in origin. On the other hand, the contractions induced by stimulation at 10, 15, and 20 Hz were not abolished by $10^{-6}M$ tetrodotoxin, suggesting that stimulation at frequencies over 10 Hz produced contractions not only by nerve stimulation but also by direct stimulation of the muscle. Therefore, in further GABA release experiments, 1-minute electrical stimulation with 1-msec pulse duration, 30-V intensity, and a frequency of 5 Hz was used.

The efflux of GABA from tissue in response to depolarizing stimuli often serves as an index of GABA release from nerve terminals into the synapses. However, elements other than nerve terminals also become depolarized during stimulation, and GABA is released from these elements. High concentrations of potassium or electrical stimulation releases [^{3}H]GABA from glial cells in the dorsal root ganglia (*4*), superior cervical ganglia (*5*), and brain (*6*). In our preparations the smooth muscle cells, nerves, blood and lymph vessels, mast cells,

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connective tissue, and intramural plexus were left intact. However, autoradiography has demonstrated that [^{3}H]GABA is mainly taken up into the myenteric plexus (*1*). To determine whether the evoked release of [^{3}H]GABA was from neurons or from the other elements—particularly glial cells in the myenteric plexus—tetrodotoxin was used to suppress neuronal depolarization. The electrically induced increase in both [^{3}H]GABA release and contractions was abolished by tetrodotoxin (Fig. 2a), indicating that the responses were neurogenic.

The presence in the medium of Ca^{2+} is required for the release of several transmitters from nerve terminals, although the release of GABA from glial elements is reportedly Ca-dependent in the dorsal root ganglia (*4*). In the present study the stimulus-evoked release of [^{3}H]GABA from the strips of colon was markedly reduced by the perfusion of Ca^{2+}-free medium (Fig. 2b). High concentrations of Mg^{2+} suppress the stimulation-induced release of neurotransmitters from nerve endings by competing with Ca^{2+} (*7*). When the colonic tissue was superfused with medium in which NaCl was reduced by 24 m*M* and replaced with $MgCl_2$ at an equimolar concentration, the electrical stimulation did not increase the release of [^{3}H]GABA or the contractions, thereby supporting the findings that the stimulus-evoked GABA release is indeed Ca-dependent. These findings provide further evidence that GABA was released from neuronal elements. Thus, the tetrodotoxin-sensitive and Ca-dependent release of GABA may originate in neuronal terminals in the cat colon. This contrasts with the observation that the stimulus-evoked release of [^{3}H]GABA from glial elements of superior cervical ganglia is tetrodotoxin-resistant and Ca-independent (*5*).

There was no difference in the degree of increase in the release of [^{3}H]GABA induced by 1 minute of electrical stimulation (30 V, 5 Hz) with pulse duration between 0.1 and 1.0 msec. Therefore, the properties of the GABA-containing neurons are similar to those of other postganglionic neurons in which the transmural stimulation for the release of acetylcholine (*8*), serotonin (*9*), adenosine triphosphate (*10*), and substance P (*11*) consisted of pulses of 0.1 to 1.0 msec at a frequency of 0.1 to 1.0 Hz.

Since the stimulus-evoked release of GABA was accompanied by contraction of the preparation, we attempted to determine whether the GABA release was due to a mechanical distortion. Superfusion with a medium containing $10^{-5}M$ nicotine or 10^{-8} or $10^{-5}M$ acetylcholine did not cause any significant change in the spontaneous release of [^{3}H]GABA but did produce contractions, indicating that nicotinic and muscarinic receptors may not be involved in the release of [^{3}H]GABA from cat colon.

It has been determined pharmacologically that GABA produces contractions, relaxations, or both in isolated intestine (*12*). GABA also causes contraction in strips of guinea pig ileum and relaxations in strips of distal colon, but not in nerve-free strips of these tissues (*13*). Also, in

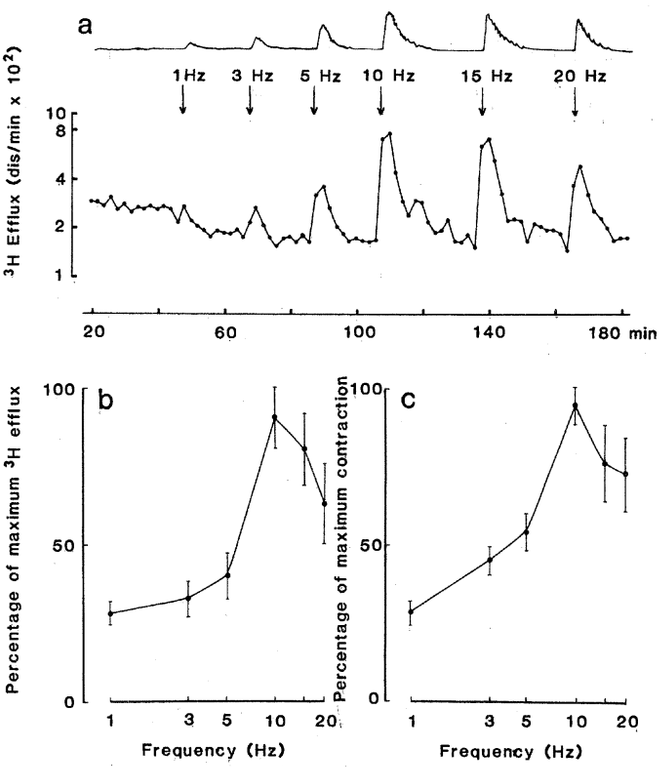

Fig. 1. Efflux of [^{3}H]-GABA and mechanical activity induced by transmural electrical stimulation of isolated cat colon. Results are those of a typical experiment and show (a) concomitant [^{3}H]GABA release and contraction of colon, (b) frequency-response curves for [^{3}H]GABA efflux, and (c) contraction. Stimulation composed of monophasic pulses (1 msec, 30 V, 1 to 20 Hz) was applied for 1 minute to a strip of colon fixed between two parallel platinum electrodes. The evoked release of [^{3}H]GABA was calculated as the ratio of evoked release of tritium to spontaneous release of tritium. Frequency - response curves are percentages of the maximum increase in [^{3}H]-GABA release and maximum contraction elicited by transmural electrical stimulation. Values are means ± standard errors for five cats.

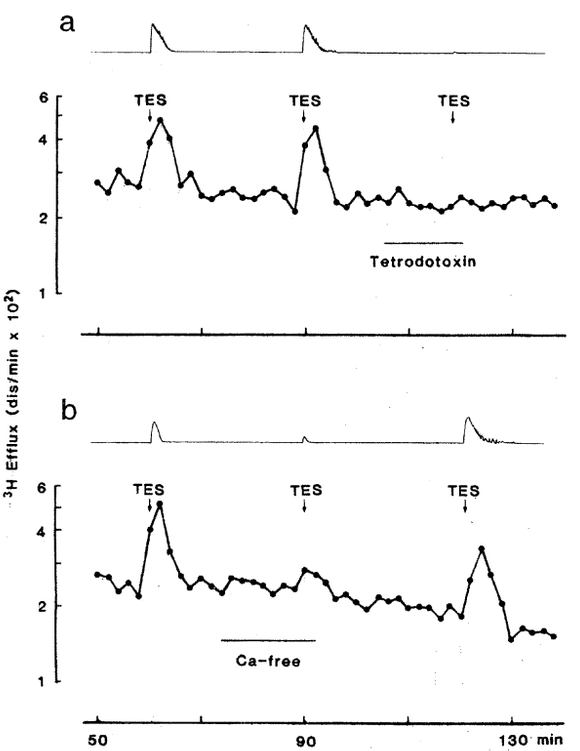

Fig. 2. Effect of tetrodotoxin (a) and Ca-free medium (b) on contraction and [^{3}H]GABA release induced by transmural electrical stimulation (*TES*). Tetrodotoxin ($10^{-6}M$) was applied 15 minutes before stimulation and again during stimulation. Calcium chloride was omitted from the perfusion medium 20 minutes before and during stimulation. Stimulation consisted of 1-msec pulses at 30 V and 5 Hz for 1 minute.

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.

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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.

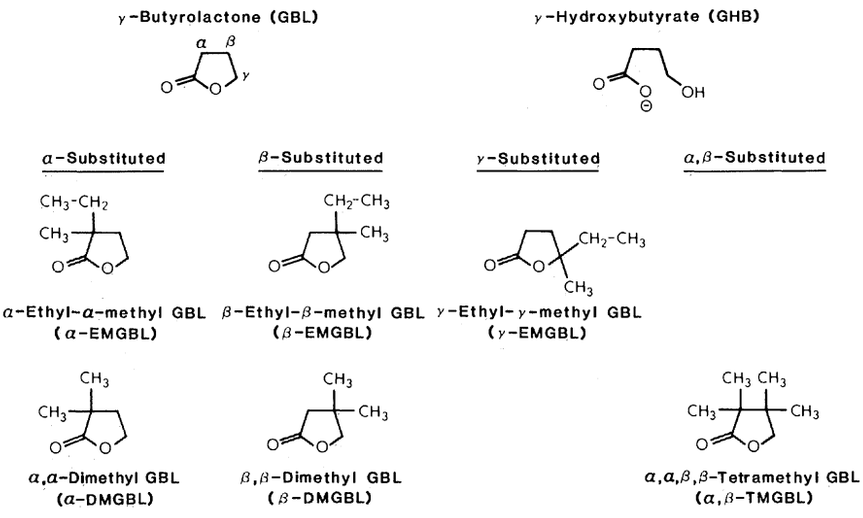

Fig. 1. Chemical structures of some alkyl-substituted GBL's.

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-

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