I. N. McCave, Ed. (Plenum, New York, 1976), p. 48. Equation 1 ignores insignificant depositional burial of dissolved species; molecular D_s values include corrections for tortuosity and porosity effects. The relation between D_s [in units of square centimeters of bulk sediment per second (cm_s^2 sec^{-1})] and the pure solution molecular diffusivity, D, is given by the expression: $D_s = D/\phi F$, where F is the formation factor [as described by F. T. Manheim, *Earth Planet. Sci. Lett.* **9**, 307 (1970)]. Surface values of ϕ and F for station A-1 are approximately 0.94 and 1.2, respectively (*5*); therefore, predicted D_s values at our study site should be approximately 0.89 D.
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12. The general equation describing the radon distribution in sediments is (*11*)

$$\frac{dC}{dt} = D_s \frac{\partial^2 C}{\partial z^2} + \lambda C_{eq} - \lambda C \quad (4)$$

where C is the measured radon concentration in atoms per cubic centimeter or disintegrations per minute per cubic centimeter of bulk sediment. The steady-state solution to Eq. 4 is

$$C = C_{eq}\{1 - \exp[-(\lambda/D_s)^{1/2}z]\} + C_0 \exp[-(\lambda/D_s)^{1/2}z] \quad (5)$$

In the bight water column, C_0 is approximately 0.5 to 2 dpm $liter^{-1}$. The radon flux across the sediment water interface is given by

$$J = -D_s(\partial C/\partial z)_{z=0} = -(\lambda D_s)^{1/2}(C_{eq} - C_0) \quad (6)$$

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14. We conducted a series of mixing experiments, using a benthic chamber system in an artificial pond. Two or three samples were taken simultaneously at set time intervals for radon analysis; 99 percent mixing was obtained in 60 minutes, and the results agreed with numerical solutions to a set of mixing equations for the system.
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18. We thank G. Miller, J. Chanton, S. Marocchi-Klump, F. Sansone, P. Crill, and K. Gruebel for assistance with field and analytical work. We are grateful to G. Mathieu and other personnel of Lamont-Doherty Geological Observatory for assistance with radon equipment design and fabrication and ^{226}Ra standards. We thank the University of North Carolina, Institute of Marine Sciences, Morehead City, for use of boats and laboratory space. Financial support was provided by grants OCE75-06199 and OCE78-9453 from the Marine Chemistry Program, Oceanography Section, National Science Foundation.

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Seizure Protection and Increased Nerve-Terminal GABA: Delayed Effects of GABA Transaminase Inhibition

Abstract. *Changes in γ-aminobutyric acid (GABA) occurring in the presence and in the absence of GABA-containing nerve terminals were estimated in rats in which the dense GABA projection to the substantia nigra was surgically destroyed on one side of the brain. The net increase in GABA of the denervated nigra was compared with that of the intact nigra at various times after a single injection of γ-vinyl-GABA, which irreversibly inhibits GABA transaminase. Total GABA reached a maximum within 12 hours, but the GABA pool associated with nerve terminals did not increase until 36 hours and peaked at 60 hours. The onset and peak of anticonvulsant activity against maximal electroshock seizures directly paralleled the time course for the increase in GABA in nerve terminals, but was not positively correlated with that independent of the terminals. This result supports the concept that elevating GABA in nerve terminals facilitates GABA-mediated synaptic transmission and predicts anticonvulsant activity.*

The recent availability of compounds that selectively alter either the concentration of γ-aminobutyric acid (GABA) or the activity of neuronal GABA receptors has provoked a resurgence of investigations into the neurotransmitter role of this amino acid (*1*). A major obstacle encountered in these studies is that of discriminating between the GABA compartment associated with nerve terminals and other compartments of GABA. Throughout the brain, synthesis, uptake, and degradation of GABA take place both in GABA-containing nerve terminals and in cells (glial and neural) that do not use this compound as a neurotransmitter (*2, 3*). As a result, the degree to which changes in endogenous GABA can be expected to influence GABA-mediated synaptic transmission is difficult to ascertain (*3*).

The relationship between elevation of brain GABA and protection against electrically or chemically induced seizures in animals illustrates this problem. Drugs that decrease brain GABA or block GABA receptors can induce seizures in a variety of species (*4*), whereas drugs that enhance GABA transmission have anticonvulsant properties (*5*). However, quantitative changes in the amount of brain GABA are often poorly correlated with susceptibility to seizures (*6*). Ideally, one would like to be able to predict the degree of seizure protection to be expected from a given elevation in brain GABA. The achievement of this goal is complicated by our inability to determine what portion of the increased GABA concentration is available for release at synaptic terminals (*3*).

The importance of this issue is underscored by the apparently discordant biochemical and pharmacological actions of γ-vinyl-GABA, a specific and irreversible inhibitor of GABA transaminase (*7*). This compound has been reported to be relatively ineffective as an anticonvulsant agent in several animal seizure tests, despite its ability to increase GABA in whole brain and in various brain regions (*8*). We hypothesized that some major portion of the GABA increase produced by this agent might be sequestered in compartments that do not directly participate in GABA-mediated synaptic transmission. We obtained evidence in support of this hypothesis by experimentally discriminating between nerve-terminal-dependent and nerve-terminal-independent changes in GABA (*9*). We also discovered, however, that γ-vinyl-GABA possesses marked anticonvulsant activity which became evident only after a 2-day latency. To our knowledge, these experiments provide the first documentation that γ-vinyl-GABA can protect rats against tonic seizures produced by maximal electroshock and show that the appearance of this action coincides with an increase in nerve-terminal-dependent GABA.

We assessed the effect of γ-vinyl-GABA on the duration of the tonic hindlimb extension component of maximal electroshock seizures. Duration of tonic hindlimb extension was unchanged 12 hours after γ-vinyl-GABA was administered (600 to 1600 mg per kilogram of body weight, injected intraperitoneally). However, 36 hours after the injection (1600 mg/kg) the duration of tonic hindlimb extension was significantly attenuated, and by 60 hours complete suppression was observed. Since antiseizure activity was maximal at 60 hours (Fig. 1), the dose-dependency of the effect was examined at this time (Fig. 2). The mean effective dose (ED_{50}) was about 900 mg/kg; the dose-effect function parallels that obtained with sodium valproate [*n*-dipropyl acetate (DPA)], a drug used to treat human epilepsy.

To examine the possibility that γ-vi-

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nyl-GABA might have short-lasting properties which mask or antagonize its anticonvulsant actions in the first 12 hours, we treated one group of rats ($N = 10$) with two injections of γ-vinyl-GABA (900 mg/kg each): one at 60 hours, and the other 12 hours before the electroshock test. The response of these rats was the same as that of rats that had received one 900 mg/kg dose 60 hours before electroshock. Thus, the second dose neither antagonized the anticonvulsant effect of the first nor added to or potentiated its action.

Given enough time, γ-vinyl-GABA also suppressed chemically induced seizures. At 60 hours after the injection (900 mg/kg), we obtained the following results: (i) complete protection against the GABA-receptor antagonist bicuculline, administered intravenously in a dose (0.25 mg/kg) that caused clonic convulsions in all control animals; (ii) a 67 percent decrease in the incidence of tonic seizures induced by a 0.5 mg/kg intravenous dose of bicuculline; and (iii) a 40 percent decrease in the incidence of clonic seizures produced by pentylenetetrazol (100 mg/kg injected subcutaneously). On the other hand, at an earlier time (4 hours) after γ-vinyl-GABA administration, Schechter *et al.* found no protection against bicuculline or pentylenetetrazol-induced seizures (*8*).

In another series of animals we examined the changes in GABA after administering γ-vinyl-GABA. In order to evaluate GABA elevation in the relative absence of GABA-containing nerve terminals, we destroyed the dense GABA-containing neural projections to the substantia nigra (SN) on one side of the brain by surgically transecting fibers between the forebrain and midbrain (*9*). The GABA content of SN, unlike that of many other brain nuclei, derives largely from nerve terminals whose cell bodies are located some distance away in the forebrain (*10*). Thus, 7 to 10 days after the operation, the GABA content of the SN in the transected hemisphere decreased by 80 to 90 percent. This decrease represents the loss of the major, if not the entire, extent of nigral nerve terminal GABA; the remaining GABA is associated primarily with glial cells and neural perikarya (*9*). Despite the denervation, the SN retains much of its integrity as evidenced by normal wet weight and protein content, as well as by its ability to specifically bind [^{3}H]GABA (*11*) and to induce contralateral turning in response to GABA-receptor activation by intranigrally applied muscimol (*9*).

The GABA content of the SN in the hemisphere contralateral to the transection is unaffected by the lesion (legend to Table 1) and, therefore, an increase in GABA in the presence of GABA-containing nerve terminals can be evaluated in this SN. Table 1 lists GABA concentrations in the SN from transected and intact hemispheres of rats with and without γ-vinyl-GABA treatment. At 12 hours after a single dose of γ-vinyl-GABA (900 mg/kg), the absolute increase in GABA in the intact SN (Δ_1)

Table 1. Time-dependent change in GABA content of the SN after intraperitoneal injection of γ-vinyl-GABA. A dose of 900 mg per kilogram of body weight was administered to male Sprague-Dawley rats (250 to 300 g) in which unilateral hemitransections had been made 7 to 10 days before (*9*). The values for intact SN with and without drug treatment were the same as those obtained in rats that had not undergone surgery. The values of Δ_1 and Δ_2 represent the differences in GABA concentration between the control and drug treatments. The ratio Δ_2/Δ_1 is an estimate of the portion of the GABA increase in the intact SN that can be accounted for by the GABA elevation in the denervated SN. Rats were killed 10 days after surgery by focused microwave irradiation of the head for 5 seconds. The SN was microdissected from a coronal slice of midbrain (*9*) and stored at −80°C until it was assayed. The GABA was measured by the enzymatic-fluorometric method of Okada *et al.* (*22*), without the 60° heating step. Proteins were assayed according to Lowry *et al.* (*23*). Mean protein content of the SN was 0.44 ± 0.01 mg. Values are means ± standard errors from six rats.

Treatment	GABA concentration (nmole per milligram of protein)				
	Intact	Δ_1	Denervated	Δ_2	Δ_2/Δ_1
Controls	81 ± 2.7		15 ± 2.0		
γ-Vinyl-GABA					
12 hours	160 ± 8.1	79	112 ± 7.3	97	1.2
36 hours	150 ± 8.8	69	71 ± 3.8*	56	.8
60 hours	127 ± 5.3*	46	39 ± 3.1*	24	.5

*Significantly different (*t*-test, $P < .01$) from values obtained in the respective hemisphere (intact or denervated) when 60 hours is compared with 36 hours and 36 hours is compared with 12 hours. All values from drug-treated rats were significantly different ($P < .01$) from respective control (vehicle injection) values.

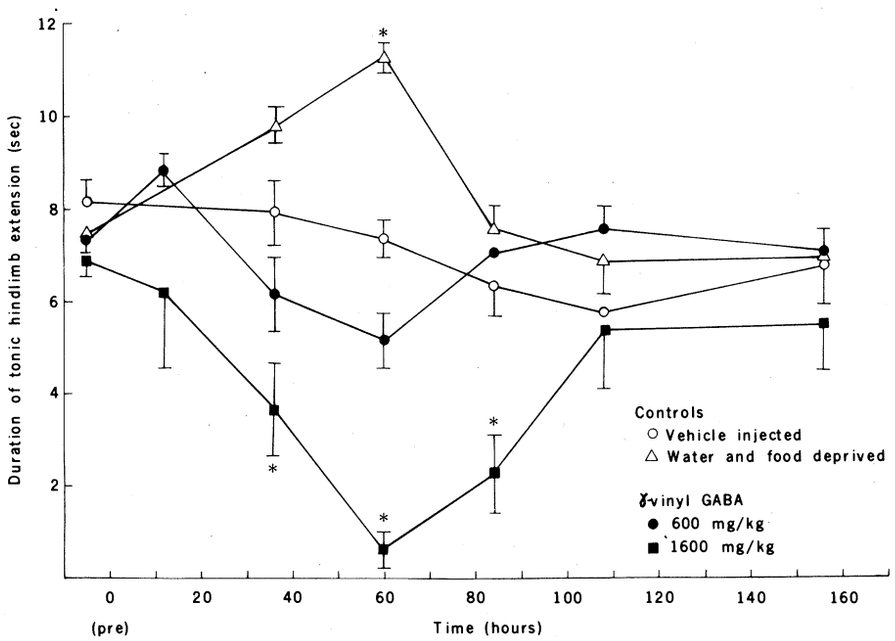

Fig. 1. Suppression of tonic hindlimb extension as a function of time after γ-vinyl-GABA treatment. Maximal electroshock was applied through corneal electrodes (*21*) to rats which had received intraperitoneal injections of drug or vehicle (distilled H_2O) 12 to 156 hours earlier. Duration of tonic hindlimb extension (seconds) was timed with a stopwatch. All animals were screened 12 hours before drug treatment ($T = 0$) to eliminate any that did not show tonic hindlimb extension. All rats in each treatment group ($N = 12$ each) were tested repeatedly at each time point. A group deprived of food and water for the first 48 hours was tested in parallel with the drug-treated group to determine the effect of decreased food intake and weight loss (10 to 15 percent decrease from initial body weight) on the duration of tonic hindlimb extension. This condition was a control for the decreased food intake and weight loss (10 percent over 2 days) observed in rats treated with γ-vinyl-GABA (1600 mg/kg). Injections of γ-vinyl-GABA were given at night (after 11:00), and maximal electroshock testing was done during the day. Asterisks denote values significantly different from those of vehicle-injected controls (*t*-test, $P < .01$).

Table 2. Relationship between suppression of tonic hind limb extension and GABA elevation. All drugs were administered intraperitoneally in the following doses: γ-vinyl-GABA, 900 mg/kg; AOAA, 30 mg/kg; and DPA, 300 mg/kg. The time after treatment at which measurements were made is indicated for each drug. Values for γ-vinyl-GABA were obtained from the data shown in Table 1. Values for DPA and AOAA were obtained from (*9*).

Treatment	Suppression (%)	GABA elevation (% over control) (*24*)		
		Total	Non-nerve terminal	Nerve terminal
γ-Vinyl-GABA				
12 hours	0	98	646	0
36 hours	28	85	373	19
60 hours	45	57	160	33
DPA (0.5 hour)	90	36	28	39
AOAA (2 hours)	18	25	100	7

was no greater than that in the denervated SN (Δ_2) (Table 1). This result indicates that the absolute magnitude of change in GABA under these circumstances is independent of GABA-containing nerve terminals.

The situation began to change 36 hours after drug treatment, at which time one-fifth of the net change in GABA in the intact SN cannot be accounted for by the change in GABA in the denervated SN (Table 1). The change is therefore probably associated with nerve terminals. This separation between the GABA concentrations in the intact and denervated tissues became more apparent at 60 hours; at this time, nearly half of the net increase in GABA in the intact SN was associated with the presence of nerve terminals.

The temporal coincidence between the development of antiseizure effects and the elevation of GABA associated with nerve terminals after γ-vinyl-GABA treatment suggests that functional augmentation of GABA-mediated synaptic transmission may be correlated with changes in nerve-terminal GABA and not with gross increases in brain tissue concentrations of GABA. To explore this possible correlation, the data from rats with transections were used to estimate the proportional changes in GABA in the compartment associated with GABA nerve terminals and in the compartment independent of these terminals (Table 2). We have made the assumption that the difference in GABA content between the denervated and intact SN represents the nigral GABA associated with nerve terminals (*9*). Values obtained with DPA and aminooxyacetic acid (AOAA) are included in our analysis to test predictability across drug treatments. Both DPA (*12*) and AOAA (*13*) interfere with GABA degradation through mechanisms that are distinct from each other and also from that by which γ-vinyl-GABA exerts its effect (*7*).

The values presented in Table 2 reveal a close positive correlation (r = .93) between the extent of suppression of tonic hindlimb extension and the increase in GABA associated with nerve terminals. On the other hand, no positive correlation exists between changes in total GABA and seizure protection. These data demonstrate that the degree of change in GABA in the compartment which appears functionally relevant (GABA-containing nerve terminals) can be concealed by changes taking place in other compartments.

The GABA-transaminase inhibitors γ-vinyl-GABA and AOAA appear to increase GABA predominantly in compartments not directly associated with GABA-containing nerve terminals. This is perhaps because these compartments contain a large amount of GABA-transaminase (*14*). Our observations are consistent with studies that have demonstrated marked effects of GABA-transaminase inhibition in nonneuronal cells and neural tissues that are not known to contain GABA terminals (*15*) as well as with subcellular fractionation studies (*16*). Since these compounds appear to affect nerve-terminal GABA only after other compartments of GABA have been elevated severalfold, it is understandable that relatively large increases in total GABA are required to elicit various GABA-related physiological effects with these drugs (*17*).

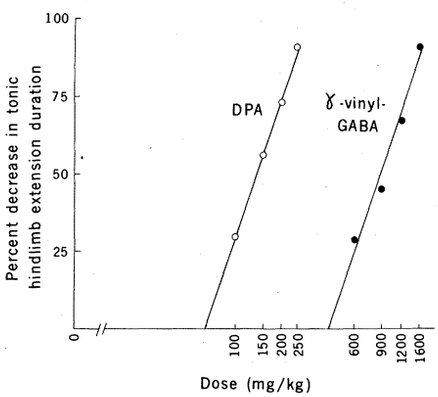

Fig. 2. Suppression of tonic hindlimb extension as a function of dose of γ-vinyl-GABA. The mean duration of tonic hindlimb extension for each drug-treated group (N = 12) was subtracted from that (8 seconds) of vehicle-injected controls and expressed as a percentage of the control mean. Data were obtained from rats tested only once, 60 hours after drug administration. The values obtained from the animals treated with 600 and 1600 mg/kg in this experiment were similar to those shown in Fig. 1. Data obtained from another group of rats (N = 10 rats per dose) 30 minutes after various doses of DPA are shown for purpose of comparison.

A curious aspect of the γ-vinyl-GABA effects on seizure activity and nerve-terminal GABA is the time course over which they develop. Since these effects occur only after some delay, at a time during which total GABA is no longer increasing, it appears that a redistribution of GABA has occurred. At present we have no explanation for how or why this could happen.

The experiments with DPA demonstrate that increases in nerve-terminal GABA can be obtained in the absence of large effects on other GABA compartments. A selective effect of DPA on nerve-terminal GABA has also been demonstrated in subcellular fractionation studies (*18*). The possibility that the anticonvulsant action of DPA is mediated through its effect on GABA has met with skepticism, in part because of the undramatic effect of this drug on total GABA (*12*). While we cannot exclude the possibility that DPA may have anticonvulsant effects mediated by factors other than GABA, our data suggest that the DPA-induced increase in nerve-terminal GABA is sufficient to predict marked anticonvulsant activity. Thus, a small increase in the pool of GABA which can be mobilized for synaptic transmission is likely to have pronounced functional effects.

In evaluating compartmentalized changes in GABA, we have examined one brain nucleus. The degree to which our data can be generalized will depend on the results of similar studies in other brain areas. We have previously reported that different brain regions show different degrees of change in GABA after AOAA and DPA (*19*). Moreover, the brain areas most affected by DPA were not those most affected by AOAA. We have recently found (*20*) that the profile of GABA elevation across brain areas after γ-vinyl-GABA more closely resembles that obtained with AOAA than that obtained with DPA. Perhaps the different regional responses to these drugs reflect variations both in the relative density of GABA-containing nerve terminals and in the turnover rate of GABA across

brain regions (*3*). It is therefore likely that the physiological and behavioral effects of these drugs may be a function of both their ability to elevate GABA in nerve terminals and their ability to exert this effect in specific brain regions.

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24. The percentages by which GABA increased in the various compartments were calculated as follows:

$$\text{Total} = 100\,\frac{(x' - x)}{x}$$

$$\text{Non-nerve terminal} = 100\,\frac{(y' - y)}{y}$$

$$\text{Nerve terminal} = 100\,\frac{[(x' - x) - (y' - y)]}{(x - y)}$$

where x is GABA concentration in the intact SN; y is GABA concentration in the denervated SN; x' is GABA concentration in the intact SN after drug treatment; and y' is GABA concentration in the denervated SN after drug treatment.
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Glutamine- and *N*-Acetylglutamate–Dependent Carbamoyl Phosphate Synthetase in Elasmobranchs

Abstract. *High levels of glutamine- and* N*-acetyl-*L*-glutamate-dependent carbamoyl phosphate synthetase activity are present in liver extracts of marine species of fish that retain high levels of urea in their tissues for the purpose of osmoregulation. The function of the synthetase in these species appears to be related to urea synthesis.*

Carbamoyl phosphate is utilized in the first step of two major metabolic pathways, one leading to biosynthesis of uridine monophosphate and the pyrimidine nucleotides and the other leading to biosynthesis of arginine or urea (or both) (*1*). At least three different kinds of enzymes catalyzing carbamoyl phosphate formation have been identified on the basis of substrate specificity (that is, the nitrogen donating substrate) and cofactor requirements. Carbamoyl phosphate synthetase I (CPSase I) is located specifically in the liver mitochondria of ureogenic animals, requires *N*-acetyl-L-glutamate (NAG) as a cofactor, and utilizes only ammonia as the nitrogen donating substrate (*1, 2*). The properties of CPSase I are related to its function in ammonia detoxification via the urea cycle (*2*). The K_m (Michaelis constant) for ammonia is relatively low (about 2 m*M*), and the requirement of NAG for CPSase I activity is considered a mechanism for regulating urea cycle activity in response to the level of the amino acid pool.

CPSase II utilizes glutamine rather than ammonia as the physiologically significant nitrogen donating substrate, and NAG is not required for catalytic activity (*1, 3*). Like other amidotransferases, CPSase II from most sources will utilize ammonia in place of glutamine as the nitrogen-donating substrate, but concentrations of at least 0.1*M* are required for full activity. The function of CPSase II is related to pyrimidine nucleotide biosynthesis in higher animals and to both arginine and pyrimidine nucleotide biosynthesis in other animal species and probably in plants.

Trammel and Campbell have reported the presence in several invertebrate species of a third type of CPSase, CPSase III (*4*). This enzyme, like CPSase II, utilizes glutamine as the nitrogen-donating substrate, but, like CPSase I, it requires NAG as a cofactor. We have found CPSase III activity in liver of largemouth bass (*Micropterus salmoides*), a freshwater teleost fish, thus establishing that CPSase III is also present in vertebrates (*5*).

The requirement for NAG suggests that the function of CPSase III may be related to the urea cycle. In the case of fish, most species show various levels of urea in their tissues and do excrete urea (*6, 7*). The level of urea in the tissues of most bony fish such as the teleosts in Osteichthyes is usually very low (0.01 to 0.03 percent) (*6, 8*). Although all five of the enzymes required for the urea cycle, including CPSase, are present in several species of teleosts, the activities are generally low (*8, 9*). In contrast, marine cartilaginous fish in the class Chon-

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