Molecular Neurobiology

ISSN0893-7648/94/9(1–3): 253–258/$5.20

Altered GABAergic and Glutamatergic Transmission in Audiogenic Seizure-Susceptible Mice

M. L. Cordero, J. G. Ortiz, G. Santiago, A. Negrón, and J. A. Moreira*

Department of Pharmacology, University of Puerto Rico, School of Medicine, PO Box 365067, San Juan, Puerto Rico 00936-5067

Abstract

The C57BL/10 SPS/sps mouse mutant are audiogenic seizure-susceptible. The enzymatic activities of glutamate decarboxylase (GAD), GABA aminotransferase (GABA-T), alanine aminotransferase (ALA-T), aspartate aminotransferase (ASP-T), and glutamate dehydrogenase (GDH) of whole brain supernatant are significantly reduced in these epileptic mice. GABA uptake is decreased in cortex, midbrain, and pons medulla. Previous studies showed the presence of two sodium-dependent GLU uptake systems in normal (SPS/SPS) mice. Glutamate U_{max} by System 1 is significantly decreased in these mice, whereas the U_{max} value for System 2 is significantly increased in the epileptic mice.

Index Entries: Glutamate; GABA; audiogenic seizures; epilepsy; uptake; release.

Abbreviations: GABA, gamma-aminobutyric acid; GLU, L-glutamic acid; SPS/SPS, normal mice; SPS/sps, epileptic (audiogenic seizure-susceptible) mice; NMDA, *N*-methyl-D-aspartate; EAA, excitatory amino acids; GAD, glutamate decarboxylase; GABA-T, GABA aminotransferase; ALA-T, alanine aminotransferase; ASP-T, aspartate aminotransferase; GDH, glutamate dehydrogenase.

Introduction

Excitatory amino acids and GABA play an important role in epileptogenesis *(1,2)*. Excitatory amino acid levels and NMDA receptor binding are increased in rat epileptic tissue *(3)*. Decreased GABA levels have been associated with generalized seizures in humans *(4)*.

The C57BL/10 heterozygote (SPS/sps) mouse mutant exhibits seizures on exposure to intense acoustic stimuli (audiogenic seizures) *(5)*. There is evidence for the supporting role of GABA and glutamate in the pathogenesis of this and other genetic models of epilepsy *(2,6,7)*. However, the molecular and cellular basis of epilepsy remains unknown. In these experiments, we examine various neurochemical parameters, such as release, binding, and uptake, in the C57BL/10 SPS/sps mice.

Methods

Animals

C57BL/10B SPS/sps mice (audiogenic seizure-susceptible) mutants of different ages were raised in our colony from homozygote (sps/sps, audio-

*Author to whom all correspondence and reprint requests should be addressed.

genic seizure-susceptible, behavioral arrest) and normal (C57BL/10 SPS/SPS, audiogenic seizure resistant, no behavioral arrest) crossings. Age- and sex-matched normal (SPS/SPS) mice served as controls.

Audiogenic Seizure Latency

Mice were placed in an acrylic chamber (36.5 × 36.5 cm) for 30 s prior to being exposed to an intense noise (electric doorbell, 95–109 dB). The noise was presented for a maximum of 90 s *(5)*.

Subcellular Fractionation

The brain was promptly removed and then homogenized in cold oxygenated (95% O_2/5% CO_2) EBSS (Earle's balanced salt solution) for the uptake studies or the specified buffer for the binding studies. The crude homogenate was centrifuged twice at 800*g* for 10 min. The supernatant was then centrifuged at 12,000*g* for 20 min. The membranes (P_2 fraction) were resuspended in buffer and promptly used.

Glutamic Acid Decarboxylase Activity Assay

(GAD, E. C. 4.1.1.16) Enzymatic activity was determined as described by Sze *(8)*. The $^{14}CO_2$ evolved was collected in filter disks (0.7 cm diameter) impregnated with hyamine. Boiled tissue blanks and reagent blanks were used in all the determinations. Radioactivity was quantified using a Beckman LS-1800 counter.

Other Enzymatic Assays

GABA-aminotransferase (GABA-T, E. C. 2.6.1.19), aspartate aminotransferase (ASP-T, E. C. 2.6.1.1), alanine aminotransferase (ALA-T, E. C. 2.6.1.2), and glutamate dehydrogenase (GDH, E. C. 1.4.1.3) activities were measured as described by Dinwoodie and Boecker *(9)*. Reagent blanks were used in all the experiments and were shown not to be different from boiled tissue blanks. Radioactivity was quantified using a Beckman 1800 liquid scintillation counter.

GABA Uptake Experiments

Crude synaptosomes from different regions were incubated at 37°C in oxygenated (95% O_2, 5% CO_2) Krebs Henseleit buffer containing 0.1 m*M* aminooxyacetic acid (AOAA) and 1.0 μ*M* ^{14}C-GABA for 10 min. Samples incubated at 0–4°C served as controls. Radioactivity was quantified using a Beckman LS-1800 counter.

Synaptosomal (^{3}H)GLU Uptake

The reaction began with the addition of whole brain synaptosomes (10–40 μg protein in a final vol of 100 μL) to samples containing varying concentrations (1–200 μ*M*) of [^{3}H]GLU in EBSS (Earle's balanced salt solution) and incubated for 5 min at 37°C. Samples incubated at 0–4°C served as controls. The reaction was terminated by placing the samples in ice followed by filtration of 100 μL through Millipore AP40 filters (0.7 cm diameter) on a Scheiller and Schuell minifold. Filters were washed twice with 100 μL cold EBSS. The radioactivity of each dry filter was quantified in a Beckman LS 1800 scintillation counter.

Glutamate uptake was linear under the experimental conditions used; 5 min of incubation and 10–40 μg of protein. Sodium dependence was examined by measuring GLU uptake in equimolar choline-containing buffer *(10)*. Glutamate uptake was not significantly affected by 100 μ*M* 2-amino-7-phosphonoheptanoic acid.

Release Experiments

Synaptosomes were incubated as described above for GABA except that the incubation time was increased to 1 h. The synaptosomes were then collected in filters, and superfused with Krebs-Henseleit buffer at 37°C. The perfusate was collected every 5 min. After obtaining a stable baseline (65 min), a high potassium (K^+, 47 m*M*) pulse was given, followed again by normal Krebs-Henseleit perfusion. The assays were performed in duplicate and have been normalized to the values of the stable baseline obtained at 65 min. Release of radioactivity could not be detected in the absence of Ca^{2+}.

Materials

2-keto [1-^{14}C] glutaric acid, sodium salt, 56 mCi/mmol, ACSII (water compatible scintillation fluid), and Spectrofluor were obtained from Amersham Corporation (Arlington Heights, IL). L-[1-^{14}C] glutamic acid, 56 mCi/mmol, and [1-^{14}C] GABA, 55 mCi/mmol, were obtained from American Radiolabelled Co. (St. Louis, MO). L-(2, 3, 4-^{3}H) GLU (250 μCi/mol) was also obtained from American Radiolabelled Chemicals. Filters, AP4002405, 24 mm (similar to Whatman GF/B) were obtained from Millipore Corporation (Cidra, Puerto Rico). All the other reagents, including *E. coli*'s glutamate decarboxylase, were obtained from Sigma Chemi-

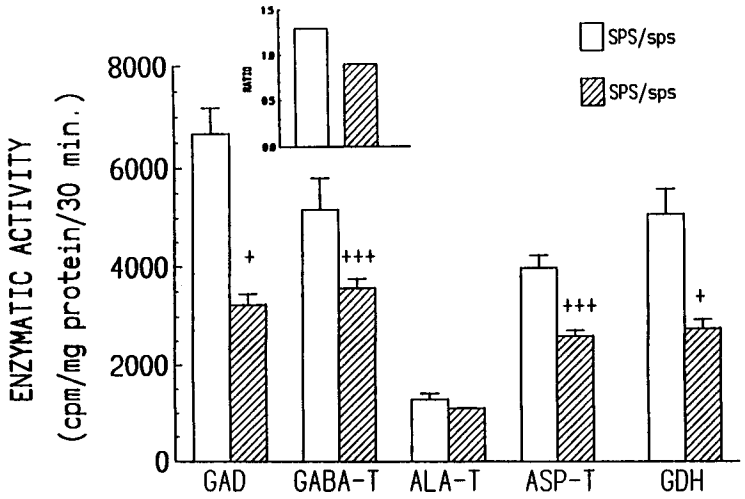

Fig. 1. Activity of glutamate decarboxylase (GAD), GABA aminotransferase (GABA-T), alanine aminotransferase (ALA-T), aspartate aminotransferase (ASP-T), and glutamate dehydrogenase (GDH) of whole brain supernatant of normal (SPS/SPS) and heterozygote (SPS/sps) mice. Each value represents the mean ± SEM of at least six different animals. (+ $p < 0.005$, +++ $p < 0.05$, relative to the normal strain) The ratio of the enzymatic activities of GAD relative to GABA-T is shown in the insert.

cal Co. (St. Louis, MO) and were of the highest purity available.

Protein Determination

The protein concentration was measured as described by Bradford *(11)* using bovine serum albumin as standard.

Kinetic and Statistical Analysis

The kinetic constants (U_{max}, maximal uptake and the apparent K_m) were estimated by linear regression analysis of the Eadie Hofstee plot (Uptake vs uptake/total concentration) using Inplot (v. 3.15) obtained from GraphPad (San Diego, CA). Statistical analysis of data was performed using Student's *t*-test with two-tailed comparison using INSTAT (v. 1.13, GraphPad).

Results

Figure 1 shows the activities of glutamic acid decarboxylase (GAD), GABA aminotransferase (GABA-T), alanine aminotransferase (ALA-T), aspartate aminotransferase (ASP-T) and glutamate dehydrogenase (GDH) in the whole mouse brain S_1 fraction (800*g* supernatant). Except for ALA-T activity, all the other enzymatic activities were significantly reduced in the epileptic mice. The ratio of enzymatic activity of GAD relative to GABA-T suggests that the synthesis rather than the degradation is mainly reduced.

GABA uptake is significantly reduced in synaptosomes from cortex, midbrain, and pons medulla of SPS/sps mice, as shown in Fig. 2. In contrast, no changes are observed in GABA uptake by hippocampal synaptosomes of epileptic mice.

Figure 3 shows the K^+-stimulated, Ca^{2+}-dependent release of radioactivity from crude synaptosomes preloaded with ^{14}C-GABA from cortex, midbrain, hippocampus, and pons medulla. In the cortex and midbrain preparations, there is no difference in the height of the normalized release among the strains. In pons medulla and hippocampal preparations, there is a modest, but not significant increase in the normalized release.

The saturation curves for [^{3}H]GLU uptake by mouse brain synaptosomes from 2-mo-old normal and epileptic mice are shown in Fig. 4. From the Eadie Hofstee analysis, shown in inserts, two high-affinity systems are identified in crude synaptosomes from normal mice. They have been defined as follows: (1) System 1 with an apparent K_m of 3.65 ± 0.67 μ*M*, and U_{max} of 1.21 ± 0.10 nmol/mg protein/5 min; and (2) System 2 with an apparent K_m of 46.78 ± 4.34 μ*M* and U_{max} of 3.69 ± 0.15 nmol/mg prot./5 min. In epileptic mice, both the apparent K_m and U_{max} values for System 2 are significantly increased (K_m of 108.24 ± 9.74 μ*M* and U_{max} of 8.39 ± 0.50 nmol/mg prot./5 min, $p < 0.0001$) [^{3}H]GLU U_{max} by System 1 is significantly decreased (0.50 ± 0.07 nmol/mg prot./5 min., $p < 0.005$). The appar-

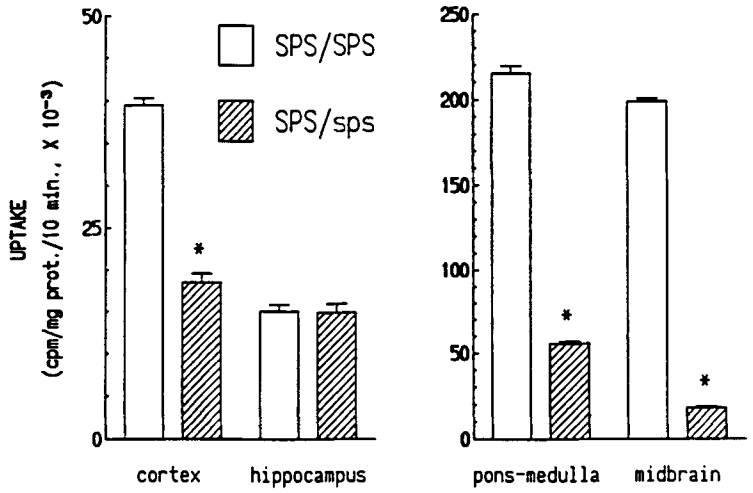

Fig. 2. GABA uptake by crude synaptosomes from cortex, midbrain, hippocampus, and pons medulla of normal (SPS/SPS) and epileptic (SPS/sps) mice. (*$p < 0.05$).

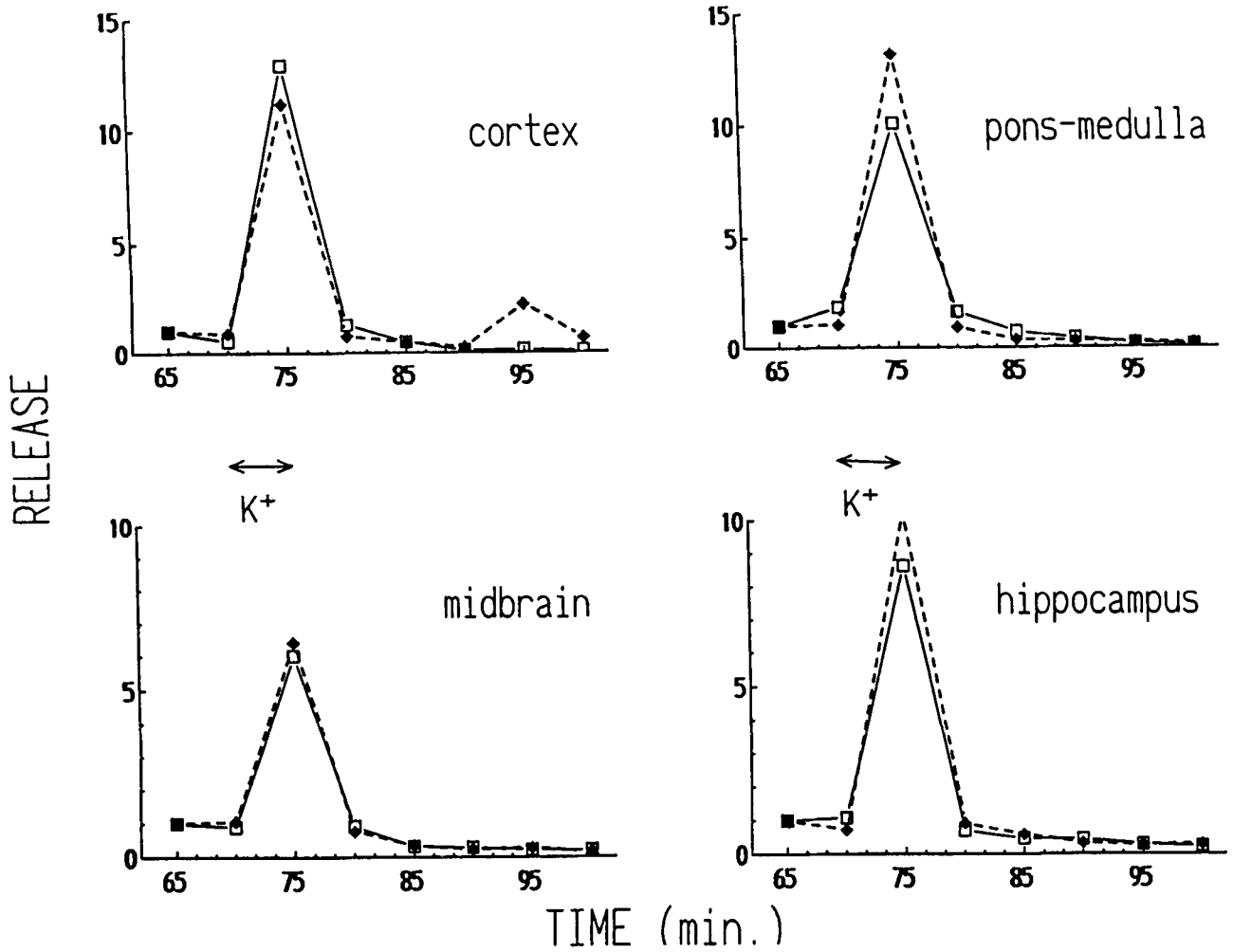

Fig. 3. K^+-stimulated, Ca^{2+}-dependent release of radioactivity from crude synaptosomes preloaded with [^{14}C-GABA] in different brain regions. A 47.0-m*M* K^+ pulse was given at 70 min. Thereafter, normal Krebs-Henseleit buffer was used to perfuse the synaptosomes.

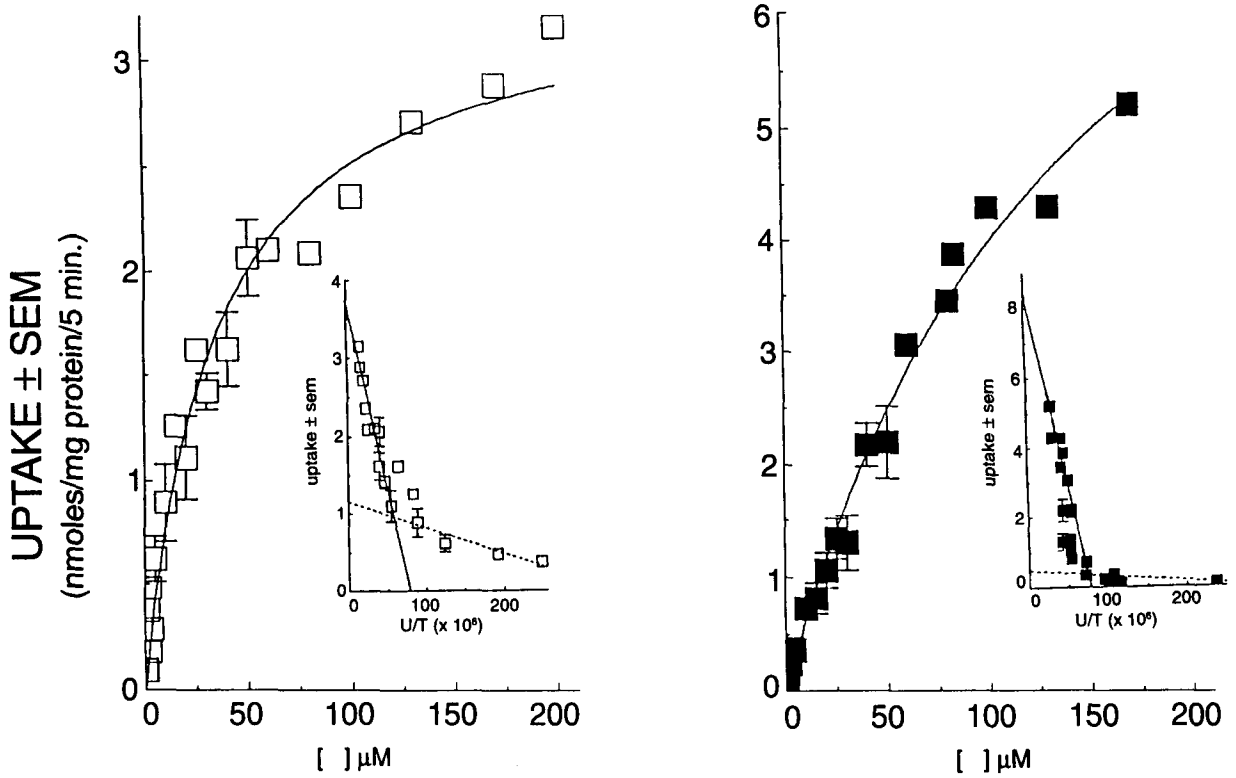

Fig. 4. Saturation curves for [^{3}H]GLU uptake by whole brain crude synaptosomes from normal (open squares) and epileptic (filled squares) mice. The corresponding Eadie Hofstee analysis (Uptake vs U/T) of [^{3}H]GLU uptake is shown in the inserts. Each value represents the mean ± SEM of at least three experiments. Dashed lines denote System 1, whereas the solid lines denote the linear regression analysis of the Eadie Hofstee plot of System 2.

ent K_m value is also reduced, but not to a significant degree, suggesting that this system is still present, but has less capacity. These changes exhibit regional specificity since glutamate U_{max} is increased in synaptosomes of cortex and midbrain of epileptic mice, whereas it is reduced in the hippocampus *(12)* and pons medulla *(13)*.

Discussion

Epilepsy may be visualized as excessive neuronal discharges. At the cellular level, this hyperexcitable state could be the result of enhanced excitatory and/or decreased inhibitory transmission.

All the alterations observed in SPS/sps mice appear to be compensatory in nature since they are in the opposite direction to what would be expected. For example, GABAergic transmission appears to be enhanced as indicated by the decreased uptake in all areas but hippocampus in the presence of unaltered release. Similarly, the reduced GAD/GABA-T ratio observed in SPS/sps mutants points to altered GABA turnover since there are no alterations in amino acid levels in SPS/sps mouse mutants (unpublished results). Similarly, SPS/sps mice appear to be attempting to reduce EAA transmission as the glutamate uptake is increased, and the enzymatic activities are reduced. However, preliminary results suggest a slight, but not significant decrease in [^{3}H]MK-801 binding and release in whole brain synaptosomes from these epileptic mice.

There is evidence for the role of excitatory amino acids (EAA) and GABA transmission in the epileptogenesis of the genetically epilepsy-prone rat (GEPR) *(3)*. Our studies are in agreement with previous results in the epileptic rats. The changes observed in GABA and glutamate uptake in pons medulla are of particular importance because the inferior colliculus *(14)* plays a key role in audiogenic seizures. The fact that audiogenic seizures are limited to the brain stem *(15)* explains the little observable change in the hippocampus.

These results support previous evidence for the role of reuptake during the initiation of seizures

(16). The recent cloning and characterization of the amino acid transporters *(17–20)* may help to elucidate the possible molecular mechanisms responsible for GABA and GLU uptake alterations in epileptic mice.

Summary

Our data demonstrate GABAergic and glutamatergic alterations in the brain of epileptic mice. Changes in GABAergic transmission are indicated by: (1) the reduction of GAD and GABA-T activities, and (2) the region-specific decrease of GABA uptake in cortex, midbrain, and pons medulla. Excitatory amino acid transmission is also affected in these mice, as indicated by a decrease in the enzymatic activities of GDH and ASP-T, and the increase in whole brain synaptosomal GLU uptake. These changes in transmission may reflect compensatory mechanism(s) to regulate the neurotransmission process during seizures.

Acknowledgments

This work was supported by the Institutional NIH/MBRS (grant no. 2 SO6 GMO8224) Program, RCMI Program, and the Animal Resource Center. M. L. Cordero was a recipient of a Minority Predoctoral Fellowship from the American Psychological Association. We are very grateful for the technical assistance of Sonia Cardona, Vladimir Vélez, Olga Claudio, and Rebeca Méndez.

References

1. Dingledine R., McBain C. J., and McNamara O. (1991) *Trends Pharm. Sci. Special Report*, 49–53.
2. Meldrum B. S. and Murugaiah K. (1983) *Eur. J. Pharmacol.* **89,** 149–152.
3. Laird H. E. and Jobe P. C. (1987) in *Neurotransmitters, and Epilepsy* (Jobe P. C. and Laird H. E., eds.), Humana, Clifton, NJ, pp. 57–94.
4. Van Gelder N. M., Sherwin A. L., and Rasmussen T. (1972) *Brain Res.* **40,** 385–393.
5. Maxson S. C., Fine M. D., Ginsburg B. E., and Koniecki D. (1983) *Epilepsia* **24,** 15–24.
6. Flavin H. J., Wieraszko A., and Seyfried T. N. (1991) *J. Neurochem.* **56,** 1007–1011.
7. Ortiz J. G., Negrón A. E., Thomas A. P., Heimer H., Moreira J. A., Cordero M. L., Aranda J., and Bruno M. S. (1991) *Exp. Neurol.* **113,** 338–343.
8. Sze P. Y. (1977) *Brain Res.* **122,** 59–69.
9. Dinwoodie R. C. and Boecker E. A. (1979) *Analyt. Biochem.* **96,** 24–33.
10. Robinson M. B., Hunter-Ensor M., and Sinor J. (1991) *Brain Res.* **544,** 196–202.
11. Bradford M. M. (1976) *Analyt. Bochem.* **72,** 248–254.
12. Cordero M. L., Ortiz J. G., and Santiago G. (1994) *Analyt. Biochem.* **78,** 44–48.
13. Ortiz J. G., Cordero M. L., and Santiago G. (1993) Workshop on Neurobiology of Epilepy, Tromso, Norway, June 26–29.
14. Faingold C. L., Naritoku D. K., Copley C. A., Randall M. E., Riaz A., Boersma-Anderson C. A., and Arneric S. P. (1992) *Epilepsy Res.* **13,** 95–105.
15. Maxson S. C. and Cowen J. S. (1976) *Physiol. Behav.* **16,** 623–629.
16. Chapman A. G. (1993) Workshop on Neurobiology of Epilepsy, Tromso, Norway, June 26–29.
17. Kanai Y. and Hediger M. (1992) *Nature* **360,** 467–469.
18. Pines G., Danbolt N., Bjoras M., Zhang Y., Bendahan A., Elde L., Koepsell H., Storm-Mathisen J., Seeberg E., and Kanner B. I. (1992) *Nature* **360,** 464–466.
19. Storck T., Schulte S., Homann K., and Stoffel W. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10,955–10,959.
20. Uhl G. R. and Hartig P. R. (1992) *Trends Pharm. Sci.* **13,** 421–425.