Altered Excitatory and Inhibitory Amino Acid Receptor Binding in Hippocampus of Patients with Temporal Lobe Epilepsy

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We examined binding to excitatory amino acid and inhibitory amino acid receptors in frozen hippocampal sections prepared from surgical specimens resected from 8 individuals with medically refractory temporal lobe epilepsy. The excitatory receptors studied included N-methyl-D-aspartate (NMDA), strychnine-insensitive glycine, phencyclidine, and quisqualate. The inhibitory receptors studied were gamma-aminobutyric acid type A (GABA$_A$) and benzodiazepine. Excitatory and inhibitory amino acid receptor binding were differentially altered in the patients with temporal lobe epilepsy in comparison to 8 age-comparable autopsy control subjects, and changes in receptor binding were regionally selective in four areas. Binding to phencyclidine receptors associated with the NMDA channel was reduced by 35 to 70% in all regions in the hippocampi of the patients. In contrast, binding to the NMDA recognition site and its associated glycine modulatory site was elevated by 20 to 110% in the cornu ammonis (CA) 1 area and dentate gyrus of the hippocampus of the patients. Binding to these sites was unaffected in area CA4. Binding to the quisqualate-type excitatory amino acid receptor was unchanged in all regions except the stratum lacunosum moleculare CA1, where it was increased by 63%. GABA$_A$ and benzodiazepine receptor binding was reduced by 20 to 60% in CA1 and CA4, but unchanged in dentate gyrus. The data indicate that excitatory and inhibitory amino acid receptors are altered in the hippocampus of patients with temporal lobe epilepsy.

Considerable evidence suggests that abnormalities of specific neurotransmitter systems play a role in epilepsy. Impairment of gamma-aminobutyric acid(GABA) ergic inhibitory neurotransmission may contribute to epilepsy: GABA type A (GABA$_A$) receptor antagonists are potent convulsants and GABAergic markers are selectively reduced in temporal lobe epilepsy (TLE) [1–8]. Excitatory amino acid (EAA) neurotransmitters such as glutamate and aspartate also may contribute to epileptic seizures and to central nervous system (CNS) injury consequent to seizures [9–13]. EAA neurotransmitters are the principal excitatory neurotransmitters in mammalian brain and participate prominently in normal hippocampal function [14]. EAA receptor agonists are also potent convulsants and neurotoxins [13, 15–17]. Local injection of EAA receptor agonists and stimulation of glutamatergic afferent pathways produce a pattern of acute and chronic morphological changes in the rat hippocampus that are similar to the

changes found in the hippocampus of patients with TLE [17–25]. EAA receptor antagonists possess anticonvulsant properties and prevent the morphological changes associated with seizures [13, 16, 26–28]. EAA receptors may be involved in the formation of epileptogenic foci since antagonists of EAA receptors suppress the development of spontaneous seizures and associated neurochemical changes produced by kindling, a model of TLE [29–33]. Direct evidence to support the role of EAA in human epilepsy, however, is limited [34–37].

The current study is based on the hypothesis that binding to EAA and inhibitory amino acid (IAA) neurotransmitter receptors may be altered in epileptic brain tissue of patients with TLE. We measured binding to receptors associated with the N-methyl-D-aspartate (NMDA) receptor/channel complex, the quisqualate-type EAA receptors, and the GABA$_A$-benzodiazepine complex IAA-type receptors in hippo-
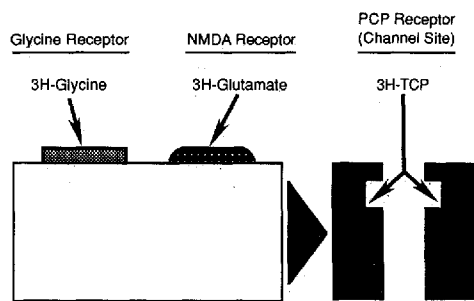
Fig 1. The receptor components that comprise the NMDA receptor/ channel complex. The NMDA recognition site can be selectively labeled with [³H]glutamate. Activation of the glycine modulatory site markedly enhances receptor/channel activation. [³H]Glycine can be used to selectively label this site. The phencyclidine (PCP) analogues, tritiated thienyl derivative of PCP (TCP) and MK-801, reduce receptor/channel activation by binding to a site within the channel. The binding site can be labeled selectively with [³H]TCP.

campal tissue resected from patients with medically refractory TLE and from comparably aged postmortem control specimens.

The regional distribution of EAA and IAA receptors in brain can be imaged and studied quantitatively by labeling each receptor with a tritiated ligand under conditions selective for each receptor type. The NMDA-type EAA receptor/channel complex is comprised of several regulatory binding sites (Fig 1) [38]. The NMDA recognition site can be selectively labeled, un-

der appropriate conditions, with [³H]glutamate. The associated glycine modulatory site can be measured with [³H]glycine. The tritiated thienyl derivative of phencyclidine (TCP) selectively labels a binding site located within the NMDA receptor–associated ionophore. The quisqualate-subtype of EAA receptors can be measured with [³H]glutamate under selective conditions. The modulatory receptors comprising the $GABA_A$ receptor/ionophore complex can be measured with [³H]muscimol to label the $GABA_A$ recognition site and [³H]flunitrazepam to label the associated benzodiazepine site [39].

Materials and Methods

We studied 8 patients (4 males and 4 females) with medically refractory TLE whose seizure focus originated unilaterally (Table 1). The anterior 2 cm of the hippocampus was resected from these patients. Control hippocampus was obtained at autopsy from 8 subjects (4 males and 4 females) who had no reported history of neurological or psychiatric disease (mean age ± standard error of mean, 46 ± 5 years; postmortem delay, 11 ± 2 hours). Hippocampal tissue was examined by a neuropathologist (P. E. M.) to determine an anatomical diagnosis, and neuronal loss was rated qualitatively.

Materials

[³H]TCP was obtained from New England Nuclear (Wilmington, DE). [³H]Glutamate, [³H]glycine, [³H]muscimol, and [³H]flunitrazepam were obtained from Amersham (Arlington Heights, IL). All the unlabeled compounds were purchased from commercial sources.

Table 1. Clinical Characteristics and Neuropathological Findings of Patients with Temporal Lobe Epilepsy

Patient No.	Sex	Age (yr)	Side Resected	Age at Seizure Onset (yr)	Initial Febrile Seizure	Duration of Frequent Seizures (yr)	Seizure Frequence at Time of Surgery	Medications at Time of Surgery	Pathology
1	F	29	L	0.5	Yes	21	4/wk, CPS 1/mo, GTC	PHT P	CA1 Neuronal loss
2	F	29	R	13	No	16	3/wk, CPS	PHT CBZ	Astrocytoma, grade 1
3	F	20	L	4	No	16	3/wk, CPS	CBZ VPA	Astrocytoma, grade 1
4	M	28	L	2	No	16	1/wk, SPS 1/mo, CPS	PHT	Neuronal loss and gliosis CA1, calcification CA4
5	M	60	L	1	Yes	40	1.5/mo, CPS 8/mo, SPS	PHT	Not available
6	F	18	R	9	No	6	8/mo, CPS	CBZ VPA	Oligodendroglioma
7	M	43	L	0.5	Yes	34	3/wk, CPS	CBZ	Not available
8	M	27	L	1	Yes	20	3/wk, CPS	CBZ	Neuronal loss in dentate gyrus

CPS = complex partial seizures; GTC = generalized tonic-clonic seizures; SPS = simple partial seizures; PHT = phenytoin; P = primidone; CBZ = carbamazepine; VPA = valproic acid.

Tissue Preparation

Once obtained, hippocampal specimens from patients with TLE and autopsy control subjects were quickly frozen on dry ice, stored at $-70°C$, and mounted on cryostat chucks. Mounted specimens were allowed to equilibrate to the temperature of the cryostat (-12 to $-20°C$) and 20-μm frozen sections were cut and thaw-mounted onto gelatin-coated slides. Binding to each type of receptor was carried out in adjacent serial sections from each specimen. Total receptor binding and nonspecific binding were determined in at least 3 sections per specimen per each receptor assay. In order to remove endogenous neurotransmitters and modulatory ligands, all sections underwent a prewash for 30 minutes at 4°C in the indicated buffer and were blown dry under a stream of air at room temperature prior to receptor labeling. The sections were incubated with radioactive ligands at subsaturating concentrations for periods of time shown previously to allow equilibrium conditions to be reached. During the incubation period, sections were placed in cytomailers containing the [³H]ligand (final volume, 8 ml); under these conditions less than 5% of free ligand is bound.

NMDA Recognition Site Binding Assay

The EAA receptor agonist [³H]glutamate was used to label the NMDA-type EAA receptor recognition site under conditions that select for binding to NMDA recognition sites [40, 41]. Sections were prewashed for 30 minutes at 4°C in 50 mM Tris-acetate (pH 7.4) to remove endogenous EAA neurotransmitters, and were dried under a stream of air at room temperature. Subsequently, sections were incubated for 45 minutes in 50 mM Tris-acetate (4°C, pH 7.4) containing 2.5 μM quisqualate and 40 nM [³H]glutamate to label NMDA recognition sites selectively. Since glutamate binds to both NMDA and quisqualate receptors, a 2.5 μM concentration of quisqualate, which saturates quisqualate receptors, was included in the incubation buffer so that [³H]glutamate would selectively label the remaining NMDA-type EAA receptors. A second group of sections was incubated in the above buffer and unlabeled glutamate (1 mM) to determine the level of nonspecific binding. Following incubation, sections were rinsed three times with 2 ml of ice-cold buffer, followed by a final rinse with 2 ml of ice-cold glutaraldehyde (49%)-acetone mixture (1:19 vol/vol), and quickly dried under a stream of warm air to minimize uneven ligand dissociation.

Glycine Modulatory Site Binding Assay

Tissue sections were prewashed in 50 mM Tris-citrate (pH 7.4, 4°C) for 30 minutes to remove endogenous ligands, and then were dried under a stream of air at room temperature. Tissue sections were then incubated in the same buffer (pH 7.4) containing 100 nM [³H]glycine and 100 μM strychnine for 35 minutes at 4°C [42]. Under these conditions, the saturating concentration of strychnine prevents [³H]glycine from labeling the inhibitory glycine receptor and provides a selective measure of the glycine modulatory sites associated with the NMDA receptor/channel complex [42]. Nonspecific binding was determined in the same buffer in the presence of 1 mM unlabeled glycine and represented less than 10% of total binding. Following incubation, sections were rinsed three times with 2 ml of ice-cold buffer, followed by a final

rinse with 2 ml of ice-cold glutaraldehyde-acetone mixture (1:19 vol/vol), and quickly dried under a warm stream of air to optimize the level of specific binding and to minimize uneven ligand dissociation.

Phencyclidine Receptor (NMDA-Associated Channel Binding Site) Binding Assay

Sections were prewashed for 30 minutes in 50 mM Tris-acetate (pH 7.4, 4°C) to remove endogenous modulatory compounds, and sections were dried under a stream of air at room temperature. Phencyclidine (PCP) receptors were labeled with the selective PCP receptor ligand [³H]TCP. Tissue sections were incubated with 30 nM [³H]TCP in 50 mM Tris-acetate plus 1 mM magnesium (Mg^{2+}) acetate for 45 minutes at 4°C (pH 7.4) [41, 43]. Addition of 1 mM Mg^{2+} to the incubation buffer previously has been shown to maximize the level of [³H]TCP binding in frozen brain sections [43]. With this method, binding equilibrium is achieved by approximately 45 minutes in the presence of 1 mM Mg^{2+}. The level of nonspecific binding was determined by incubating sections in the same buffer containing 20 μM unlabeled TCP. The level of nonspecific binding was minimized by subsequently rinsing the sections, after the incubation, three times for 1 minute each with ice-cold buffer. Sections were then rapidly dried under a stream of warm air.

Quisqualate-Type Receptor Binding Assay

Sections were prewashed for 30 minutes in 50 mM Tris-hydrochloric acid (HCl) (pH 7.4, 4°C) to remove endogenous ligands and then dried under a stream of cool air. The quisqualate subtype of EAA receptors was labeled with [³H]glutamate in the presence of a saturating concentration of NMDA to prevent [³H]glutamate binding to the NMDA site. This method selectively labels quisqualate receptors in human brain tissue [40, 44]. Tissue sections were incubated for 45 minutes in 50 mM Tris-HCl (pH 7.4) containing 100 μM NMDA and 2.5 mM calcium chloride ($CaCl_2$) at 4°C. Addition of 2.5 mM $CaCl_2$ to the incubation medium selectively maximizes binding to the quisqualate subtype of EAA receptors. The level of nonspecific binding was determined by incubating additional sections in the same incubation buffer plus 1 mM unlabeled glutamate. Subsequently, tissue sections were rinsed three times with 2 ml of ice-cold buffer and one time with ice-cold glutaraldehyde-acetone solution (1:19 vol/vol) and rapidly dried under a stream of warm air. Under these conditions, binding in this assay represents mainly postsynaptic neuronal, non-NMDA-type quisqualate receptors [44]. In contrast to binding in membranes, quisqualate receptor binding in the presence of $CaCl_2$ in frozen tissue sections represents postsynaptic high-affinity neuronal binding sites rather than sequestration sites [44].

GABA Receptor Binding Assay

Tissue sections were prewashed for 30 minutes in 50 mM Tris-citrate (pH 7.0, 4°C) to remove endogenous ligands and were dried under a stream of cool air. $GABA_A$ receptors were labeled with the selective $GABA_A$ receptor ligand [³H]muscimol [45]. Sections were incubated for 30 minutes in 50 mM Tris-citrate (pH 7.0, 4°C) containing 50 nM [³H]muscimol. The level of nonspecific binding was deter-

mined in the presence of 100 μM GABA. Following incubation, sections were rinsed four times with 2 ml of ice-cold buffer and rapidly dried under a stream of warm air.

Benzodiazepine Receptor Binding Assay

Sections were prewashed three times for 5 minutes each at 4°C in 50 mM Tris-citrate (pH 7.0) to remove endogenous ligands and dried under a stream of cool air. Benzodiazepine receptors associated with $GABA_A$ receptor/channel complexes were labeled with the selective benzodiazepine receptor ligand [³H]flunitrazepam [46, 47]. Sections were incubated for 30 minutes in 50 mM Tris-citrate (pH 7.0, 4°C) plus 10 nM [³H]flunitrazepam. Nonspecific binding was determined in the presence of the benzodiazepine receptor ligand clonazepam (1 mM). Following incubation, sections were rinsed two times for 5 minutes each in ice-cold buffer and rapidly dried with a stream of warm air.

Data Collection and Analysis

Tissue sections were apposed to tritium-sensitive film (LKB Ultrafilm 3H; Pharmacia Co, Bromma, Sweden) for 1 to 8 weeks. A set of radioactive standards (American Radiochemicals, St Louis, MO; ARC 3H) calibrated against brain pastes with known amounts of tritium was exposed with each film. Quantitative analysis of the resulting autoradiograms was performed densitometrically with a microcomputer-based video densitometer system (Imaging Research, St Catharines, Ontario, Canada) [45]. Optical density values were converted to pmol/mg of protein values with a computer-generated polynomial regression analysis that compared film densities produced by the tissue sections to those of radioactive standards. Ten readings per area were averaged and means of averaged readings from 3 to 5 sections per patient per receptor assay were compared to corresponding values for control subjects. Four hippocampal regions were examined: stratum lacunosum moleculare and stratum pyramidale of cornu ammonis 1 (CA1), area CA4 (hilus), and stratum moleculare of the dentate gyrus. Other hippocampal areas were not consistently present in resected tissue from patients with TLE. Receptor binding data represent specific binding (total minus nonspecific binding) and are presented as mean ± SEM in density units (fmol/mg of protein). Statistical comparisons were made with one-way analysis of variance (ANOVA).

Results

Table 1 contains a summary of the clinical characteristics and neuropathological findings of the 8 patients with TLE. The mean age (± SEM) of the patients with TLE at the time of surgery was 32 ± 5 years compared to 46 ± 5 years for the autopsy control subjects (n = 8). The range of ages in both groups was similar (TLE, 18 to 60 years; controls, 22 to 61 years). Four of the 8 patients with TLE were male. In 6 of the 8 patients with TLE, seizures originated from the left hemisphere. The initial age of onset of seizures occurred in the first postnatal year in 4 of the 8 patients with TLE and these seizures were all associated with febrile convulsions. The duration of the seizure disorder ranged from 6 to

40 years (mean ± SEM, 21 ± 4 years). The patients with TLE were taking single or multiple anticonvulsant drugs (see Table 1). At the time of surgery, the patients received multiple different medications, including thiamylal (Surital), fentanyl, droperidol, and labetalol, as well as the anesthetics nitrous oxide, isoflurane (Forane). One patient received morphine, and another, lorazepam. Although hippocampal neuronal loss was not examined quantitatively in this study, qualitative analysis of the hippocampi in the patients with TLE by a neuropathologist (P. E. M.) revealed mild astroglial proliferation and loss of pyramidal and granule cells in the majority of patients. Severe cell loss in the hippocampal subfields and in the hilus, typical of Ammon's horn sclerosis, was not evident in any of the patients. Three patients had extrahippocampal temporal lobe tumors: 2, grade 1 astrocytomas; and 1, oligodendroglioma.

NMDA Recognition Site Binding

Regionally selective increases in binding to the NMDA recognition site were observed in the surgical hippocampal specimens compared to autopsy control brains (Table 2, Figs 2 and 3). Significant mean increases in NMDA recognition site binding were found in stratum lacunosum moleculare and stratum pyramidale of hippocampal area CA1, and in stratum moleculare of the dentate gyrus but not in the CA4 subfield of the hippocampi of patients with TLE, compared to autopsy control subjects. Elevated levels of NMDA recognition site binding were not found in all TLE specimens: 1 TLE specimen exhibited levels of NMDA receptor binding lower than the mean level in autopsy control subjects. The interspecimen variability in receptor binding was greater in the TLE group than in the autopsy control subjects.

Glycine Modulatory Site Binding

Selective laminar changes in [³H]glycine binding were observed in the CA1 hippocampal subfield (see Table 2 and Fig 3). In the TLE group, glycine modulatory site binding was significantly increased in stratum lacunosum moleculare but the level of binding in stratum pyramidale was equivalent to the level in autopsy control subjects. Binding in stratum moleculare in the dentate gyrus of TLE specimens was significantly elevated relative to binding in autopsy control subjects. In contrast, equivalent binding densities were observed in hippocampal subfield CA4 of TLE and control groups. The variation in [³H]glycine binding in both TLE and autopsy groups was greater than for any of the other binding assays and the degree of variation in the TLE group was especially pronounced. In fact, [³H]glycine binding densities in several patients with TLE were below the corresponding mean binding densities of the autopsy control subjects.

Table 2. Excitatory and Inhibitory Amino Acid Receptor Binding in Hippocampus of Patients with Temporal Lobe Epilepsy (TLE)[a]

Receptor Binding	Hippocampal Region			
	Stratum Lacunosum Moleculare of CA1	Stratum Pyramidale of CA1	CA4	Stratum Moleculare of Dentate Gyrus
NMDA				
Control	175 ± 15	480 ± 46	96 ± 13	288 ± 43
TLE	365 ± 48[b]	702 ± 84[c]	145 ± 51	610 ± 71[d]
	(+108%)	(+46%)	(+51%)	(+111%)
Glycine (strychnine-insensitive)				
Control	469 ± 45	1093 ± 72	304 ± 33	675 ± 67
TLE	801 ± 137[c]	1231 ± 186	344 ± 95	1065 ± 162[c]
	(+71%)	(+13%)	(+13%)	(+57%)
Phencyclidine				
Control	122 ± 18	373 ± 27	112 ± 10	247 ± 29
TLE	79 ± 10	209 ± 25[d]	33 ± 7[d]	163 ± 23[c]
	(−35%)	(−44%)	(−71%)	(−34%)
Quisqualate				
Control	298 ± 25	487 ± 29	201 ± 16	520 ± 40
TLE	486 ± 81[c]	598 ± 89	174 ± 49	589 ± 72
	(+63%)	(+23%)	(−14%)	(+13%)
GABA$_A$				
Control	3250 ± 281	4843 ± 357	1279 ± 122	4603 ± 350
TLE	1803 ± 279[b]	3180 ± 382[b]	533 ± 183[b]	3933 ± 431
	(−45%)	(−34%)	(−58%)	(−15%)
Benzodiazepine				
Control	966 ± 92	1161 ± 68	480 ± 45	1197 ± 40
TLE	790 ± 93	820 ± 87[b]	262 ± 77[c]	1107 ± 86
	(−18%)	(−29%)	(−45%)	(−7%)

[a]Values represent mean ± SEM (fmol bound/mg of protein). Values in parentheses represent percent change in mean receptor binding in hippocampus of patients with TLE relative to control subjects.
[b]$p < 0.01$.
[c]$p < 0.05$.
[d]$p < 0.001$.

Phencyclidine Receptor (Channel Site) Binding
[^{3}H]TCP binding to the NMDA-associated ionophore was consistently decreased in all four hippocampal regions of specimens from patients with TLE, compared with autopsy control values (see Table 2, Figs 2 and 3). The largest decrease was observed in the CA4 subfield, with smaller decreases in other hippocampal regions. Variation in PCP receptor binding between specimens was equivalent for TLE and autopsy control groups and was the smallest observed among the different binding assays. PCP binding densities were uniformly lower in specimens from patients with TLE than corresponding mean densities of the autopsy control group.

Comparison of Relative Changes in NMDA, Glycine, and Phencyclidine Receptor Binding
Figure 4 illustrates the relative changes in binding to three components of the NMDA receptor/channel complex within individual patients with TLE. In stratum lacunosum moleculare of CA1, the pattern of relative changes in NMDA, glycine, and PCP receptor binding was consistent between patients with TLE. The elevations of NMDA binding paralleled the changes in

glycine receptor binding in patients with TLE. PCP receptor binding was consistently reduced in patients with TLE. Similar relative changes were observed in the other hippocampal areas examined.

Quisqualate-Type Receptor Binding
[^{3}H]Glutamate binding to quisqualate-preferring receptors in stratum lacunosum moleculare of CA1 was significantly elevated in patients with TLE, compared with autopsy specimens (see Table 2 and Fig 3). A similar trend was observed in stratum pyramidale of CA1, but the results were not statistically significant. However, equivalent binding densities were observed between specimens from patients with TLE and autopsy control subjects in the CA4 subfield and in stratum moleculare of the dentate gyrus. Interspecimen variation in binding densities was greatest in the TLE group, compared with the autopsy group.

GABA$_A$ Receptor Binding
The changes in IAA receptor binding in the hippocampus of patients with TLE were different from those in EAA binding (Figs 5 and 6; see Table 2). GABA$_A$ receptor binding was significantly reduced in both lami-
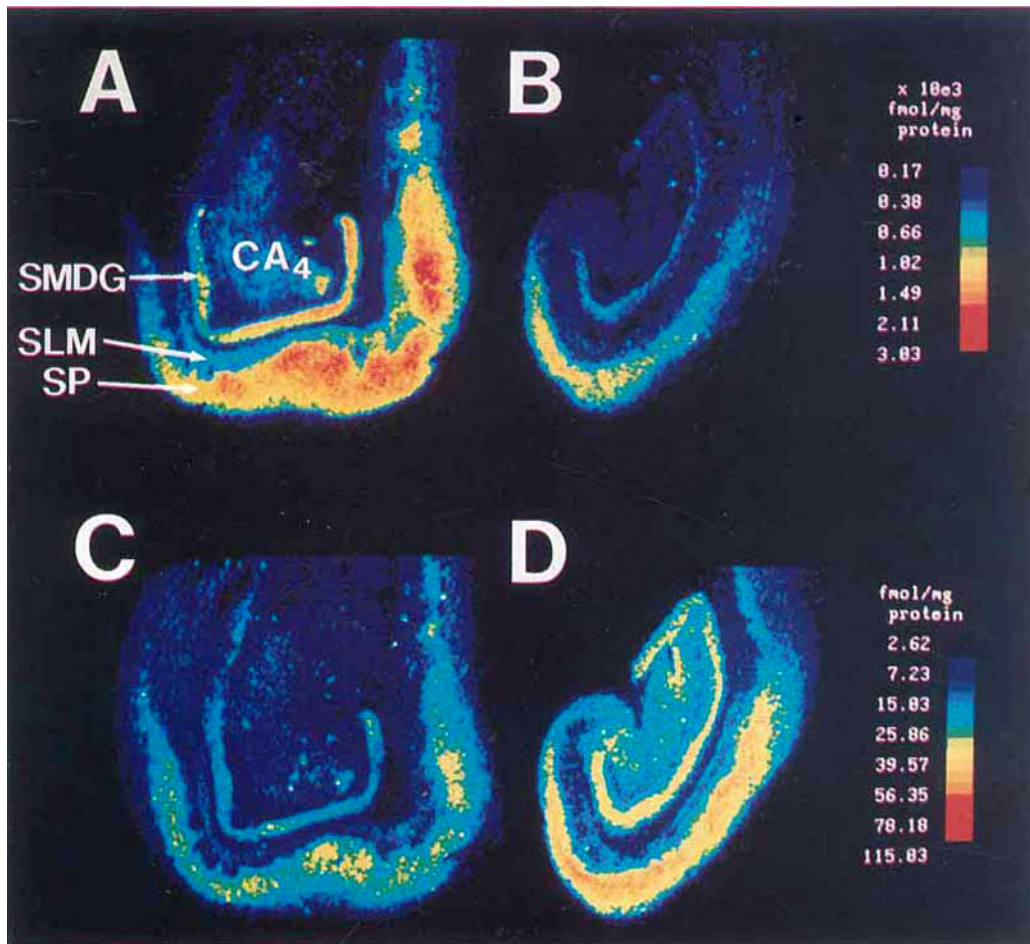
Fig 2. Quantitative autoradiograms of NMDA (A, B) and phencyclidine (PCP) (C, D) receptor binding in the hippocampus of patients with temporal lobe epilepsy (TLE) (A, C) and control subjects (B, D). NMDA receptors were labeled with 40 nM [³H]glutamate in 50 mM Tris-acetate plus 2.5 µM quisqualate. PCP receptors were labeled with 40 nM [³H]TCP (tritiated thienyl derivative of PCP) in 50 mM Tris-acetate and 1 mM magnesium acetate. Note that the color scale bars for NMDA and PCP receptor binding are different. NMDA and PCP receptor binding have similar regional distributions in control specimens.
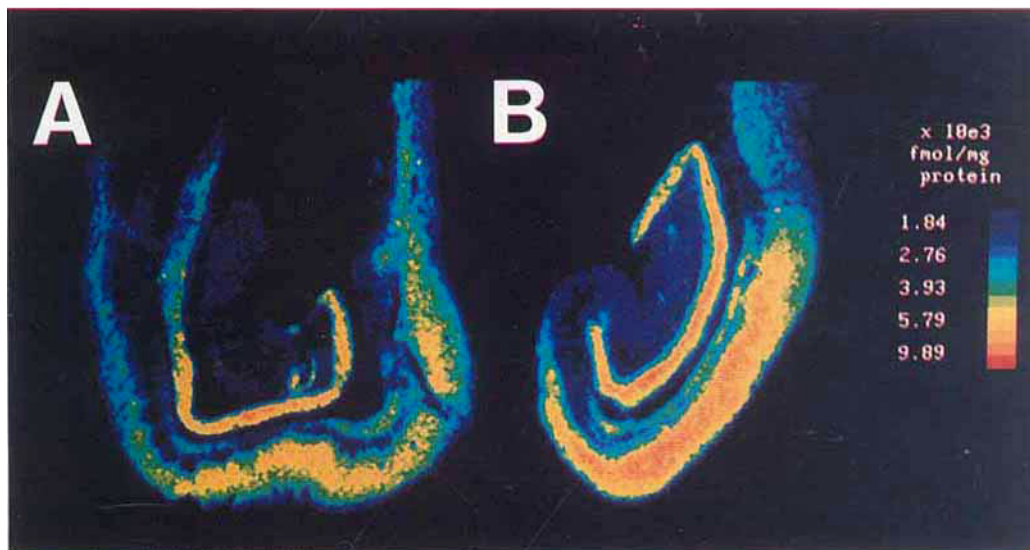
Fig 5. Quantitative autoradiograms of GABA_A receptor binding in the hippocampus of patients with temporal lobe epilepsy (TLE) (A) and autopsy controls (B). GABA_A receptors were labeled with 50 nM [³H]muscimol in 50 mM Tris-citrate.
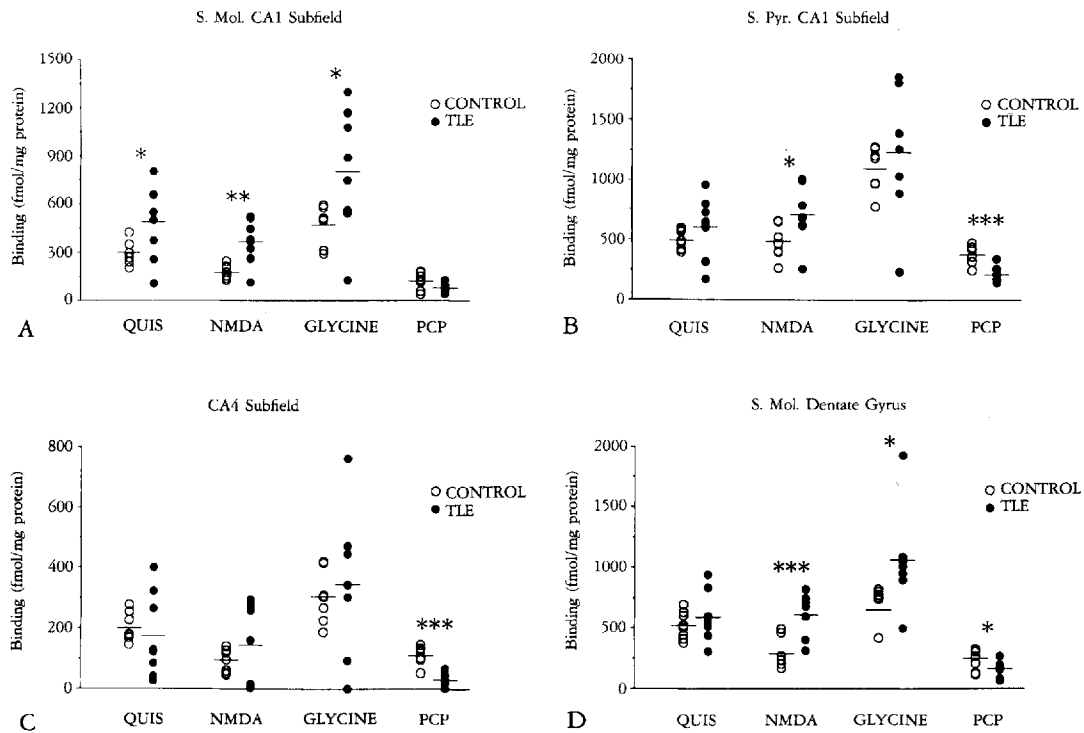
Fig 3. Histograms of excitatory amino acid receptor binding. (A) Stratum lacunosum moleculare of CA1, (B) stratum pyramidale of CA1, (C) CA4, and (D) stratum moleculare in the dentate gyrus of hippocampi from patients with chronic temporal lobe epilepsy (TLE) and from age-matched autopsy control subjects. Binding to quisqualate-type glutamate receptors (QUIS) and to three receptors comprising the NMDA receptor channel complex (NMDA recognition site, glycine modulatory site, and phencyclidine [PCP] receptor) was measured in adjacent frozen sections with in vitro receptor autoradiography. Data represent binding densities in hippocampal regions from individual patients. Horizontal lines indicate the mean values for each group. Hippocampi from 8 patients with TLE and from 8 autopsy control subjects were used for each receptor assay. *p < 0.05, **p < 0.01, ***p < 0.001, TLE versus control, one-way ANOVA.

nae of the CA1 subfield and in the CA4 subfield in the hippocampus of patients with TLE, compared with autopsy control subjects. Binding densities, however, were equivalent between the groups in the stratum moleculare of the dentate gyrus. The degree of GABA$_A$ receptor binding variability between specimens was similar for both TLE and autopsy control groups.

Benzodiazepine Receptor Binding

The regional changes in benzodiazepine receptor binding between TLE and autopsy groups paralleled the regional changes in GABA$_A$ receptor binding (see Table 2, and Figs 5 and 6); however, there was a nonsignificant trend for the magnitude of these changes to be smaller. [^{3}H]Flunitrazepam binding to benzodiazepine
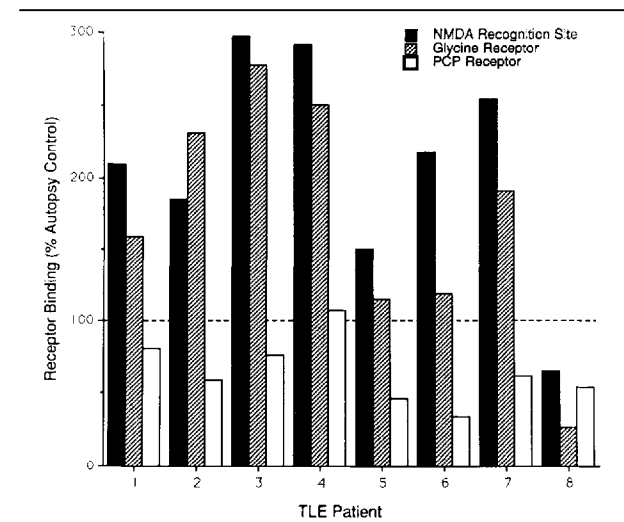

Fig 4. Comparison of relative binding to NMDA, glycine, and phencyclidine (PCP) receptors within temporal lobe epilepsy (TLE) specimens. Mean receptor binding in stratum moleculare in CA1 is expressed as a percent of mean binding in autopsy controls. The patient numbers correspond to the patient numbers listed in Table 1.
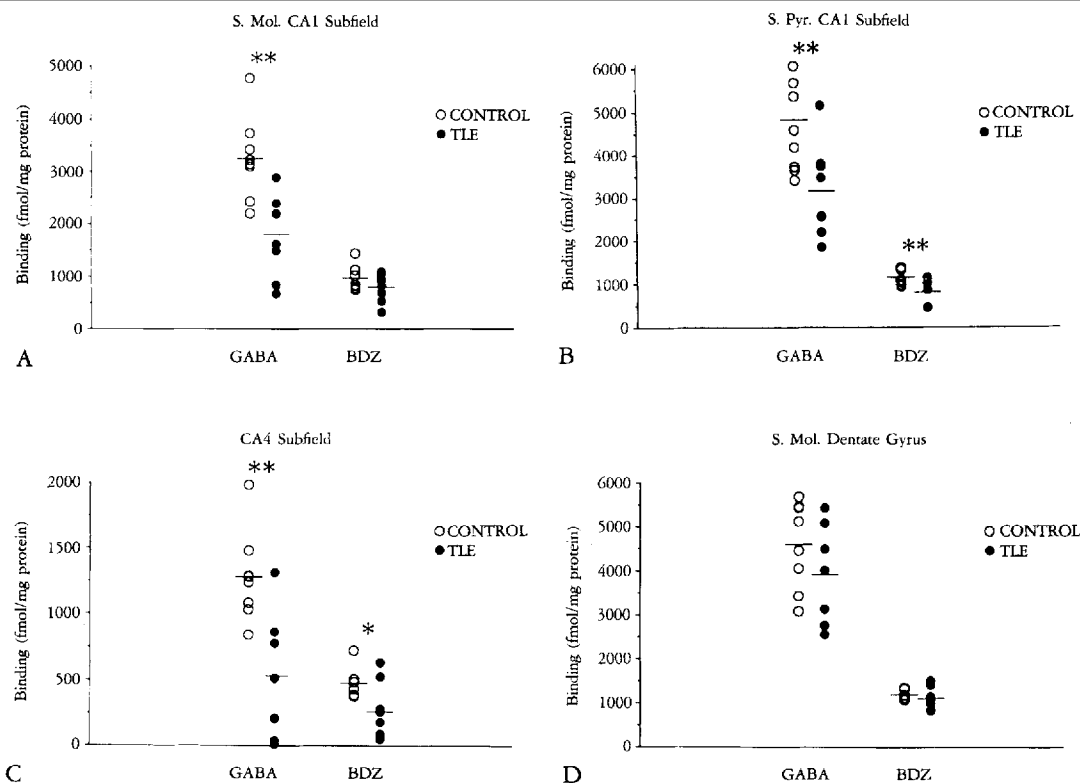
Fig 6. Histograms of inhibitory amino acid (IAA) receptor binding (GABA$_A$ and benzodiazepine [BDZ] receptors) in four hippocampal regions from patients with temporal lobe epilepsy (TLE) and from age-matched autopsy controls (n = 7/group). IAA receptors were labeled in sections adjacent to those used to label excitatory amino acid receptors (see Fig 3). Data points represent binding densities in individual patients and horizontal lines indicate mean binding densities/group. (A) Stratum lacunosum moleculare of CA1. (B) Stratum pyramidale of CA1. (C) CA4. (D) Stratum moleculare of the dentate gyrus. *p < 0.05, ** p < 0.01, ***p < 0.001.

receptors was significantly reduced in stratum pyramidale of CA1 (−29%) with a similar trend in stratum lacunosum moleculare of CA1. Binding densities were similar between groups in stratum moleculare of the dentate gyrus. A significant decrease in binding was observed in TLE CA4 subfield (−45%), compared to autopsy controls. The variability in binding between specimens was similar in TLE and control groups.

Discussion

We examined the hypothesis that EAA and IAA neurotransmitter receptor binding are altered in hippocampal tissue removed from patients with TLE. The hippocampal tissue examined in these studies contained pathological evidence of neuronal loss but not advanced mesial temporal sclerosis. Using quantitative receptor autoradiography, we found that EAA and IAA receptor binding were differentially altered in the

hippocampus of patients with TLE, compared with autopsy control subjects. The observation that binding to IAA (GABA$_A$ and benzodiazepine) receptors was reduced is consistent with previous observations [4]. Similarly, the reduction in binding to the PCP receptor site located within the excitatory NMDA receptor channel was reported in a recent preliminary communication [48]. The novel observation made in this study is that the density of NMDA-type EAA receptor sites and glycine modulatory sites was increased in the same tissue in which binding to the PCP channel receptor was reduced. Thus, we found two possible neurotransmitter receptor alterations that could contribute to enhanced neuronal excitability: a reduction in IAA receptor binding and an increase in the ratio of NMDA and glycine-type EAA receptors to PCP-type receptors within the NMDA receptor–operated channel. Although this is the first report of an increase in the ratio of NMDA receptors to PCP receptors in hippocampus from patients with TLE, a similar alteration in the NMDA receptor/channel complex was observed in animal experiments, described below [49, 50]. This change could contribute to enhanced vulnerability of the TLE hippocampus to progressive neuronal injury.

The experimental methods and design of this study have some limitations. Both the small number of specimens and the inherent problems of neuropathological variability associated with studies of chronic neurologi-

cal brain disorders limit the interpretation of the data. Because of the relatively small number of specimens per group, the effects of possible confounding variables such as the effects of postmortem delay on receptor binding in autopsy control specimens or the effects of chronic medication on receptor binding in patients with TLE could not be directly determined. Some evidence suggests that effects of postmortem delay do not substantially alter the results. A previous study with a larger number of autopsy specimens demonstrated no correlation between the postmortem delay period (0 to 16 hours) and the density of EAA and IAA receptor binding [51]. Also, the EAA and IAA receptor binding changes observed in our study were in opposite directions and were regionally selective. This argues against a generalized effect of postmortem delay.

The greater mean age of autopsy control subjects compared with patients with TLE may contribute to the apparent elevation of receptor binding in TLE patients since in a previous study receptor binding densities tended to decrease up to 10% per decade in humans [51]. Although these age-related differences may contribute to the observed changes, they cannot explain the entire increase in NMDA and glycine receptor binding in patients with TLE (50 to 100% increase) or the decreased binding to PCP receptors and $GABA_A$ or benzodiazepine receptors.

The effects of medications may be confounding factors. Rinsing tissue sections prior to receptor labeling should remove perioperative or chronic anticonvulsant medications from the tissue of patients with TLE. Only 1 patient received a medication (morphine) that directly alters EAA and IAA receptor binding [52]. It is likely, however, that chronic anticonvulsant treatment contributed to the changes in receptor binding. Although none of the patients received anticonvulsant compounds whose site of action is at EAA or IAA receptors (except one who received primidone in combination therapy), chronic treatment may indirectly cause regulatory changes in these receptors. Although there is no experimental evidence to support this idea, the effects of chronic anticonvulsant treatment cannot be ruled out.

Another problem was the limited amount of hippocampal tissue available from patients with TLE. This made it impossible to perform detailed kinetic studies to assess whether the changes in equilibrium binding represented changes in receptor affinity or alterations in receptor numbers. In addition, the severity of regional neuronal loss in TLE specimens could not be reliably assessed in the frozen tissue sections. It is unlikely that differences between groups in endogenous neurotransmitters and neuromodulations that bind to EAA and IAA receptors could account for the observed changes in receptor binding since the tissue was prerinsed prior to receptor labeling under conditions

that have been demonstrated to minimize potential differences in residual endogenous compounds. However, the binding could reflect the presence of very adhesive molecules such as polyamines. If this is the case, our in vitro observations could reflect physiologically significant alterations in the state of receptors in living tissue.

NMDA Recognition Site Binding

The pattern of NMDA recognition site binding observed in autopsy control specimens was similar to the pattern of binding observed in previous studies of human and nonhuman primate hippocampus [53–55]. The regionally selective elevation of NMDA receptor binding within the CA1 subfield in stratum lacunosum moleculare and stratum pyramidale in TLE specimens corresponds to the hippocampal fields that receive the majority of EAA neurotransmitter inputs [14]. The enhanced binding in TLE specimens compared to autopsy controls can be related to several mechanisms or combination of mechanisms. The apparent increase in NMDA receptor binding in patients with TLE may represent a selective loss of these receptors in the autopsy control specimens as a result of postmortem delay; however, this seems unlikely based on previous studies [51, 54]. Alternatively, the enhanced binding may reflect regulatory changes secondary to altered neuronal activity, neuronal injury, adaptive reorganization of neuronal circuits, and/or chronic anticonvulsant treatment. Studies in experimental animals demonstrated that excitotoxic injury or abnormal functional activity in the hippocampus is associated with axonal sprouting and synaptic rearrangement [56–58], and such synaptic rearrangements appear to occur in the hippocampus of patients with TLE [24, 59–61]. Whether these rearrangements are associated with an increase in NMDA receptor binding is not known. We found in a series of animal experiments that the number of NMDA receptor (recognition) sites increases rapidly by as much as 100% in response to treatment with drugs that block the NMDA-operated channel [49, 62].

Previous studies of NMDA receptor (recognition site) binding in human brain tissue from patients with epilepsy yielded conflicting results. NMDA receptor binding was elevated in two preliminary reports of NMDA receptor binding in patients with TLE. In one study, the maximal amount of ligand bound to the NMDA receptor binding was increased 2.5-fold in homogenates prepared from the epileptic focus in temporal cortex compared to nonepileptic temporal cortex of patients with TLE [63]. In a second study, NMDA receptor binding was increased in the entorhinal cortex of TLE specimens compared to autopsy controls, as determined by quantitative receptor autoradiography [64]. Two other preliminary studies suggested that

the NMDA recognition site binding is reduced in the hippocampus of patients with TLE. Hosford and colleagues [48] reported reduced NMDA-sensitive [³H]glutamate binding in the CA3 subfield of patients with TLE from Ammon's horn sclerosis. In the second study [64], reductions in NMDA receptor binding were reported in the CA1, CA3, and CA4 hippocampal subfields of patients with TLE from Ammon's horn sclerosis. The differences in results may reflect the severity of the neuropathology in the patients examined. In contrast to the extensive neuronal loss in TLE specimens (i.e., Ammon's horn sclerosis) examined in the studies reporting NMDA receptor loss [48, 64], the severity of pathology in our study was not as marked and none of our patients had Ammon's horn sclerosis. Three of the 8 TLE specimens in our study contained low-grade gliomas.

Glycine Modulatory Site Binding
Previous studies of EAA receptor binding in TLE did not examine strychnine-insensitive [³H]glycine binding. The distribution of glycine modulatory site binding paralleled the distribution of NMDA recognition site binding in the hippocampus of autopsy control specimens, which is consistent with previous reports of colocalization of these receptors in human brain [65]. The regionally selective receptor binding changes observed in the hippocampus of patients with TLE paralleled, in general, the increases in NMDA recognition site binding. One exception was in stratum pyramidale of CA1, where only binding to the NMDA recognition site was elevated. The coordinate changes in these two binding sites are consistent with the idea that these two sites are components of the same receptor/channel complex (see Fig 1). The results suggest that these two sites are simultaneously upregulated in the hippocampus of patients with TLE despite neuronal injury and cell loss.

Phencyclidine Receptor (Channel Site) Binding
The topography of [³H]TCP binding in autopsy control specimens was similar to the pattern of binding to the other two components of the NMDA receptor complex (NMDA and glycine receptors), which is consistent with the idea that these receptors comprise a receptor complex (see Fig 1). The distribution of [³H]TCP binding was similar to the pattern described in previous reports on human hippocampus [66, 67]. Reduced [³H]TCP binding was found in all four hippocampal regions of patients with TLE compared to control subjects, in contrast to the more selective regional changes found in binding to NMDA and glycine sites. The same factors already mentioned (anticonvulsant treatment, neuronal cell loss and synaptic reorganization, receptor downregulation, and postmortem autolysis in control specimens) could have contributed to

these changes. A previous study suggested that postmortem autolysis does not contribute to the results [51]. Chronic anticonvulsant treatment could have contributed to the changes and cannot be ruled out. However, none of the anticonvulsants the patients had taken are known to have effects at the NMDA receptor/channel complex.

Change in the Ratio of NMDA to Phencyclidine Binding Sites
Binding to the PCP receptor site within the NMDA-operated ion channel is reduced even though binding to the associated NMDA and glycine receptor sites is elevated in the same tissue. This change might be related either to an increase in NMDA and glycine sites not associated with NMDA-operated channels or to an apparent change in the ratio of NMDA and glycine receptor sites to PCP channel sites per receptor/channel complex [68]. There are several experimental precedents for a change in the quantitative relationship between NMDA receptor sites and PCP channel sites. [³H]TCP binding is modulated by NMDA and glycine receptor activation [69, 70]. The effective coupling between these receptors may be reduced in patients with TLE as has been demonstrated in patients with Alzheimer's disease [71–73], such that the remaining endogenous glutamate and glycine in the tissue sections is less effective at increasing [³H]TCP binding. It is also possible that multiple genetic forms of NMDA receptor/channel complexes exist, each with a different ratio of NMDA, glycine, and PCP receptors [42]. Our data could reflect an alteration in the relative numbers of these different forms expressed in selected regions of TLE hippocampus.

A third possibility is that the reduction in [³H]TCP binding and increased binding to the NMDA recognition site reflect a reaction to neuronal injury within the hippocampus of patients with TLE and functional expression of cryptic NMDA receptors [14, 22–25, 53–55, 66, 74, 75]. Damage to dendrites of principal hippocampal neurons could result in a loss of NMDA receptor/channel complexes, reflected in a loss of [³H]TCP binding sites. The enhanced NMDA binding could reflect functional NMDA receptor upregulation secondary to reduction in PCP channel activity. This hypothesis is supported by our experimental observation that blockade of PCP channels by MK-801 results in a prompt increase in NMDA receptors in rodents [49].

Quisqualate-Type Receptor Binding
The regional distribution of quisqualate receptor binding in the hippocampus of control specimens was similar to the pattern described previously [53]. The moderate elevation or maintenance of quisqualate receptor binding in the hippocampus of patients with TLE de-

spite cell loss suggests that these sites are preserved or possibly upregulated in TLE. Interpretation of these data has the same limitations as that of the data concerning NMDA receptor binding. These data are consistent with findings in several preliminary reports of increased quisqualate receptor binding in hippocampus from patients with epilepsy. In one preliminary study of patients with TLE who had Ammon's horn sclerosis, there was significantly increased quisqualate receptor binding in stratum moleculare of the dentate gyrus and maintained binding in other hippocampal subfields despite extensive cell loss [48]. In a second preliminary study, [^{3}H]α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) binding to a subtype of quisqualate receptors was enhanced in entorhinal cortex, maintained in stratum moleculare of the dentate gyrus, and reduced in CA1, CA3, and CA4 in the context of marked cell loss in the hippocampus of patients with TLE and with Ammon's horn sclerosis [64].

GABA$_A$ and Benzodiazepine Receptor Binding

The regionally selective reductions in GABA$_A$ and benzodiazepine receptor binding found in patients with TLE are consistent with previous reports of reductions in GABAergic synaptic markers and selective injury and loss of hippocampal neurons [4, 22–25]. Some studies demonstrated no alteration in GABA$_A$ and benzodiazepine receptor binding in patients with TLE or other forms of epilepsy [63, 76]. It is possible that the reduction of GABA$_A$ and benzodiazepine binding in our study may reflect secondary downregulation resulting from neuronal injury or chronic anticonvulsant therapy. The relative contribution of neuronal loss to the decrease in GABA$_A$ and benzodiazepine receptor binding in specimens from patients with TLE is not addressed by this study. It is unclear from this study whether the trend for a greater reduction in benzodiazepine receptor binding than GABA$_A$ receptor binding in patients with TLE represents differential receptor regulation or an artifact of the binding assays.

Possible Implications of Altered Excitatory and Inhibitory Amino Acid Receptor Binding

The increased NMDA and glycine receptor binding and reduced IAA receptor binding observed in TLE specimens could predispose the hippocampus to neuronal hyperexcitability and possibly promote further seizures and neuronal injury. NMDA receptor activation is involved in burst discharges in hippocampal neurons [35, 77–80]. Sustained bursting may result in excessive calcium entry and cytotoxicity [11, 12, 81–83]. Excitotoxic injury in the hippocampus is associated with axonal sprouting and synaptic rearrangement [56, 57] and these processes may be regulated by EAA receptor activation [84]. Such synaptic rearrangements are likely to occur in TLE [24, 59–61]. The increased

binding to NMDA and glycine modulatory receptors in TLE may be related to an adaptive regulatory response to neuronal injury. In experimental paradigms, an increase in the ratio of NMDA receptors to PCP receptors is associated with an increase in susceptibility to neuronal damage from intracerebral injections of the excitotoxic glutamate analogue NMDA [49]. If this occurs in humans, it could contribute to the development of an epileptogenic focus over time. This could be an ongoing, cycling process involving neuronal injury and adaptive synaptic reorganization [23, 24, 56–61].

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