Glutamate Currents in Morphologically Identified Human Dentate Granule Cells in Temporal Lobe Epilepsy

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Isokawa, Masako, Michel Levesque, Itzhak Fried, and Jerome Engel, Jr. Glutamate currents in morphologically identified human dentate granule cells in temporal lobe epilepsy. J. Neurophysiol. 77: 3355-3369, 1997. Glutamate-receptor-mediated synaptic transmission was studied in morphologically identified hippocampal dentate granule cells (DGCs; n = 31) with the use of whole cell patch-clamp recording and intracellular injection of biocytin or Lucifer yellow in slices prepared from surgically removed medial temporal lobe specimens of epileptic patients (14 specimens from 14 patients). In the current-clamp recording, low-frequency stimulation of the perforant path generated depolarizing postsynaptic potentials that consisted of excitatory postsynaptic potentials and phase-inverted inhibitory postsynaptic potentials mediated by the γ-aminobutyric acid-A (GABA_A) receptor at a resting membrane potential of -62.7 ± 2.0 (SE) mV. In the voltage-clamp recording, two glutamate conductances, a fast α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-receptor-mediated excitatory postsynaptic current (EPSC; AMPA EPSC) and a slowly developing N-methyl-D-aspartate (NMDA)-receptor-mediated EPSC (NMDA EPSC), were isolated in the presence of a GABA_A receptor antagonist. NMDA EPSCs showed a voltage-dependent increase in conductance with depolarization by exhibiting an N-shaped current-voltage relationship. The slope conductance of the NMDA EPSC ranged from 1.1 to 9.4 nS in 31 DGCs, reaching up to twice the size of the AMPA conductance. This widely varying size of the NMDA conductance resulted in the generation of double-peaked EPSCs and a nonlinear increase of the slope conductance of up to 37.5 nS with positive membrane potentials, which resembled “paroxysmal currents,” in a subpopulation of the neurons. In contrast, AMPA EPSCs, which were isolated in the presence of an NMDA receptor antagonist (2-amino-5-phosphonovaleric acid), showed voltage-independent linear changes in the current-voltage relationship and were blocked by 6-cyano-7-nitroquinolxaline-2,3-dione. The AMPA conductance showed little variance, regardless of the size of the NMDA conductance of a given neuron. The average AMPA slope conductance was 5.28 ± 0.65 (SE) nS in 31 human DGCs. This value was similar to AMPA EPSC conductances in normal rat DGCs (5.35 ± 0.52 nS, mean ± SE; n = 55). Dendritic morphology and spine density were quantified in the individual DGCs to assess epileptic pathology. Dendritic spine density showed an inverse correlation (r² = 0.705) with a slower rise time and a longer half-width of the excitatory postsynaptic potentials mediated by the NMDA receptor. It is concluded that both AMPA and NMDA EPSCs contribute to human DGC synaptic transmission in epileptic hippocampus. However, a wide range of changes in the slope conductance of the NMDA EPSCs suggests that the NMDA-receptor-mediated conductance could be altered in human epileptic DGCs. These changes may influence the generation of chronic subthreshold epileptogenic synaptic activity and give rise to pathological excitation leading to epileptic seizures and dendritic pathology.

INTRODUCTION

Glutamate or analogue excitatory amino acids are the principal excitatory neurotransmitters in the mammalian CNS (Watkins and Evans 1981). In the hippocampus, two different types of glutamate receptors, the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, each linked to different classes of ion channels, are coactivated on the release of glutamate from presynaptic terminals (Bekkers and Stevens 1989). Functional properties of AMPA and NMDA receptor channels have been studied extensively in embryonic hippocampal neurons in culture (Jahr and Stevens 1987), in acutely dissociated cells (Gibb and Colquhoun 1992), and in slices (Hestrin et al. 1990; Keller et al. 1991). However, to date there is no report describing synaptic currents mediated by the glutamate receptors in adult human hippocampal neurons.

The presence of AMPA, kainate, and NMDA receptors in the human hippocampus has been reported in postmortem brains with the use of antibodies to functional glutamate receptor subunits (Bockers et al. 1994; Breese and Leonard 1993) and receptor autoradiography (Hosford et al. 1991). Extracellular and intracellular recordings from human hippocampus showed field responses and synaptic potentials that were differentially sensitive to both AMPA and NMDA receptor antagonists (Isokawa et al. 1991; Masukawa et al. 1991; Urban et al. 1990; Williamson and Spencer 1995). In these studies, hippocampal specimens were obtained from epileptic patients, and intracellular recordings indicated that excitatory postsynaptic potentials (EPSPs) were increased in both amplitude and duration in dentate granule cells (DGCs) and that this increase was sensitive to an NMDA receptor antagonist. In addition, DGCs that were acutely dissociated from human epileptic hippocampus showed prolonged NMDA receptor channel openings (Lieberman et al. 1996). On the basis of these reports, in the present study we investigated physiological and pharmacological properties of synaptically generated glutamate currents in DGCs with the use of whole cell patch-clamp recordings in human hippocampal slices, which were prepared from surgically excised temporal lobe tissue in epileptic patients. Cell morphology was visualized by the intracellular injection of tracers.
through recording pipettes to identify cell type and to demonstrate
epilepsy-associated morphological changes. The severity of
epileptic pathology in each recorded DGC was assessed by dendritic morphology and spine density because these anatomic parameters can represent epilepsy-associated cell degeneration (Isokawa and Levesque 1991; Scheibel et al. 1974). Dendritic changes were quantified in single DGCs individually, and correlated with glutamate-mediated synaptic responses of corresponding DGCs.

Human hippocampus was obtained from diseased surgical specimens; normal specimens were not available for ethical reasons. Nonsclerotic (nonepileptic by definition) human hippocampus such as tumor-bearing specimens, which could serve as controls, were not available either. However, these tissue samples are not necessarily an ideal control tissue because they have been involved in epileptic processes and have essentially been kindled (Wuarin et al. 1992). In addition, all patients were on a variety of anticonvulsant medication depending on disease state, specific to patients’ seizure histories. Possible effects of these anticonvulsants on the present recordings could not be ruled out. These technical and ethical limitations of conducting neurophysiological studies on human brain specimens make it difficult to address human-specific and/or disease-specific components in our findings. Nevertheless, parallel recordings from normal rat hippocampal slices under the identical experimental protocols can help to interpret human data to advance our understanding of functional properties of glutamate receptors in excitatory synaptic transmission in diseased human hippocampal specimens.

METHODS

Hippocampal specimens and patient selection

Human hippocampal specimens were obtained from 14 patients who underwent en bloc resection of the mesial temporal lobe for surgical treatment of medically intractable seizures. Selection criteria for surgical treatment included inpatient telemetry electroencephalographic evaluation of both ictal and interictal periods, neuropsychological assessment, a fluoroexoxyglucose positron emission tomography scan, and diagnostic imaging by magnetic resonance and angiography (Engel et al. 1981). In the present group of patients, there was evidence of a medial temporal origin in duration, isolated from ground (Grass stimulation isolation unit). The distance between recording and stimulating electrodes was ≥300 μm. Slices were used for data acquisition only when field responses had I) an unfluctuating amplitude of ≥2 mV, 2) consistent waveforms of population EPSPs and population spikes, and 3) reproducibility when stimulus intensity was <0.5 mA. The same electrode and stimulus parameters were used for the perforant path stimulation during whole cell recording. Stimulus current intensity was 1–10 μA for whole cell recordings and 100–500 μA for field responses.

For pharmacological identification of glutamate receptors, the following compounds were added to the ACSF: bicuculline methiodide (BMI; 10 μM, Sigma) as an antagonist to the γ-aminobutyric acid-A (GABA_A) receptor; 2-amino-5-phosphonovaleric acid (APV; 50 μM, Tocris Cookson) as an antagonist to the NMDA receptor; and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 5 μM, Tocris Cookson) as an antagonist to the AMPA-kainate receptor. Stock solutions of APV (50 mM) and bicuculline (20 mM) were made by dissolving the compounds into double-distilled water. For final working solutions, 100 μl of APV (50 mM) was mixed with 100 ml of ACSF, and 100 μl of BMI (20 mM) was mixed with 200 ml of ACSF. Stock solution of CNQX was made to a concentration of 250 μM in ACSF, and was further diluted to a final working concentration of 5 μM in ACSF. We performed a control experiment by mixing 100 μl of distilled water into 100 or 200 ml of ACSF, and confirmed no changes in field responses or EPSPs/excitatory postsynaptic currents (EPSCs).

Whole cell patch-clamp recording

Borosilicate glass capillaries (1.5 mm OD, WPI) were used to prepare patch pipettes for whole cell recordings with the use of a two-stage pipette puller (Narishige PP-88). Patch electrodes were filled with one of the following solutions (composition, in mM): 1) 130 CsF, 10 tetraethylammonium, 5 NaCl, 1 MgCl\(_2\), 1 CaCl\(_2\), 11 ethylene glycol-bis-(β-aminoethoxy ethyl)-N,N,N’,N”-tetraacetic acid (EGTA), and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.2 or 2) 130 potassium gluconate, 10 KCl, 2 MgCl\(_2\), 10 HEPES, and 10 EGTA, pH 7.2. In solution 1, cesium was chosen as the main cation to block potassium conductances, and fluoride was chosen as the main anion because it has been shown to reduce calcium currents (Kay et al. 1986; Kostyuk et al. 1975) and improve the duration of the recordings (Hestrin et al. 1990). Biocytin (0.1%, Sigma) or Lucifer yellow (dipotassium salt, 0.2–0.4%, Sigma) was added to these solutions for intracellular staining. Pipette resistance was 4.5–5 MΩ when filled.
with internal solution. When Lucifer yellow was used, pipette resistance was increased to 10–20 MΩ.

Seal formation and the establishment of the whole cell configuration were made in a current-clamp mode by applying a brief negative pressure to a recording pipette during the application of 30-ms negative current pulses of 0.12 nA with a frequency of 10 Hz (Blanton et al. 1989). Seal resistance was calculated by measuring a voltage deflection at the end of the pulses (1.7 ± 0.08 GΩ, mean ± SE, in 31 human DGCs; 1.82 ± 0.05 GΩ, mean ± SE, in 55 rat DGCs), which had not reached a plateau. Thus the actual seal resistance was estimated to be substantially higher than these values. In all DGCs of both human and rat hippocampi that were analyzed in the present study, adequate seal resistance (>9 GΩ) was initially achieved and maintained for 15–20 s before the whole cell configuration was established. Immediately after the establishment of the whole cell recording, a resting membrane potential (V_m) was measured, and input impedance was calculated by applying the same current pulses for seal formation. No negative DC was applied to stabilize the recording during the establishment of the whole cell configuration or during the measurements of the above parameters. Subsequently, the cells were voltage clamped at a V_m of −70 mV in a continuous mode (Axoclamp 2A, Axon Instruments). Access resistance was monitored indirectly by observing a reduction in response amplitude during recording. Whenever EPSP/EPSC amplitude was reduced by >20% during the stimulation under a given experimental condition, recording was stopped, and reopening of the cell membrane under the pipette was made to reestablish the whole cell mode, or otherwise the recording was discontinued. In most cases, however, when the whole cell configuration was made well at the beginning, the cells could be held without losing response amplitude, probably with an aid of fluoride and a sufficient concentration of a calcium chelator in the pipette. Data were stored on a video cassette recorder (Unitrade) and analyzed with a digital oscilloscope (Nicolet 4094).

**Visualization of human DGCs**

Biocytin or Lucifer yellow was introduced into a single DGC by passive diffusion during whole cell recording. Slices were fixed with 4% paraformaldehyde after the recording. When Lucifer yellow was used, slices were whole mounted on a glass slide and examined under epifluorescent microscopy. When biocytin was injected, slices were cut into 30-μm sections and processed for visualization with the use of avidin-biotin complex reagent (Vec-Stain ABC kit, Vector Laboratories). Neuroanatomic data were determined to be satisfactory when the number of dendritic branches and spines was counted. Spines membrane voltage across all the cells, the above value was analyzed in the present study, adequate seal resistance (>9 GΩ) was initially achieved and maintained for 15–20 s before the whole cell configuration was established. Immediately after the establishment of the whole cell recording, a resting membrane potential (V_m) was measured, and input impedance was calculated by applying the same current pulses for seal formation. No negative DC was applied to stabilize the recording during the establishment of the whole cell configuration or during the measurements of the above parameters. Subsequently, the cells were voltage clamped at a V_m of −70 mV in a continuous mode (Axoclamp 2A, Axon Instruments). Access resistance was monitored indirectly by observing a reduction in response amplitude during recording. Whenever EPSP/EPSC amplitude was reduced by >20% during the stimulation under a given experimental condition, recording was stopped, and reopening of the cell membrane under the pipette was made to reestablish the whole cell mode, or otherwise the recording was discontinued. In most cases, however, when the whole cell configuration was made well at the beginning, the cells could be held without losing response amplitude, probably with an aid of fluoride and a sufficient concentration of a calcium chelator in the pipette. Data were stored on a video cassette recorder (Unitrade) and analyzed with a digital oscilloscope (Nicolet 4094).

**Results**

**EPSPs**

Whole cell patch-clamp recordings were obtained in current-clamp mode from 31 human DGCs in 14 hippocampi in 14 patients (input impedance: 197.8 ± 17.9 MΩ, mean ± SE; resting V_m: −62.9 ± 1.24 mV, mean ± SE; action potential amplitude: 90.3 ± 5.47 mV, mean ± SE). These values were not different from those obtained from normal rat DGCs (n = 55 in 33 rats; input impedance: 210.3 ± 12.1 MΩ, mean ± SE; resting V_m: −62.1 ± 2.05 mV, mean ± SE; action potential amplitude: 91.4 ± 6.59 mV, mean ± SE).

Depolarizing postsynaptic potentials (DPSPs) were generated during low-frequency stimulation of the perforant path. DPSP duration was measured from the point at which the membrane voltage deviated positive from the resting level to the point at which the positively deviated membrane voltage returned to the resting level. When DPSPs had a single peak (Fig. 1A1), the average duration was 90.2 ± 12.1 (SE) ms with a resting V_m of −62.3 ± 1.8 (SE) mV in 19 of the 31 DGCs. On the other hand, when DPSPs had multiple peaks (Fig. 1B1), DPSP duration was substantially longer (n = 12). In these neurons, DPSP duration ranged from 122 to 663 ms at V_m = −63.1 ± 2.1 (SE) mV. In contrast, in rat DGCs (n = 55), DPSP duration was uniformly consistent, with an average duration of 58.3 ± 6.3 (SE) ms at V_m = −62.8 ± 2.37 (SE) mV.

In DGCs, GABA_A-receptor-mediated inhibitory postsynaptic potentials (IPSPs) could be present as inverted IPSPs and contribute to the generation of DPSPs near the resting potential. Thus we isolated EPSPs in the presence of BMI (10 μM) in the extracellular ACSF solution. In the presence of BMI, EPSPs were shorter in duration than DPSPs in all 31 DGCs, confirming our hypothesis that GABA_A-receptor-mediated IPSPs generated depolarizing synaptic responses near the resting potential in our human DGCs. However, the dichotomy in the duration, which was observed in DPSPs, remained in EPSPs even though the GABA_A-receptor-mediated IPSP component was removed from DPSPs. In 19 DGCs, the average duration of EPSPs was 71.8 ± 5.7 (SE) ms at V_m = −63.9 ± 2.7 (SE) mV. Although the present measurement of EPSP duration at the resting V_m is not as well controlled as measurement of durations at a given (identical) membrane voltage across all the cells, the above value was significantly shorter than that for the remaining 12 neurons, whose EPSPs showed an average duration of 135.2 ± 19.5 (SE) ms at V_m = −63.1 ± 3.3 (SE) mV in 9 neurons, and 597.0 ± 42.9 (SE) ms at V_m = −55.3 ± 2.0 (SE) mV in 3 neurons (t = 5.22, P < 0.01; unpaired 2-tailed t-test; note that the average resting V_m was more negative for the neurons that showed shorter-duration EPSPs). These findings indicate that the variability observed in the DPSP duration is...
FIG. 1. Two types of synaptic responses were observed in human dentate granule cells (DGCs) in response to single perforant path stimulation (pulse duration: 200 μs; 0.1 Hz). A1: single peak postsynaptic potentials (PSPs) recorded in control artificial cerebrospinal fluid (ACSF). Increased intensity of stimulation increased amplitude of excitatory PSPs (EPSPs), eliciting an action potential. A2–A4: EPSPs were isolated in presence of 10 μM bicuculline methiodide (BMI). This concentration was subthreshold for eliciting epileptiform activities with low intensities of stimulation. A5 and A6: higher intensity of stimulation produced multiple burst firings in BMI-containing ACSF. B1: PSPs with substantially longer duration. This type of PSP showed multiple peaks, and stronger stimulation elicited multiple action potentials from these PSPs. B2: application of BMI isolated an EPSP (arrowhead). B3: slight increase in stimulus intensity generated second EPSP peak in addition to initial EPSPs (arrowhead). Multiple burst firings were elicited from second EPSP. B4 and B5: latency for onset of second EPSP was voltage dependent, whereas that of initial EPSP (arrowhead) was voltage independent. Calibrations—voltage scales: 10 mV; time scales: 20 ms (A1), 100 ms (A2–A6 and B1–B5). V_m, membrane potential.

unlikely to be originated in the GABA_A IPSP duration, but rather it is related to glutamate mediated EPSP durations.

When EPSPs showed prolonged durations, they were composed of two components: an initial EPSP and a slowly developing late EPSP. A slowly developing late EPSP required a higher stimulus current, showed higher amplitude and longer duration, and was elicited subsequent to an initial EPSP (Fig. 1, B2 and B3, arrowheads). Multiple action potentials could be generated from the late EPSP component. The latency for the late EPSP onset was membrane voltage dependent. When the cells were hyperpolarized, the onset was delayed (Fig. 1, B4 and B5). On the other hand, the latency for the initial EPSP (Fig. 1, B4 and B5, arrowheads) was not affected by the cell membrane voltage. Although multiple action potentials could be elicited from the DGCs that did not exhibit prolonged duration of EPSPs when the application of BMI and a stronger stimulation were combined, no initial EPSPs could be separated from these cells (Fig. 1, A5 and A6).

In normal rat DGCs, the application of 10 μM BMI isolated EPSPs with an average duration of 39.2 ± 8.8 (SE) ms at V_m = −61.1 ± 3.07 (SE) mV (n = 55). No epileptic discharges were elicited with this concentration of BMI in the rat DGCs. The EPSP durations were uniform, and no dichotomy in the EPSP duration was observed. At the end of the recording of EPSPs, all human DGCs (n = 31) and rat DGCs (n = 55) were voltage clamped for the recording of glutamate currents.

Glutamate currents

Postsynaptic currents (PSCs) were recorded in control ACSF in 31 human DGCs (Fig. 2A) and 55 rat DGCs (Fig. 2B). When cesium was used as a main cation in the re-
FIG. 2. Postsynaptic currents (PSCs) were recorded in voltage-clamp mode in human DGCs (A) and normal rat DGCs (B) in response to low-frequency single perforant path stimulation in normal ACSF. A1: series of PSC traces shown at holding $V_m$ from $-90$ to $+10$ mV in a human DGC. PSCs were biphasic at potentials between $-40$ and $-10$ mV, indicating presence of $>1$ current comprising PSC (intrapipette solution contained cesium as a main cation). Calibration: 100 ms, 100 pA. A2: early glutamate-mediated excitatory PSCs (EPSCs; ●) and late γ-aminobutyric acid (GABA)-mediated inhibitory PSCs (IPSCs) (*) plotted separately to show linear current-voltage (I-V) relationships and differing reversal potentials. Early component was measured 7 ms after stimulation, and late component was measured 25 ms after stimulation. B1: series of PSCs recorded from a normal rat DGC at various holding potentials ranging from $-90$ to $+10$ mV. Synaptic currents were biphasic at potentials between $-70$ and $-50$ mV (intrapipette solution contained potassium as a main cation). Calibration: time scale, 100 ms; current scale, 100 pA. B2: I-V relationship plotted for current traces shown in B1. Two current components were measured; a glutamatergic current was measured 7 ms after stimulation, and a GABAergic current was measured 25 ms after stimulation.

CORDING pipette, the PSC was comprised of a fast-rising and slowly decaying inward current at a holding potential more negative than $-40$ mV in both human and rat DGCs. At holding potentials between $-30$ and $0$ mV, the PSC became biphasic, with an early inward current component (measured at 7 ms after stimulation) and a late outward current component (measured at 25 ms after stimulation). A representative example of current traces for holding potentials ranging from $-90$ to $+10$ mV is shown in a human DGC in Fig. 2A1, and current-voltage (I-V) curves for the early (7 ms) and late (25 ms) PSC components are shown in Fig. 2A2. Kinetics of these two PSC components and their I-V relations were similar in normal rat DGCs in the present study (data not shown). When cesium was replaced with potassium in the intrapipette solution, it changed only the reversal potential of the late current component by shifting it negatively in human and rat DGCs. Reversal potentials for the late component were $-49.0 \pm 4.85$ (SE) mV with CsF in 19 human DGCs and $-58$ mV with potassium gluconate in one human DGC. On the other hand, in the rat DGCs, reversal potentials for the late component were $-52.4 \pm 4.17$ (SE) mV ($n = 456-6/9k13$$ju15 08-05-97 10:27:38 neupas LP-Neurophys downloaded from http://jn.physiology.org/ by 10.220.33.4 on May 13, 2017
5) with potassium gluconate. A series of current traces and corresponding I-V curves to show this change are depicted in a normal rat DGC in Fig. 2, B1 and B2, respectively. These PSC profiles indicated that there were at least two types of currents in the PSC with differing reversal potentials. Because the chloride reversal potential is close to the resting $V_m$ in hippocampal DGCs and because cesium is empirically known to depolarize the cell to shift the chloride reversal potential to around $-45 \text{ mV}$, it is likely that the late current component is a GABA$_A$-mediated inhibitory PSC (IPSC). The average slope conductance for the late current component in human DGCs was $5.27 \pm 1.21 (\text{SE}) \text{ nS} (n = 19)$ in control ACSF. This value was slightly lower than the average slope conductance for the late current component in normal rat DGCs in the present study ($6.90 \pm 0.48 \text{ nS}, \text{ mean } \pm \text{ SE}; n = 23$). The late current component was blocked by $10 \ \mu\text{M BMI}$, supporting the hypothesis that this current component represented GABA$_A$ IPSCs. A detailed analysis for the GABA$_A$ IPSC in human DGCs is reported by Isokawa (1996a). When DGCs were held at a positive $V_m$ above $0 \text{ mV}$, PSCs were uniformly outward regardless of cesium or potassium in the intraperiaiput solution.

EPSCs were isolated by BMI ($10 \ \mu\text{M}$) in the external ACSF. A series of current traces for representative EPSCs with membrane holding potentials ranging from $-90$ to $+30 \text{ mV}$ is shown in a human DGC in Fig. 3A. In 27 of 31 human DGCs, current traces similar to those in Fig. 3A were observed. On the basis of previous reports in rat hippocampal neurons (Hestrin et al. 1990; Keller et al. 1991; Randall et al. 1990), two EPSC components, i.e., fast AMPA and slow NMDA EPSCs, were separated by their differing latencies of peak currents. The AMPA EPSC was measured 7 ms after the perforant path stimulation, and the NMDA EPSC was measured 25 ms after the stimulation for rat DGCs and 50 ms after the stimulation for human DGCs. A longer latency for the measurement of the NMDA EPSC in the present group of human DGCs was based on the observation that the NMDA EPSC had a distinct peak at $\sim 50$ ms after the stimulation in a subpopulation of the present group of human DGCs. The I-V relationships for the AMPA and NMDA EPSCs are shown in Fig. 3B for a human DGC and in Fig. 3C for a normal rat DGC. In normal rat DGCs, NMDA EPSCs showed a voltage-dependent increase in conductance above $-30 \text{ mV}$ of the $V_m$, exhibiting an N-shaped I-V relation (Fig. 3C, ○). Once the NMDA receptor/channel complex was activated with depolarizing $V_m$ above threshold, the slope conductance was similar between AMPA and NMDA EPSCs. In contrast, in human DGCs (27 of 31 DGCs), NMDA EPSCs showed large variance in slope conductance ranging from $1.1$ to $9.7 \text{ nS}$, and exhibited different I-V relationships from those reported in rat DGCs, as shown in Fig. 3B. The membrane voltage for generating negative conductance shifted negative (left in the plot), indicating that a membrane voltage more negative than the membrane voltage required for the NMDA receptor activation in normal rat DGCs can activate the NMDA receptor in human epileptic DGCs. In addition, in 6 of the 27 human DGCs, NMDA-receptor-mediated EPSCs showed a larger slope conductance ($8.06 \pm 0.46 \text{ nS}, \text{ mean } \pm \text{ SE}$) than AMPA-receptor-mediated EPSCs ($5.54 \pm 0.28 \text{ nS}, \text{ mean } \pm \text{ SE}$) ($t = 4.70; P < 0.001; \text{unpaired 2-tailed} \ t$-test). However, no differential reversals of currents were observed in the early AMPA EPSC component or the late NMDA EPSC component. EPSCs reversed at an average $V_m$ of $3.75 \pm 1.51 (\text{SE}) \text{ mV}$ in the human DGCs and at $+4.00 \pm 1.02 (\text{SE}) \text{ mV}$ in the rat DGCs.

In four human DGCs, EPSCs isolated in the presence of $10 \ \mu\text{M BMI}$ generated dual peaks at $V_m$ depolarized above $-30 \text{ mV}$ (Fig. 4A). I-V relationships for the first peak and the second peak are shown in Fig. 4B. The first EPSC peak showed a linear I-V relationship, and the current reversed at $9 \pm 1.35 (\text{SE}) \text{ mV}$ (Fig. 4B, ∗). The average slope conductance for the first EPSC peak was $3.25 \pm 0.66 (\text{SE}) \text{ nS}$ ($n = 4$). The second EPSC peak became clear with a depolarized $V_m$ above $-30 \text{ mV}$, and the current reversed at $12.0 \pm 3.63 (\text{SE}) \text{ mV}$. However, the peak amplitude of the second EPSC abruptly (nonlinearly) increased as $V_m$ became further positive, exhibiting large variance in the slope conductance at different $V_m$ among cells ranging from $4.4$ to $37.5 \text{ nS}$. There was a region of negative slope conductance for the second EPSC at membrane voltages more negative than $-30 \text{ mV}$ (Fig. 4B, ●). The rise time and peak latency for the second EPSC were sensitive to the stimulus intensity (Fig. 4, C and D), and a decay time for the second EPSC was prolonged with positive $V_m$ (Fig. 4A). The application of an NMDA receptor antagonist, APV ($50 \ \mu\text{M}$), blocked the second EPSC peak (Fig. 4E), providing pharmacological confirmation that the second EPSC was NMDA receptor mediated. These observations suggested that the NMDA-receptor-mediated EPSCs showed a wide range of changes in the slope conductance in human epileptic DGCs, including a prominent increase in some neurons.

**Pharmacological isolation of the AMPA-receptor-mediated EPSCs**

The AMPA EPSC was isolated by bath applying APV ($50 \ \mu\text{M}$). A representative example of the AMPA-receptor-mediated current is shown in Fig. 5A, with holding potentials ranging from $-80$ to $+40 \text{ mV}$. The EPSC decayed more quickly in the presence of APV, and the I-V relationship for the peak EPSC was linear, exhibiting a voltage-independent property of the AMPA EPSC (Fig. 5B). The current reversed at $11.5 \pm 2.36 (\text{SE}) \text{ mV}$, CNQX ($5 \ \mu\text{M}$) was applied to determine whether this EPSC component was mediated by AMPA receptors. CNQX completely blocked this EPSC component (data not shown). Slope conductance of the AMPA EPSC, measured in the presence of APV, was $5.28 \pm 0.65 (\text{SE}) \text{ nS}$ on average in 31 human DGCs. There was no significant difference in the AMPA slope conductance between neurons that generated single-peak EPSCs ($n = 27$) and dual-peak EPSCs ($n = 4$). This finding suggested that the AMPA EPSC was not a major factor in generating the various degrees of prolongation of excitatory postsynaptic responses in human epileptic DGCs. In contrast, in normal rat DGCs ($n = 55$), the average slope conductance for the AMPA-receptor-mediated EPSC was $5.35 \pm 0.52 (\text{SE}) \text{ nS}$ in the presence of APV and bicuculline. This suggested that AMPA EPSCs were similar in their electrophysiological properties, such as voltage independence and slope conductances, in the present group of human epileptic DGCs and in normal rat DGCs.
HIPPOCAMPAL GLUTAMATE CURRENTS IN HUMAN EPILEPSY

FIG. 3. A: EPSCs were isolated by 10 μM BMI. Series of current traces with V_m ranging from −90 to +30 mV shown in a human DGC. Because BMI blocked the GABA_A IPSC component, there were no biphasic profiles in these current traces. B: 2 I-V plots, 1 for the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) EPSC (∗) and the other for the N-methyl-D-aspartate (NMDA) EPSC (○), shown in human DGC. AMPA component was measured 7 ms after perforant path stimulation, and NMDA component was measured 50 ms after stimulation. Slope conductance of NMDA EPSC was larger than that of AMPA EPSCs in the present group of human DGCs. C: AMPA and NMDA EPSCs isolated in a rat DGC. AMPA EPSC was measured 7 ms after stimulation, and I-V curve for this EPSC showed a linear I-V relationship (●). Late NMDA component was measured 25 ms after stimulation and showed a membrane-voltage-dependent, N-shaped relationship (○). Both currents reversed near 0 mV, and slope conductances for the AMPA and NMDA EPSCs were similar above −20 mV.

Dendritic morphology

Intracellular injection of tracers revealed distinct morphology in both axons and dendrites in human epileptic DGCs. Quantitative analyses of the DGC axon, the mossy fiber, in human single DGCs have been reported in detail in the surgically removed specimens from epileptic patients (Iso-kawa et al. 1993). In the present study, dendritic morphologies were studied and analyzed as to their branches, shafts, and spines because they can show epileptic pathology (Iso-kawa and Levesque 1991; Scheibel et al. 1974). Figure 6 shows representative examples of human DGCs visualized by biocytin or Lucifer yellow. As stated in METHODS, neurons were selected for the quantification of dendritic morphology when dendritic spines and shafts were clearly visible in both proximal and distal dendrites. In most cells, dendrites formed fan-shaped dendritic trees in the molecular layer (Fig. 6a). In addition to the dendrites in the molecular layer, one or more basal dendrites were observed in some DGCs in the hilus together with the axon. The presence of basal dendrites was previously reported in primate and human DGCs in autopsy specimens (Seress and Mrzljak 1987). Dendritic branches had spines consistently from proximal parts to distal ends (Fig. 6g). The proximal dendritic shafts that were covered with densely distributed fine spines are shown in Fig. 6b. In some neurons, however, spine density was considerably lower in the proximal dendrites, and individual spines had larger heads and longer necks (Fig. 6, c and d). There were no simple and/or systematic rules found for the alterations in spine shapes and lowered spine densities in relation to the proximal and distal axis of dendritic trees in the present group of human DGCs. There were a group of neurons whose dendrites lost spines and dendritic shafts became irregular (Fig. 6e). In the cell shown in Fig. 6e, few spines were identified in the proximal dendrites (double arrowheads), and more spines were present in distal branches (arrowhead). In addition, aggregates of stubby protrusions appeared at the proximal dendritic shaft replacing regularly distributed individual spines. In these aggregates, no clearly identifiable spines or spine heads were observed. In other cells, dendritic spines were protruded from ‘‘swollen’’ shafts forming a complex dendritic...
FIG. 4.  

A: in a subpopulation of human DGCs, when EPSCs were isolated by 10 μM BMI, 2 current peaks were generated in the EPSC with $V_{m}$-s ranging from $-30$ to $+50$ mV.  

B: $I-V$ relationships for 1st EPSC peak (average peak latency: 19 ms; *) was linear, but $I-V$ curve for 2nd EPSC peak (average peak latency: 85 ms; ●) showed membrane-voltage-dependent changes with a negative slope conductance below $-30$ mV of membrane voltage.  

C and D: 2 EPSC peaks showed differing sensitivity to stimulus intensity. latency was shortened in 2nd EPSC peak when stimulus intensity was increased $V_{m}$ $-30$ mV (C), $+30$ mV (D).  

E: 2nd EPSC peak was 2-amino-5-phosphonovaleric acid (APV) sensitive. Top traces in each pair (marked BMI): dual peak EPSCs before application of APV. Bottom traces in each pair (marked BMI + APV): blockade of 2nd EPSC peak by APV. Blockade was tested at both negative ($-40$ mV) and positive ($+40$ mV) $V_{m}$-s.
FIG. 5. A: AMPA-receptor-mediated EPSC was isolated by 50 μM APV and 10 μM bicuculline. Current traces were obtained from neuron that showed dual peak EPSCs shown in Fig. 4. EPSC decayed much faster in presence of APV. B: I-V plot for APV-insensitive EPSC component. curve was linear, exhibiting voltage-independent relationship. Similar results were obtained from human epileptic DGCs that showed only single peak in EPSC.

structure (Fig. 6, arrowhead in Fig. 6f and inset in g). In the cell shown in Fig. 6f, three proximal dendritic branches had substantial numbers of spines from swollen shafts, and the remaining one branch (left) had very few spines. It was not the case that dendritic spine loss and swellings started to occur at distal portions of all the dendrites and systematically moved toward proximal dendrites. Spine density was variable depending on individual dendritic branches (Fig. 6, h and i). Both dendrites belonged to the cell shown in Fig. 6g. The dendrites in Fig. 6h had regularly distributed spines.
In contrast, the dendrite in Fig. 6i showed heavy nodulation with very few spines (arrowhead). When dendritic shafts bore large swellings, the swellings had a densely stained contour on one side and a very thin membranous structure on the other side, which made them look like lacuna. In this particular dendrite in Fig. 6i, spines and swellings were mutually exclusive. No spines protruded from swollen shafts; unlike seen in Fig. 6f. Again, these changes did not appear to start from distal ends of dendrites spreading toward proximal parts. Instead, reduction in spine density and the formation of swelling or lacunae were restricted to local spots in the dendritic shafts and not be generalized to the entire dendritic trees. Two closely located branches could show contrasting features, i.e., one with a full of spines and the other with the loss of spines and the presence of swellings, at the same proximal-distal axis of the dendritic tree.

These data suggest that the mechanisms underlying these pathological changes must be operating locally.

Changes in dendritic morphology were quantified in 15 human epileptic DGCs that best satisfied the criteria for data analysis (see Visualization of human DGCs). In these neurons, the total number of spines and swellings was counted, and the total dendritic length was measured by summing individual branch lengths. In addition, dendritic height, dendritic domain, and number of dendritic branches were measured. Table 1 summarizes the results from these 15 DGCs. In the remaining neurons that did not meet the criteria for this quantitative analysis, several good spots were selected for quantification and used to support our conclusions that were derived from the 15 DGCs. As shown in the table, when DGCs had spines consistently along dendritic shafts, the spine density was \(0.610 \pm 0.042\) (SE) spines per micrometer, and the swelling density was negligible (0.045 per \(\mu m\)). This value for spine density was in agreement with those reported in Golgi stained rat CA1 pyramidal cells (0.55–1.2 spines/\(\mu m\)) (Gould et al. 1990; Lacey 1985; Wenzel et al. 1972).

In neurons with an average spine density of 0.610, summed dendritic length was >6 mm, and the height of the dendritic tree exceeded 1 mm. However, when spine density declined (0.279 \(\pm 0.045\) spines/\(\mu m\), mean \(\pm\) SE), dendritic length and height were also reduced (Table 1, middle column). This reduction in dendritic domain was observed without any appreciable increase in swelling density. There are, however, assumptions inherent in our measurements. First, these measurements were not corrected for fixation-induced shrinkage. Thus the total dendritic length and dendritic height could be underestimated. Second, our measurements were made in two dimensions, despite the fact that dendrites had three-dimensional structure. Thus invisible spines and/or swellings behind the shaft were uncounted. Consequently, the spine/swelling density in the present study could be underestimated. Indeed, Trommald et al. (1995) estimated that, when two-dimensional analysis was used, the detected amount was 65–73% of the total that was estimated by direct three-dimensional structural analysis, because three-dimensional analysis can detect hidden spines and swellings undetected in the present analysis.

Morphological changes were correlated with physiological findings. Table 1, bottom row, shows the duration of EPSPs recorded from the total of 31 human DGCs in the whole cell recording. The neurons were grouped on the basis of their spine density, and the average duration of the EPSP was calculated in each group. The duration of EPSP was significantly longer in neurons that had reduced spine density compared with neurons that showed more densely distributed spines (\(t = 5.22; P < 0.01\), unpaired 2-tailed \(t\)-test). In the selected 15 neurons, which were used for the quantification of anatomic changes, simple regression was calculated between the spine density and the EPSP duration (Fig. 7). There was a significant correlation between EPSP duration and spine density (\(r = -0.84, P < 0.005\)), suggesting that synaptically mediated, prolonged excitation was accompanied by changes in dendritic spine morphology.

**Discussion**

Prolonged excitatory postsynaptic depolarization and the underlying currents

The present study showed the generation of prolonged excitatory synaptic responses in human DGCs in hippocampal slices prepared from surgically resected specimens from epileptic patients. Voltage-clamp analysis indicated that the NMDA-subtype glutamate-receptor-mediated currents are likely to mediate this prolonged response.

Similar to rat DGC EPSCs recorded in the present study and those reported by Keller et al. (1991), the human epileptic DGCs revealed dual components of glutamatergic EPSCs, i.e., the fast CNQX-sensitive AMPA conductance and the slow APV-sensitive NMDA conductance. In normal rat DGCs, a dominant conductance mediated by AMPA receptors displayed a linear \(I-V\) relation, and a smaller conductance mediated by the NMDA receptor displayed a region of negative slope conductance below \(-30\) mV of \(V_m\). Because of the considerably smaller size of the NMDA conductance in comparison with the AMPA conductance, when both conductances were allowed to be generated, the whole EPSC peak current resulted in a linear \(I-V\) relationship. In
TABLE 1. Quantification of dendritic alterations and the duration of EPSPs in human DGCs

<table>
<thead>
<tr>
<th></th>
<th>Neurons With Full Spines</th>
<th>Neurons With Less Spines</th>
<th>Neurons Without Spines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine density</td>
<td>0.610 ± 0.042 μm</td>
<td>0.279 ± 0.045 μm</td>
<td>0.042 ± 0.021 μm</td>
</tr>
<tr>
<td>Swelling density</td>
<td>0.045 ± 0.005 μm</td>
<td>0.047 ± 0.011 μm</td>
<td>0.171 ± 0.060 μm</td>
</tr>
<tr>
<td>Summed dendritic length</td>
<td>6.024.7 ± 642.2 μm</td>
<td>3.018.0 ± 195.4 μm</td>
<td>1.666.0 ± 11179.9 μm</td>
</tr>
<tr>
<td>Dendritic height</td>
<td>1.164.9 ± 629.1 μm</td>
<td>406.3 ± 65.5 μm</td>
<td>368.0 ± 920.0 μm</td>
</tr>
<tr>
<td>Dendritic domain</td>
<td>61.1 ± 6.0°</td>
<td>51.9 ± 8.4°</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of branches</td>
<td>11.8 ± 1.18</td>
<td>11.0 ± 1.31</td>
<td>5.2 ± 1.82</td>
</tr>
<tr>
<td>Duration of EPSPs (whole cell)</td>
<td>71.8 ± 5.7 ms (N = 19)</td>
<td>135.2 ± 19.5 ms (N = 9)*</td>
<td>No data</td>
</tr>
</tbody>
</table>

(\(V_m = -63.9\) mV ± 2.7)

Values are means ± SE. In these 3 groups of neurons (5 neurons for each group), intracellular staining was excellent: somata, primary, secondary, and tertiary axons and dendrites were clearly visible. All spines and swellings were counted, and all dendrites were measured in length under the magnification of \(×1,000\)–1,200. Spine and swelling density were calculated by dividing the total number of spines and swellings by the total summed length of the dendrites. In the remaining neurons, several spots (dendritic areas) that showed satisfactory staining were selected to take measurements and calculate the average density. These measurements were used for the calculation of the average excitatory postsynaptic potential (EPSP) durations in all human dentate granule cells (DGCs) studied in the present study \((N = 31)\). N/A, not available. \(V_m\), membrane potential. * Duration of EPSPs in these neurons that showed reduced density of spines was significantly longer than that of the neurons that showed normal density of spines \((t = 5.22; P < 0.01, \text{unpaired 2-tailed } t\text{-test}).

contrast, in the human epileptic DGCs, differing proportions of increase in the NMDA-receptor-mediated conductance over the AMPA-receptor-mediated conductance were detected. This change was reflected in the \(I-V\) relation. The relative contribution of the nonlinear component increased, deviating from linearity in the peak \(I-V\) relationship of the whole EPSC peak current. Thus the slope conductance of the NMDA-receptor-mediated EPSC increased and exceeded the slope conductance of the AMPA-receptor-mediated EPSC. The larger fraction of synaptic NMDA component in the glutamatergic EPSC was unique to the human epileptic DGCs, and was not observed in normal rat DGCs in the present study. This difference could be due to the fact that the human DGCs were from epileptics, because a similar increase in the whole cell NMDA conductance has been reported in the kindling model (Mody and Heinemann 1987), in the kainate model (Wheat et al. 1991), and in the pilocarpine model (Isokawa and Mello 1991).

Interestingly, in normal rat hippocampal neurons in slices (Keller et al. 1991) and cultures (Bekkers and Stevens 1989), DGCs have a higher slope conductance of the NMDA-receptor-mediated EPSC compared with pyramidal cells (Hestrin et al. 1990). It may be that DGCs naturally have a larger proportion of NMDA-receptor-mediated conductance relative to AMPA-receptor-mediated conductance in their glutamatergic EPSCs when compared with hippocampal pyramidal cells. Although it is unlikely that the NMDA-receptor-mediated conductance is prominently involved in normal synaptic transmission in the pyramidal cells, Lambert and Jones (1989) reported that the NMDA receptor does contribute to normal perforant path synaptic transmission in DGCs. This may explain a physiological role of higher slope conductance in the NMDA-receptor-mediated synaptic current in DGCs.

Single-channel conductance of the NMDA receptor channel is reported not to be different in hippocampal DGCs after kindling (Kohr et al. 1993). Thus the frequency of opening and/or the voltage-dependent properties of activation may be responsible factors for the possible increase of whole cell NMDA conductance in human epilepsy (Lieberman et al. 1996). The present finding of relatively negative membrane voltage for inducing NMDA conductances in the epileptic human DGCs supports the hypothesis that the NMDA receptor channel may be more easily activated at negative membrane voltages in epileptic DGCs. Alternatively, receptor insertion and redistribution (Wahlestedt et al. 1993) may be a morphological substrate for the increase of the whole cell NMDA conductance. A recent study of the distribution of NMDA receptors in hippocampal neurons reported that the NMDA receptor was highly mobile and rapidly recruited in specific synaptic locations (Benke et al. 1993). This suggests that the distribution of the NMDA receptor can change during synaptic remodeling, and that the ratio between the NMDA receptor and the AMPA receptor at a given dendritic patch could vary. Although this type of change can be detected when individual NMDA receptors are visualized with fluorescent dye-conjugated blockers (Benke et al. 1993), it is difficult to detect electrophysiologically by the recording of whole cell currents from neuronal somata. Small local predominance of a given receptor subtypes for the generation of local dendritic EPSCs may not well be reflected in somatic recordings unless the alteration is substantial. In addition, whole cell recordings of EPSCs from the soma can suffer from inadequate voltage and space clamp in dendrites, which also makes it difficult to assess properties of EPSCs generated at a local dendritic site (but see Hestrin et al. 1990 and Wheat et al. 1991 for evidence showing that somatic whole cell recordings can achieve satisfactory space clamp in hippocampal neurons). Because of its slow deactivation, once NMDA receptors are activated, the currents that are mediated by the receptor appears to contribute substantially to DGC depolarization and the subsequent calcium entry.

Alterations of dendrites and dendritic spines

Neuronal degeneration, i.e., hippocampal sclerosis, has been identified in human epileptic brains (Alzheimer 1898; Brown 1973; Chaslin 1891; Falconer et al. 1955; Sommer 1880; Spielmeyer 1930) among other pathologies such as
DGC axon sprouting (Houser et al. 1990; Isokawa et al. 1993; Sutula et al. 1989) and granule cell layer dispersion (Houser 1990). More specifically, alterations of dendritic morphology in epileptic brains such as loss of dendritic spines and the formation of swellings in dendritic shafts were reported in the cortex (Belichenko et al. 1994) and hippocampus (Isokawa and Levesque 1991; Scheibel et al. 1974) of epileptic patients. Although it was difficult to rule out the possibility that a metabolic failure could be a major cause for the pathology (Brierley and Graham 1984) in these human specimens, the findings are supported by many animal models such as the alumina cream model of epilepsy (Franceschetti et al. 1990; Westrum et al. 1964), the isolated cortical slab model (Gruner et al. 1974), the iron model (Reid et al. 1979), the bicuculline model (Collins and Olney 1982), the electrical stimulation model (Olney et al. 1983), the kindling model (Geinisman et al. 1992; Hosokawa et al. 1995; Nishizuka et al. 1991), the kainate model (Phelps et al. 1991; Pyapali and Turner 1994), and the pilocarpine model (Isokawa and Mello 1991). In a recent study of hippocampal slice cultures, pathological changes developed in dendrites and spines of pyramidal cells grown in a medium containing bicuculline (Muller et al. 1993). A large number of swollen and vacuolated dendrites was observed and a massive reduction in the number of dendritic spines was detected. However, when the culture was returned to normal medium, the surviving neurons reestablished healthy morphology and a normal complement of dendritic spines. Because the metabolism of the slices was not disturbed in these cultures, metabolic failure could not be considered a major factor for inducing these pathological changes. Instead, the study suggested that dendritic swelling and spine loss could be induced in the hippocampus where GABA inhibition was reduced (Drakew et al. 1995). In the pilocarpine model of chronic epilepsy, the frequency of spontaneous seizures was reported to progress in time during a period of 280 days, with a concomitant decrease in the time constant of DGCs (Isokawa 1996). Because local dendritic deformities can be identified at early stages in this model, and because neuronal time constant can be used as an indicator for cell degeneration (Isokawa 1997), this study suggested that dendritic deformities could progress in a chronic epilepsy model. Local alterations in dendritic morphology, induced by a neurotoxic effect of glutamate on confined dendritic spots, may create variations in the distribution of AMPA and NMDA receptors depending on the degree of spine pathology. Such a change might, in concert with other pathological changes such as mossy fiber reorganization (Masukawa et al. 1992) and changes in cell density (O’Connor et al. 1996), modulate the whole cell glutamate currents in epileptic DGCs, leading them to epileptic hyperexcitability.

We thank W. L. Byerly at the University of Southern California for assistance in the whole cell patch-clamp recording and D. Finch for critical reading of our manuscript.

This work was conducted under the guidance of Declaration of Helsinki and UCLA Human Subject Protection Committee and was supported by National Institute of Neurological Disorders and Stroke Grant NS-02808.

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Received 7 June 1996; accepted in final form 4 February 1997.

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