Electrophysiological Characterization of “Giant” Cells in Stratum Radiatum of the CA3 Hippocampal Region

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Savić, Nataša and Marina Sciancalepore. Electrophysiological characterization of “giant” cells in stratum radiatum of the CA3 hippocampal region. J Neurophysiol 85: 1998–2007, 2001. Whole cell patch-clamp recording and intracellular staining with biocytin allowed the morphological and electrophysiological characterization of “giant” cells, studied in stratum (st.) radiatum of the CA3 region in 17- to 21-day-old rat hippocampal slices. These neurons had extensive dendritic arborization, a triangular soma, and a bipolar vertical orientation with axons directed to the pyramidal layer or extended into the st. oriens. Giant cells had significantly higher input resistance and shorter action potentials compared with CA3 pyramidal cells. Evoked action potentials were typically followed by an afterdepolarizing potential (ADP). During depolarizing current injection, most (80%) of recorded giant cells displayed a regular firing pattern (maximum steady-state firing rate, ~30 Hz) characterized by a modest early accommodation, whereas irregular firing was observed in the remaining 20% of giant cells. Hyperpolarizing current pulses induced a slow inward rectification of the electrotonic voltage responses, blocked by 2 mM external Cs⁺. N-methyl-D-aspartate (NMDA) and non-NMDA–mediated excitatory postsynaptic currents (EPSCs) measured under voltage clamp were distinguished on the basis of their voltage dependence and sensitivity to specific NMDA and non-NMDA glutamate receptor blockers. Non-NMDA EPSCs possessed a linear current-voltage relationship. EPSCs elicited by st. lucidum stimulation were reversibly reduced (mean, 23%) by the group II metabotropic glutamate receptor agonist (2S, 1′R, 2′R, 3′R)-(2, 3-dicarboxyl-cyclopropyl)-glycine (DCG-IV, 1 μM). GABA_A-mediated postsynaptic currents were subject to paired-pulse depression that was inhibited by the GABA_A antagonist CGP 55845A (5 μM). We conclude that CA3 giant cells represent a particular class of hippocampal neuron located in st. radiatum that shares only some morphological and physiological properties with principal cells.

INTRODUCTION

Visualization of individual neurons in brain slice preparations allows the identification of nonpyramidal neurons localized in an intact circuit. In the stratum (st.) radiatum of hippocampal slices, large triangular cells with bipolar orientation clearly appear different from the rest of the cells in the same layer. For this reason, they have been often discarded by other scientists (Frerking et al. 1998; Semyanov and Kullmann 2000) as ectopic pyramidal cells. These cells closely resemble the hippocampal interneurons of the inferior region described by Amaral and Woodward (1977) and later found to be GABA immunoreactive (Woodson et al. 1989) and also those observed in the CA1 region by Lang and Frotscher (1990). Recently, these atypical so-called “giant” cells have been described in the CA1 subfield (Maccabberi and McBain 1996), found to express α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtypes 2/3 (Gulyás et al. 1998) and to exhibit a direct form of N-methyl-D-aspartate (NMDA)-dependent long-term plasticity (LTP) (Maccabberi and McBain 1996). These factors suggested that they may be included among the principal neurons whose excitatory nature has been confirmed by electron microscopy (Gulyás et al. 1998). An afterdepolarizing potential (ADP) was also typically found to follow individual evoked action potentials of CA1 st. radiatum giant cells (Gulyás et al. 1998); such intrinsic depolarizing afterpotentials have been described in different brain regions and found to be responsible for burst firing initiation (Deisz 1996; Jensen et al. 1996; Viana et al. 1993; Wong and Prince 1981).

The main aim of the present work was to investigate whether giant cells, recently described in the CA1 region (Gulyás et al. 1998; Maccabberi and McBain 1996), were also present in the CA3 subfield, and to study their intrinsic membrane properties as well as their synaptic physiology. To fulfill this goal, whole cell patch-clamp recordings and neuronal staining were combined in hippocampal brain slices. Giant cells were identified in the CA3 subfield, sharing some morphological and physiological properties with CA3 pyramidal cells.

METHODS

Slice preparation

Hippocampal slices were prepared from the brains of young (17- to 21-day-old) Wistar rats as previously described (Edwards et al. 1989). In brief, rats were decapitated under anesthesia (5% urethan ip), and their brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 126 NaCl, 3.5 KCl, 2 CaCl₂, 1.2 NaH₂PO₄, 1.3 MgCl₂, 25 NaHCO₃, and 25 glucose, gassed with 95% O₂-5% CO₂ (pH 7.3). After bisecting the brain, the tissue was immersed in cold (2–4°C), oxygenated ACSF solution. Transverse slices (250 μm thick) were cut with a vibrating microslicer (Vibracut, FTB, Weinheim, Germany) and incubated in ACSF for 1 h at 32°C before use. Giant cells in the st. radiatum were visually identified using infrared differential interference contrast (IR-DIC) videomicroscopy.

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Whole cell recordings

Tight-seal whole cell recordings were obtained from giant cells with patch pipettes (3–4 MΩ) filled with (in mM) 130 K-glucuronate, 10 KCl, 10 N-2-hydroxyethylpiperezine-N'-2-ethanesulfonic acid (HEPES), 0.4 ethylene glycol-bis(β-aminoethyl ether)-N,N',N′-tetraacetic acid (EGTA), 1 MgCl₂, 0.3 Na GTP, and 2 Na₂ATP (pH 7.3). Brain slices were constantly superfused at 2 ml/min with ACSF. Ca²⁺-free ACSF was prepared by omitting CaCl₂ and replacing it with 3.7 mM MgCl₂ plus 1 mM EGTA. Voltage responses were recorded at room temperature (22–24°C) in bridge mode with an Axoclamp 2B amplifier (Axon Instruments, low-pass filtering at 10 kHz). Capacitance neutralization and resistance compensation were applied by monitoring the voltage response onset to a current step at high time resolution. Access resistance measured with the use of the bridge compensation circuit of the amplifier ranged from 10 to 25 MΩ.

An EPC-7 patch-clamp amplifier (List, Darmstadt, Germany) was used for recording excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) in voltage-clamp mode at 32°C. To improve the space clamp, synaptic currents were recorded with pipette solutions in which cesium methanesulfonate and CsCl substituted K-glucuronate and KCl, respectively. The stimulating pipette, filled with standard ACSF, was placed ~150–200 μm away from the recorded neuron either in st. lucidum or st. radiatum. Stimuli of 4–8 V, 40 μs duration were delivered at 0.05 Hz from an isolated pulse generator (Grass Instruments, Quincy, MA). The stimulation strength was set to evoke 25% of the maximal synaptic response. The rectification index of EPSCs mediated by AMPA receptors was quantified by measuring the ratio of EPSC chord conductances at +30 and −70 mV, assuming a reversal potential of 0 mV.

Histology

Biocytin (0.2%, Sigma) was added to the pipette solution just before use. Injected cells were visualized using the avidin-biotinylated horseradish peroxidase complex reaction (ABC; Vector Laboratories, Burlingame, CA) with cobalt and nickel-intensified 3,3′-diaminobenzidine as chromogen. After slice embedding in glycerol, neurons were viewed under light microscopy. Micrographs of neurons were taken at different focal depths. After scanning each photo, the pictures were digitally overlaid and image processed using custom-prepared software to reconstruct the whole neuronal morphology observable in a 250 μm-thick slice.

Data analysis

Data were acquired and digitized with an A/D interface (Digidata 1200, Axon) and analyzed using commercial software (pClamp6, Axon Instruments, Foster City, CA). Cell input resistance (Rm) was estimated from the apparently linear portion of the steady-state voltage-current relationships obtained by measuring the amplitude of five averaged voltage responses (≤15 mV from resting potential) to hyperpolarizing current injection. The slower membrane time constant (τ0) was determined by fitting either single or double exponential functions to the averaged electrotonic response (≤10 mV) obtained by hyperpolarizing current injection (150 ms). Single action potentials were elicited by 3 ms depolarizing pulses at spike threshold intensity. A single spike just at the end of current injection was evoked to avoid interference by the capacitative transient. The parameters used to characterize action potentials were firing threshold, amplitude (measured from the threshold to the peak), and duration (measured at the half-amplitude). The afterhyperpolarizing potential (AHP) amplitude was measured from the peak to the baseline. The maximum steady-state firing rate was obtained by averaging the frequency for the last five inter-spike intervals of a train evoked by the largest current injected before onset of spike failure. The accommodation rate was quantified by measuring the instantaneous discharge frequency between the first two spikes (f_initial), 150 ms after the beginning of the discharge (f_final), and at the end of the stimulus (f_final) in the train of spikes after injection of 100 pA for 500 ms. Early and late accommodation was calculated according to (f_final – f_initial) and (f_final – f_initial)/f_initial respectively (Cauli et al. 1997). Paired-pulse depression (PPD) of evoked IPSCs was expressed as a ratio of the amplitude of the second IPSC to that of the first.

The liquid junction potential was estimated as +10 mV. Under current clamp, the membrane potential was not corrected for this potential difference; in voltage-clamp experiments this was taken in account during the acquisition of experimental data. Statistical difference between data was assessed with Student’s t-tests applied to the raw data (P < 0.05). All the results are presented as means ± SD.

Drugs

Drugs were applied via bath superfusion using a three-way tap system. 3,3′-Diaminobenzidine (DAB), tetrodotoxin (TTX), CsCl, (2S,1′R), 1′R, 3′R)-2-(2,3-dicarboxyl-cyclopropyl)-glycine (DCG-IV), choline chloride, and bicuculline methiodide (BIC) were purchased from Sigma (St. Louis, MO); 6,7-dinitroquinoxaline-2,3-dione (DNQX) and d-2-amino-5-phosphonopentanoate (AP-5) were obtained from Tocris (Bristol, UK). All other chemicals were from Merck. COP 55845 A was a generous gift from Novartis Pharma AG (Basel, Switzerland).

RESULTS

Morphology

The unique morphology of giant cells allowed their easy visual identification in the st. radiatum of CA3 hippocampal slices (Fig. 1A). A large triangular body and two prominent apical dendrites distinguished these neurons from the heterogeneous population of cells present in this layer. Giant cells were further differentiated electrophysiologically on the basis of the action potentials generated in response to a brief depolarizing pulse. In all the giant cells studied a characteristic ADP followed single action potentials. A subset of cells (60%) were loaded with biocytin and confirmed the identification of cells on the basis of IR-DIC and afterdepolarizing properties. CA3 giant cells showed a characteristic somatic triangular shape and were characterized by two main dendritic branches, parallel to the CA3 pyramidal cell apical dendrites, arising from the soma and extensively distributed in the st. radiatum and lacunosum-moleculare. Thinner branches originated from the main dendrites (Fig. 1B). Basal dendrites were not commonly observed. An accurate measurement of cell body size carried out in 15 cells yielded a longer axis mean = 27 ± 3 (SD) μm, and shorter axis mean = 10 ± 1.8 μm. Labeled axons originated from the basal pole of the soma and penetrated into the pyramidal cell layer only (n = 7) or also extended to the st. oriens (n = 3, Fig. 1A, see arrows). To allow a comparison with the principal neurons, a photomicrograph of CA3 pyramidal cell is included in Fig. 1C. Its soma was localized in the pyramidal layer, its apical dendrites projected to st. radiatum, and the basal dendrites were present with varicose swellings (Lang and Frotscher 1990) radiating in st. oriens. The axons were seen to extend into the st. oriens.

Intrinsic membrane properties of giant cells

Current-clamp recordings were obtained from a total of 41 giant cells located in st. radiatum of the CA3 hippocampal system. 3,3′-Diaminobenzidine (DAB), tetrodotoxin (TTX), CsCl, (2S,1′R), 1′R, 3′R)-2-(2,3-dicarboxyl-cyclopropyl)-glycine (DCG-IV), choline chloride, and bicuculline methiodide (BIC) were purchased from Sigma (St. Louis, MO); 6,7-dinitroquinoxaline-2,3-dione (DNQX) and d-2-amino-5-phosphonopentanoate (AP-5) were obtained from Tocris (Bristol, UK). All other chemicals were from Merck. COP 55845 A was a generous gift from Novartis Pharma AG (Basel, Switzerland).
region in slices obtained from young [postnatal days 17 to 21 (P17–21)] rats. The basic membrane properties of giant cells were measured at resting membrane potential at room temperature (22–24°C) and compared with those observed in CA3 pyramidal cells under the same experimental conditions (Table 1). The input resistance was measured by taking the regression slope of the voltage-current ($V-I$) relationship (Fig. 2A). Giant cells had significantly ($P < 0.05$) higher input resistance ($R_{in}$) than pyramidal cells (595 ± 224 MΩ vs. 307 ± 98 MΩ), whereas the membrane time constant and resting potential were similar. All giant cells fired overshooting TTX-sensitive action potentials. Single action potentials had a mean amplitude of 76 ± 7.5 mV, a value similar to that observed in CA3 pyramidal cells. Action potential duration at half-amplitude was, however, significantly shorter compared with pyramidal cells (1.1 ± 0.1 ms vs. 1.66 ± 0.4 ms). In common with pyramidal cells, giant cells did not fire spontaneously at the resting membrane potential.

At resting potential, a single evoked action potential was followed by a fast afterhyperpolarizing potential (fAHP) and an ADP. In 40% of the cells, a small amplitude medium afterhyperpolarizing potential (mAHP) (Storm 1989) was also observed (mean amplitude, 2.2 ± 0.3 mV; mean duration, 176 ± 67 ms, $n = 17$, Fig. 2B). The ADP appeared following the fAHP

**TABLE 1. A comparison of the electrophysiological properties of giant cells and CA3 pyramidal neurons recorded at 22–24°C**

<table>
<thead>
<tr>
<th>Recorded Neurons</th>
<th>Giant cells</th>
<th>Pyramidal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>$-57 \pm 1.2$</td>
<td>$-58 \pm 1.2$</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>$595 \pm 224^*$</td>
<td>$307 \pm 98$</td>
</tr>
<tr>
<td>Membrane time constant, ms</td>
<td>$67 \pm 23$</td>
<td>$71 \pm 3.3$</td>
</tr>
<tr>
<td>Firing threshold, mV</td>
<td>$-35 \pm 3.2$</td>
<td>$-35 \pm 2.0$</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>$76 \pm 7.5$</td>
<td>$80 \pm 4.6$</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>$1.1 \pm 0.1^*$</td>
<td>$1.7 \pm 0.4$</td>
</tr>
<tr>
<td>Rectification ratio</td>
<td>$1.2 \pm 0.1$</td>
<td>$1.1 \pm 0.01$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Number of cells in Giant cells is 28 and in Pyramidal cells is 9. AP, action potential. * Significantly different from pyramidal cells ($P < 0.05$).
notch, when spike repolarization reached a voltage level of approximately \(-50\) mV (mean, \(-51 \pm 2.6\) mV, \(n = 28\)).

**Firing properties of giant cells**

The firing properties of giant cells were examined by depolarizing the cell with long-lasting (500 ms) current pulses of increasing intensity. As shown in Fig. 3, A and C, all the giant cells exhibited a firing pattern clearly different from that observed in CA3 pyramidal cells (Fig. 3D). Eighty percent of the giant cells (\(n = 33\)) recorded displayed regular firing (Fig. 3A), whereas an irregular firing pattern was observed in the remaining 20% of cells (Fig. 3C, \(n = 8\)). In Fig. 3, A and C, on the right panels, the differences between the firing properties of the regular and irregular giant cells were quantified for representative cells. In case of the irregular firing cells (Fig. 3C), it was hard to obtain a good fit with linear regression.

Spike frequency accommodation was studied in the regularly firing giant cells (\(n = 13\)) by injecting 500 ms-long depolarizing current pulses (50–150 pA, Fig. 3B). The plot of the spike sequence versus the mean of instantaneous firing frequency for various injected currents showed that the number of spikes increased with the current strength. A modest early accommodation (mean, 43 ± 5.5%, \(n = 13\)) occurred over the first few spikes; late accommodation was lower (mean, 15 ± 4.5%, \(n = 13\)). The maximum steady-state firing rate was 29 ± 6 Hz (\(n = 23\)).

As shown in Fig. 4, the mAHP and also a slow AHP (sAHP) (Storm 1990) were observed after trains of action potentials (mean amplitude and duration, 7.1 ± 2.4 mV, 365 ± 45 ms and 3.4 ± 1.3 mV, 2.9 ± 0.5 s, respectively, \(n = 18\), Fig. 4A, control). As found in pyramidal cells (Lancaster and Adams 1986), the sAHP clearly increased in amplitude and duration with the number of spikes in the train (data not shown). Superfusion of a Ca\(^{2+}\)-free solution decreased the amplitude of the mAHP (40 ± 3%, \(n = 5\)) and induced a block of the sAHP (Fig. 4A, \(n = 5\)). A clear increase in cell firing rate (38 ± 6%, \(n = 5\)) was also commonly observed after blocking calcium influx (Fig. 4B).

**FIG. 3.** Comparison of firing patterns in giant cells and CA3 pyramidal cells. Trains of action potentials were evoked from resting membrane potential by long (500 ms) depolarizing current pulses. Giant cells displayed either regular (A) or irregular (C) firing patterns, clearly different from the strongly accommodating discharge observed in pyramidal cells (D). In the right panels in A and C, plots of inter-spike intervals (ISI; normalized to the 1st ISI) vs. spike sequence are shown. The ISI relationship of irregularly firing cell was difficult to fit with linear regression. B: time course of instantaneous firing frequency of each action potential in the train. Numbers in abscissa represent the sequence of action potentials. Data were obtained by injecting long (500 ms) depolarizing pulses of increasing current intensity (50–150 pA, in 25-pA steps). Filled circles and bars represent mean firing frequency (\(n = 13\)) and SD, respectively. Note the presence of early accommodation.
Inward rectification

Responses evoked by hyperpolarizing current pulses displayed slow inward rectification that was blocked after 5 min superfusion of CsCl (2 mM, \( n = 4 \), Fig. 5), suggesting the activation of a hyperpolarization-activated cationic conductance \( (I_h) \) (Halliwell and Adams 1982). The rectification ratio (calculated as the ratio between the membrane potential measured at the peak, \( V_{\text{max}} \), and at the end of a 500 ms long hyperpolarizing current injection that brought the cell \( V_{\text{max}} \) to \(-100 \text{ mV}\)) was 1.18 ± 0.1 (Table 1).

Excitatory postsynaptic responses

EPSCs were evoked in giant cells at 32°C by electrical stimulation of local afferent fibers in st. lucidum (where most mossy fiber terminals are located) (Blackstad et al. 1970) and st. radiatum [where commissural/associational (Co/A) fibers are present] (Amaral and Witter 1989) in the presence of the GABA\(_A\) receptor antagonist, BIC (10 \( \mu \text{M}, \) Fig. 6). EPSCs consisted of two components that could be distinguished on the basis of their voltage dependence and their sensitivity to specific AMPA/kainate and NMDA glutamate receptor (GluR) blockers. Thus EPSCs elicited at \(-80 \text{ mV}\) holding potential showed faster decay kinetics compared with those evoked at \(+10 \text{ mV}\) (mean \( \tau = 3.1 ± 0.8 \text{ ms} \) at \(-80 \text{ mV}, n = 10\) vs. \(142 ± 17 \text{ ms} \) at \(+10 \text{ mV}, n = 10\)). Non-NMDA EPSCs were pharmacologically isolated in the presence of the NMDA antagonist AP-5 (50 \( \mu \text{M} \)). As shown in Fig. 6A after application of AP-5, the amplitude of the EPSC evoked at \(-80 \text{ mV} \) was almost unchanged (mean \(6 ± 3.1\% \) change, \( n = 7\)), whereas a clear reduction of EPSC amplitude was observed at \(+30 \text{ mV}\) (mean \(63 ± 14\% \) of control, \( n = 7\)) with the block of the late component. Averaged responses of non-NMDA EPSCs at \(-70 \text{ mV} \) showed fast rise and decay time (means: \(1.18 ± 0.22 \text{ ms} \) and \(3.13 ± 0.6 \text{ ms}, n = 10\), respectively). A representative example of the \( I-V \) relationship of peak EPSCs amplitude in

![FIG. 4. Block of Ca\(^{2+}\) influx affects the giant cell AHPs and increases the firing discharge. A: depolarizing current pulses evoked trains of action potentials followed by a mAHP and slow AHP (sAHP). Ca\(^{2+}\)-free solution strongly decreased the mAHP and blocked the sAHP with a concomitant increase in the firing discharge (B).](image)

![FIG. 5. Giant cell shows slow inward rectification. Negative current pulses (500 ms duration) of increasing intensity were injected. Superimposed hyperpolarizing voltage responses showed a typical slow depolarizing “sag”; a depolarizing rebound characterized the offset of the current pulse. Bath application of 2 mM CsCl blocked the sag and, partially, the postpulse rebound.](image)
control (in the range from −80 to +50 mV) is shown in Fig. 6B (●). The I-V curve became almost linear after superfusion of AP-5 (∆). The mean rectification ratio of AMPA-mediated EPSCs (between conductances at +30 mV and at −70 mV) was 0.9 ± 0.1 (n = 12), whereas the mean reversal potential was around 0 mV (n = 6).

In hippocampal CA3 pyramidal cells, activation of presynaptic group II mGluRs depresses mossy fiber but not Co/A fiber EPSCs (Kamiya et al. 1996). We investigated the possible role of presynaptic mGluRs in the modulation of non-NMDA EPSCs recorded in giant cells after stimulation of st. lucidum and/or st. radiatum afferents. As shown in Fig. 6C, the group II–selective mGluR agonist DCG-IV (1 μM), applied in the bath in the presence of the NMDA receptor antagonist AP-5 (50 μM), significantly (P = 0.004) and reversibly reduced EPSCs evoked by stimulation of st. lucidum (mean depression, 23.2 ± 5%, n = 9). At the concentration used, DCG-IV neither produced any detectable inward current nor changed the membrane Rm (means, 500 ± 72 MΩ and 515 ± 60 MΩ in the absence and presence of DCG-IV, respectively, n = 9) thereby excluding any postsynaptic effect on giant cells. In contrast, DCG-IV did not significantly affect the EPSC evoked by stimulation of st. radiatum (mean 2 ± 0.7% reduction in amplitude, n = 4), indicating that mGluRs 2/3 selectively modulate glutamatergic responses at mossy fiber–giant cell synapses.

NMDA-mediated EPSCs were pharmacologically isolated in the presence of the non-NMDA receptor antagonist DNQX (20 μM). As shown in Fig. 7A, EPSCs evoked at −80 mV were almost completely blocked (mean, 95 ± 5.2%, n = 5) by DNQX application, whereas EPSCs evoked at +30 mV were less reduced in amplitude (mean, 37 ± 16%, n = 5) and duration (mean, 8 ± 10%, n = 5). In Fig. 7B the I-V relationship for the EPSCs measured in control at the peak (□) and 50 ms after the peak (○) is shown. The I-V relationship of the late EPSC (○) had a region of negative slope conductance between −80 and −20 mV, typical of NMDA receptor–mediated EPSCs (Mayer et al. 1984). Moreover, NMDA currents recorded after superfusion of DNQX (●) overlapped the values of EPSC peaks measured at a later phase (○). Pharmacologically isolated NMDA currents recorded at +30 mV had a mean rise time of 7 ± 2.1 ms and mean decay time of 197 ± 21 ms (n = 5). Decay times of 96 and 878 ms were observed in NMDA-dependent EPSCs in CA1 giant cells by Kirson and Yaari (2000); however, these recordings were made in the presence of 5 μM glycine to facilitate NMDA receptor function, and therefore decay times may have been influenced by reduced desensitization (Mayer et al. 1989).

**GABA-mediated postsynaptic responses**

IPSCs in giant cell were elicited by stimulation of st. radiatum afferents (n = 9) and pharmacologically isolated in the presence of AP-5 (50 μM) and DNQX (20 μM). The short latency (mean, 2 ± 0.4 ms, n = 9) and short rise time (mean, 1.6 ± 0.5 ms) of IPSCs indicated their monosynaptic nature. As shown in Fig. 8A, IPSCs were abolished by BIC (10 μM, n = 4), indicating that they were mediated by GABA<sub>A</sub> receptors. The I-V relationship of IPSCs was mostly linear over the range from −90 to +10 mV (Fig. 8B), and the reversal potential value was around −60 mV (n = 4), close to the calculated Nernst equilibrium potential for chloride ions (E<sub>Cl</sub> = −61 mV) under our conditions. When two stimuli of identical strength were applied in close succession to the same input, the second IPSC was clearly depressed (PPD, n = 7). When PPD was tested by changing the inter-stimulus interval from 0.05 to 2 s, a maximal depression (mean, 27 ± 2.3%, n = 3) was observed at 150–200 ms (Fig. 8C).

In hippocampal pyramidal cells, GABA regulates its own release via GABA<sub>A</sub> autoreceptors, which are responsible for frequency-dependent depression of inhibitory transmission (Deisz and Prince 1989). To investigate whether presynaptic GABA<sub>B</sub> receptors were responsible for depression of GABA release onto giant cells and modification of the extent of PPD, the specific GABA<sub>B</sub> antagonist CGP 55845 A (5 μM) was
added to the bath, and its effect on PPD was examined at an inter-stimulus interval of 200 ms (Fig. 8D). In four cells tested, no change in amplitude (157 ± 62 pA vs. 156 ± 66 pA) of the first IPSC was observed after 5 min of CGP superfusion, whereas a significant increase (mean 56 ± 24% of control) of the second IPSC amplitude occurred, reversing the PPD (25 ± 9%) to a paired-pulse facilitation (PPF, 12 ± 6.6%). These results indicated that presynaptic GABA_B receptors negatively regulate GABA release at the inhibitory synapses on giant cells.

**DISCUSSION**

The present paper describes for the first time the morphology and basic electrical/synaptic properties of a peculiar class of nonpyramidal hippocampal cell found in the st. radiatum of the CA3 subfield.

**Morphology**

The large triangular soma and extensive dendritic arborization were the major discriminating factors that characterized the giant cell type among the heterogeneous population of st. radiatum interneurons (Maccarelli and McBain 1996; McMahon and Kauer 1997). Post hoc identification by histological processing confirmed their similarity with the “inferior region interneurons” described by Amaral and Woodward (1977) subsequently shown to be GABA immunoreactive (Woodson et al. 1989), and also with the neurons found in the st. radiatum of the CA1 region (Lang and Frotscher 1990, their Fig. 7, a and c) and with the giant cells observed recently in the CA1 area (Gulyás et al. 1998; Maccarelli and McBain 1996). Giant cells differed from pyramidal cells in their soma location in st. radiatum and by their rarely observed basal dendrites (Gulyás et al. 1998), commonly described in pyramidal cells (Jonas et al. 1993). In CA3 giant cells, the axons were found to arise from the basal pole of the soma, penetrate the pyramidal cell layer, or extend in st. oriens. In the CA1 region, giant cell axons have been found to contact st. oriens interneurons and extrahippocampal areas such as the olfactory bulb (Gulyás et al. 1998). Further studies will be necessary to find the local targets of CA3 giant cell axons as well as the possible long-range projections.

**Membrane properties of giant cells**

The basic membrane properties of CA3 giant cells were similar to those found in pyramidal cells except for the shorter duration action potential and higher $R_m$, features typically found in interneurons (Lacaille et al. 1987; Williams et al. 1994). A specific potassium conductance, involved in action potential repolarization (Rudy et al. 1999), could be responsible for the shorter action potentials observed in giant cells. A higher $R_m$ would also result in larger and faster synaptic potentials (Miles 1990). The input resistance of CA3 pyramidal cells (407 MΩ) was higher than that found in adult guinea pig CA3 cells by nystatin patch method (135 MΩ) (Spruston and Johnston 1992) but not so far from that measured in 19- to 21-day-old rats by Major et al. (1994) (190 and 260 MΩ in initial and postwashout responses, respectively).

The input resistance and membrane time constant of CA3 giant cells were larger than the values found for giant cells located in the CA1 region (Gulyás et al. 1998). A similar relationship exists for CA3 and CA1 interneurons (Chitwood et al. 1999) as well as for CA3 pyramidal neurons and other principal cells of the hippocampal formation (Spruston and Johnston 1992). The differences could reflect different density and distributions of voltage-dependent conductances open at resting (Major et al. 1994; Spruston and Johnston 1992).

As with CA1 giant cells (Gulyás et al. 1998), individual action potentials of CA3 giant cells were followed by a characteristic ADP that appeared after the fAHP notch, at −50 mV. Similar characteristics were reported for the intrinsic ADP observed in dentate gyrus granule cells (Zhang et al. 1993), spinal motoneurons (Harada and Takahashi 1983), and dorsal ganglion neurons (White et al. 1989).

**FIG. 7.** Properties of NMDA-mediated EPSCs in giant cells. A: EPSCs evoked from a holding potential of −80 and +30 mV before and during 6,7-dinitroquinazoline-2,3-dione (DNQX; 20 μM). EPSCs were almost completely blocked by DNQX at −80 mV, whereas only a decrease in amplitude and duration was observed at +30 mV. Each trace is the average of 10 individual EPSCs. B: I-V relationship for the EPSCs measured at the peak (○) and 50 ms after the peak (□) in control extracellular solution. The I-V relationship for the late component almost overlapped that observed in the presence of DNQX (where EPSC amplitude was measured at the peak, ●). Note the rectification of the NMDA component at negative potentials. The EPSC reversal potential was around 0 mV.
In hippocampal pyramidal cells (Wong and Prince 1981), in thalamic neurons (Jahnsen and Llina’s 1984), as well as in neocortical cells (Deisz 1996), the ADP has been reported to amplify subthreshold signals leading to burst firing. Conductances underlying the ADP can also be targets for the modulation of the firing pattern by specific neuromodulatory substances under physiological and pathological conditions.

In contrast to pyramidal cells, a regular firing pattern was observed in giant cells characterized by a modest accommodation. The maximum firing rate resembled that observed in neocortical regularly spiking interneurons (Erisir et al. 1999). An irregular firing pattern was also observed in a minority of giant cells. Similar firing behavior has been observed in st. radiatum nonpyramidal neurons by Chitwood and Jaffe (1998); however, these cells have not been fully characterized.

**Synaptic inputs**

Studying properties of the synaptic inputs on giant cells would aid in the understanding of the role of these cells in the hippocampal circuit. The extensive dendritic arborization and the critical position of giant cells in the hippocampal region suggested that they are contacted by the afferents to the apical dendrites of CA3 pyramidal cells.

**GLUTAMERGIC INPUTS.** Glutamatergic EPSCs mediated by NMDA and non-NMDA receptors were recorded in giant cells on stimulating st. lucidum or st. radiatum fibers. In accordance with the anatomical evidence of Amaral and Woodward (1977), our results suggested that mossy fibers make excitatory synapses with giant cells, possibly on st. lucidum dendrites. In the presence of AP-5, stimulation of st. lucidum induced non-NMDA–mediated glutamatergic synaptic responses in giant cells, that were partially reduced (21%) by the group II selective mGluR agonist DCG-IV. mGluR agonists have previously been found to affect glutamatergic synapses from mossy fibers onto CA3 pyramidal cells (Kamiya et al. 1996) as well as from mossy fibers onto st. lucidum interneurons (Maccaferrì et al. 1998; Tóth and McBain 1998). Non-NMDA–mediated EPSCs, recorded in CA3 giant cells and supposed to originate from mossy fibers and/or Co/A pathways, showed a linear I-V relationship. Linear AMPA-mediated glutamatergic responses...
have also been found in CA1 giant cells (Kirson and Yaari 2000), which express GluRs 2/3 (Gulyás et al. 1998) as well as in hippocampal pyramidal cells (Colquhoun et al. 1992; Jonas et al. 1993). Inwardly rectifying, calcium-permeable AMPA receptors have been associated with the low presence of GluR2 expression (Jonas and Burnashev 1995), which dominates in GABAergic interneurons (Jonas et al. 1994). This suggests that CA3 giant cells, as those located in the CA1 region (Gulyás et al. 1998; Kirson and Yaari 2000) possess some physiological properties reminiscent of the principal cells. NMDA-dependent LTP occurred at excitatory synapses onto CA1 st. radiatum giant cells (Maccarelli and McBain 1996) later defined excitatory principal cells (Gulyás et al. 1998). NMDA-independent LTP has been extensively studied at mossy fibers–CA3 pyramidal cell synapses (Johnston et al. 1992; Zalutsky and Nicoll 1990). LTP has not been found at the excitatory synapses onto hippocampal interneurons (Laezza et al. 1999; Maccarelli and McBain 1996). The possibility of synaptic plasticity occurring at the excitatory synapses between mossy fibers and CA3 giant cells would potentiate the signal processing in the CA3 region.

GABAergic Inputs. Significant BIC-sensitive GABA_A-mediated synaptic responses with a reversal potential close to E_Cl were elicited in giant cells, pharmacologically isolated in AP-5 and DNQX. Our results also demonstrated that presynaptic activation of GABA_A receptors could down-regulate transmitter release inducing PPD. Frequency-dependent PPD, mediated by GABA_B autoreceptors, has been found in hippocampal pyramidal cells (Thompson and Gahwiler 1989) as well as in lacunosum-molecular interneurons (Williams et al. 1994) and neocortical neurons (Deisz and Prince 1989). Furthermore, PPD has been found to be highly dependent on the probability of release during the first response (Lambert and Wilson 1994).

In conclusion, st. radiatum CA3 giant cells may be considered as neurons that, although similar to pyramidal cells, share with interneurons some common physiological properties such as high-input resistance, short action potential duration, and absence of strong accommodation.

Although this work reveals excitatory and inhibitory inputs of CA3 giant cells with distinct presynaptic control mechanisms, the identification of their target cells would be necessary to understand the functional role of giant cells within the hippocampal circuit.

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