Medium Afterhyperpolarization and Firing Pattern Modulation in Interneurons of Stratum Radiatum in the CA3 Hippocampal Region

NATAŠA SAVIĆ, PAOLA PEDARZANI, AND MARINA SCIANCELEPORE

Neuroscience Programme and Istituto Nazionale Fisica della Materia Unit, International School for Advanced Studies (SISSA), 34014 Trieste, Italy; and Max-Planck Institut für experimentelle Medizin, Department of Molecular Biology of Neuronal Signals, 37075 Göttingen, Germany

Received 7 August 2000; accepted in final form 18 January 2001

Savić, Nataša, Paola Pedarzani, and Marina Sciancalepore. Medium afterhyperpolarization and firing pattern modulation in interneurons of stratum radiatum in the CA3 hippocampal region. J Neurophysiol 85: 1986–1997, 2001. Stratum (st.) radiatum interneurons represent a heterogeneous class of hippocampal cells with as yet poorly characterized physiological properties. Intracellular staining with biocytin, in situ hybridization, and patch-clamp recording have been combined to investigate the morphological and electrophysiological properties of these cells in the CA3 hippocampal region in young rats [postnatal days 10 to 21 (P10–21)]. Labeled cells presented a heterogeneous morphology with various soma shapes, often found multipolar, and dendritic arborizations confined to st. radiatum. The passive membrane properties of these st. radiatum interneurons showed instead no significant differences between P10 and P21. Low resting potential, high-input resistance, and short time constants characterized CA3 st. radiatum interneurons, which were silent at rest. Action potentials, elicited by brief current pulses, were lower and shorter than in pyramidal cells and followed by a Ca2+-dependent medium-duration afterhyperpolarizing potential (mAHP). Prolonged depolarizing current injection generated trains of action potentials that fired at constant frequency after a slight accommodation. The maximum steady-state firing rate was 31 ± 4 (SD) Hz. Hyperpolarizing current pulses revealed a prominent inward rectification characterized by a “sag,” followed by a depolarizing rebound that triggered action potentials. Sag and anodal break excitation were blocked by Cs+ suggesting that they were mediated by a hyperpolarization-activated cation conductance (Ih). In the presence of tetrodotoxin and tetraethylammonium, bifasic tail currents were elicited in voltage clamp after a depolarizing step inducing Ca2+ influx. Tail currents presented a fast Ca2+-activated and apamin-sensitive component (IAMP) and were further reduced by carbachol. The presence of IAMP was consistent with the high expression level of the apamin-sensitive SK2 subunit transcript in CA3 st. radiatum interneurons as detected by in situ hybridization. Different pharmacological agents were shown to affect the afterhyperpolarizing potential as well as the firing properties of st. radiatum interneurons. Exposure to Ca2+-free solutions mainly affected the late phase of repolarization and strongly reduced the mAHP. The mAHP was also attenuated by carbachol and by apamin, suggesting it to be partly mediated by IAMP. Reduction of the mAHP increased the interneuron firing frequency. In conclusion, st. radiatum interneurons of CA3 hippocampal region represent a class of nonpyramidal cells with action potentials followed by an AHP of relatively short duration, partially generated by apamin and carbachol-sensitive conductances involved in the regulation of the cell firing rate.

Address for reprint requests: M. Sciancalepore, Biophysics Sector, International School for Advanced Studies (SISSA), Via Beirut 2-4, 34014 Trieste, Italy (E-mail: marinas@sissa.it).

1986
0022-3077/01 $5.00 Copyright © 2001 The American Physiological Society www.jn.physiology.org

INTRODUCTION

Interneurons account for 10–12% of the total hippocampal cell population and are responsible for GABAergic inhibition, which controls the excitability of hippocampal neural circuits (Kandel et al. 1961; Nicoll et al. 1990). Furthermore, networks of GABAergic interneurons are responsible for cortical rhythmic activity such as synchronous γ (Whittington et al. 1995) and θ (Cobb et al. 1995; Tóth et al. 1997) oscillations. Membrane properties of different subclasses of interneurons in stratum (st.) oriens (Lacaille and Williams 1990; Lacaille et al. 1987; McBain 1994), st. pyramidale (Buhl et al. 1994, 1996; Gulyás et al. 1993; Knowles and Schwartzkroin 1981; Miles et al. 1996), st. lacunosum-moleculare (L-M) (Khazipov et al. 1995; Lacaille and Schwartzkroin 1988), and at the border between st. radiatum and L-M (Kawaguchi and Hama 1987; Kunkel et al. 1988; Williams et al. 1994) have been investigated. In particular, interneurons such as basket and axoaxonic cells in st. pyramidale and vertical cells in st. oriens generate short action potentials followed by fast afterhyperpolarizations (AHPs) responsible for sustained high-frequency firing (Buhl et al. 1994, 1996; Lacaille and Williams 1990; Lacaille et al. 1987); conversely, stellate cells in st. L-M possess longer action potentials (followed by a fast AHP) and fire at a slower rate (Lacaille and Schwartzkroin 1988; Williams et al. 1994). A heterogeneous population of interneurons is known to be present in the hippocampal st. radiatum (Maccarelli and McBain 1996; McMahon and Kauer 1997). These cells contain glutamate decarboxylase (GAD), the enzyme synthesizing the neurotransmitter GABA (Frotscher et al. 1984; Ribak et al. 1978; Woodson et al. 1989) and are therefore presumably inhibitory. Surprisingly, although there are reports concerning the morphology (Gulyás et al. 1993; McMahon and Kauer 1997) and synaptic input (Laezza et al. 1999; Maccarelli and McBain 1996; McBain and Dingledeine 1993; McMahon and Kauer 1997) of these cells, a detailed study of the basic electrophysiological properties of interneurons located in this region is still lacking.

Potassium currents involved in the action potential repolarization have been studied in interneurons of st. oriens-alveus (Zhang and McBain 1995), in parvalbumin-positive interneurons in st. pyramidale (Du et al. 1996), and in st. L-M (Chap-
man and Lacaille 1999). Little is known about the potassium conductances underlying action potential AHPs in the various subclasses of interneurons present in the hippocampus. In st. oriens interneurons, different Ca2+-dependent K+ conductances have been suggested to underlie an iberiotoxin-sensitive fast AHP (fAHP) and a medium-duration AHP, sensitive to block voltagedependent sodium currents and fast potassium currents (Coetzee et al. 1999), respectively. To date, the only data concerning the K+ currents underlying the AHP, recorded in the voltage-clamp mode, are related to the recent report on st. L-M interneurons by Aoki and Baraban (2000).

The aim of the present work was to investigate the intrinsic membrane properties of st. radiatum interneurons in the CA3 hippocampal region. By combining electrophysiological techniques, pharmacological tests and in situ hybridization, we characterized the properties of the currents underlying the AHP. Potassium currents sensitive to apamin and carbachol were found to contribute to the AHP, playing a fundamental role in shaping the firing pattern.

METHODS

Slice preparation

Hippocampal slices were prepared from the brain of young (10-21-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 into low temperature (2-4°C) oxygenated ACSF solution. Transverse slices (250 μm thick) were cut with a vibrating microslicer (Vibracut, FTB, Weinheim, Germany) with cobalt and nickel-intensified 3,3'-diaminobenzidine as chromogen. After slice embedding in glycerol, neurons were viewed under light microscope. Microphotographs of neurons were taken at different focal depths. After scanning each photo, the pictures were overlaid to reconstruct the whole neuronal morphology observable in a 250 μm-thick slice.

In situ hybridization

In situ hybridization was performed on brain sections (10-16 μm) from male rats using 35S-labeled antisense and sense oligonucleotide probes according to the procedure described in detail (Stacker and Pedrazzani 2000). Every SK channel probe was tested on sections obtained from four different animals, two as sagittal and two as coronal cuts. For each SK channel subunit, at least two antisense oligonucleotides corresponding to the 5′ and 3′ regions with no significant similarity to the other known SK channel subunits were chosen (SK1: 5′-GGCTTCGACGTCTCGACACCTCATGCTGATGCTGTGCTT-3′ and 5′-CAGTGCCCTTTGTTGGCGCTG-3′; SK2: 5′-AGGGCCACGTGTTGGATAGATTGCCTAGCCAC-3′ and 5′-CTTTTTCGCTGGACTTAGTGC-3′; SK3: 5′-CGATGACGGGCGCGAGAATCAGCGTGGACTTAGTCC-3′ and 5′-TAGCAGGGGCCAGTGGAGAGCATACGAC-3′). The sense oligonucleotides had complementary sequences to the second oligonucleotide listed for each channel and were used to control for general background on adjacent sections. Specificity of the observed signals was confirmed in three ways: 1) hybridization patterns were obtained with each pair of antisense oligonucleotides; 2) hybridization with a mixture of the same labeled and nonlabeled oligonucleotide in 100- to 500-fold excess did not result in detectable signal (Fig. S6); 3) a mixture of labeled oligonucleotide specific for a certain SK subunit and nonlabeled oligonucleotides for other SK subunits resulted in the same hybridization pattern as obtained with the specific antisense oligonucleotide alone. For cellular resolution, selected slides were dipped in photographic emulsion (Kodak NTB2) and developed after 12-20 wk. Brain structures were identified according to Paxinos and Watson (1986).

Whole cell recordings

Tight-seal whole cell recordings were obtained from neurons with cell bodies located in st. radiatum of the CA3b region (Lorente de Nó 1934) at room temperature (22-24°C). Large neurons with a triangular shape were excluded from the sample of interneurons, because of their presumed excitatory origin (Gulyás et al. 1998). Patch pipettes had resistances of 3-4 MΩ and were filled with the following pipette solution (in mM): 130 K-glucenate, 10 KCl, 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 0.4 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 1 MgCl2, 0.3 Na GTP, and 2 Na2ATP (pH 7.3). In current-clamp recordings, voltage responses were recorded 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. In voltage clamp, currents were recorded with an EPC-7 List amplifier, sampled every 0.2 ms and filtered at 2 kHz. Three action potentials are shown in the figures. Different parameters were used to characterize single action potentials: firing threshold, amplitude (measured from the threshold to the peak), and duration (measured at the half-amplitude), mAHF amplitude was measured from the peak to the baseline. The maximum steady-state firing rate was obtained by averaging the instantaneous frequency for the last five intervals of a train evoked by the largest current before onset of spike failure. Accommodation rate was quantified by measuring the instantaneous discharge frequencies between the first two spikes of a train of spikes after injection of 100 pA for 500 ms. Early and late accommodations were calculated according to (f(t) - f(final))/(f(final) - f(final)) and (f(t) - f(final))/(f(final)) respectively (Cauli et al. 1997). To quantify inward rectification, the membrane potential was measured at the peak and at a current of 500 μA of hyperpolarizing current injection that brought the cell to −100 mV, and the corresponding rectification ratio was calculated.

All potential values obtained in current-clamp mode were not

Data analysis

Input resistance (Rin) was estimated from the apparently linear portion of the steady-state voltage-current relationships obtained by measuring the amplitude of voltage responses (±15 mV) to hyperpolarizing current pulses. The slower membrane time constant (τm) was determined by fitting either single or double exponential functions to the average of five membrane responses (±10 mV) obtained by hyperpolarizing current injection (150 ms). At holding potential ranging from −52 to −54 mV (close to the resting potential) 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. Averages of three action potentials are shown in the figures. Different parameters were used to characterize single action potentials: firing threshold, amplitude (measured from the threshold to the peak), and duration (measured at the half-amplitude). mAHF amplitude was measured from the peak to the baseline. The maximum steady-state firing rate was obtained by averaging the instantaneous frequency for the last five intervals of a train evoked by the largest current before onset of spike failure. Accommodation rate was quantified by measuring the instantaneous discharge frequencies between the first two spikes (f1,50), and at the end of the stimulation (f_final) in a train of spikes after injection of 100 pA for 500 ms. Early and late accommodations were calculated according to (f_final - f_initial)/f_initial and (f_final - f_final)/(f_final), respectively (Cauli et al. 1997). To quantify inward rectification, the membrane potential was measured at the peak and at a current of 500 μA of hyperpolarizing current injection that brought the cell to −100 mV, and the corresponding rectification ratio was calculated.

All potential values obtained in current-clamp mode were not...
corrected for the measured liquid junction potential (10 mV), and therefore the values of membrane potential should be considered 10 mV more negative.

Potassium currents underlying the mAHP were studied in voltage clamp. To avoid problems in voltage control, they were studied as tail currents elicited from a holding potential of −50 mV after a depolarizing step (100 ms) of 60 mV (see, for example, Pedarzani and Storm 1993). The time-dependent decay of the tail currents was fitted between 80 and 20% of peak using single or bi-exponential functions. pCLAMP (Axon Instruments) software was used to perform voltage and current acquisition and analysis. Statistical parameters were assessed with Student’s t-tests applied to the raw data (P < 0.05).

Unless otherwise mentioned, all results are presented as means ± SD.

**Drugs**

3,3′-Diaminobenzidine (DAB), tetraethylammonium chloride (TEA), carbacol, and CsCl were purchased from Sigma (St. Louis, MO); apamin from Latoxan (Valence, France); and 6,7-dinitroquinoxaline-2,3-dione (DNQX) from Tocris (Bristol, UK) and tetrodotoxin (TTX) from Affinity Research Product (Exeter, UK). All other chemicals were from Merck. Drugs were applied via bath superfusion using a three-way tap system.

**RESULTS**

**Morphology**

Fifteen interneurons ([postnatal day 10 to 12 (P10–12)] and seven interneurons P17–21 with cell bodies localized in st. radiatum of the CA3b region were morphologically identified with biocytin injection and also investigated electrophysiologically. The boundary between st. radiatum and L-M was distinguished due to the darker appearance of st. L-M (Lacaille and Schwartzkroin 1988). Interneurons showed variable soma shapes (round, stellate, fusiform, or ovoid, see Fig. 1, A and B) of small size (approximately 10–12 μm in maximum diameter). Most of them were multipolar (Fig. 1B) with variable patterns of dendritic arborization, often restricted to st. radiatum. Due to the multipolar orientation, their axons were difficult to see, and they were often cut during slicing. Whenever identifiable, axons were confined to st. radiatum (n = 5) or directed toward the CA3 pyramidal layer (n = 2). Varicosities were often observed along the axons in st. radiatum. Dye coupling was observed in three slices (obtained from different rats at P10–12); in one case involving three cells (Fig. 1C); in the others, two cells. Dye-coupled interneurons showed various somata shapes and dendritic arborizations. We never observed dye coupling in older (P17–21) st. radiatum interneurons (n = 7).

**Membrane properties**

Current-clamp recordings were obtained from a total of 53 interneurons located in the CA3b region of st. radiatum. Despite the heterogeneous morphology of interneurons, membrane properties appeared to be similar and, for this reason, were pooled together. Little spontaneous synaptic activity and no spontaneous firing were present at P10–21. Basic membrane properties of interneurons were measured at resting membrane potential. They differed from those found in CA3 pyramidal cells and did not show significant changes with postnatal development between P10 and P21 (Table 1). A representative sample of an action potential and its afterpotentials elicited by a brief current pulse is shown in Fig. 2A.

**Afterpotentials**

Single action potentials were followed by a mAHP, which appeared as a direct continuation of the spike repolarization (Fig. 2A). Mean amplitude and duration of mAHP following a single spike were 6.5 ± 2.1 mV and 232 ± 69 ms, respectively (mean ± SD, n = 20). A slow AHP (sAHP, lasting more than 1 s) (Sah 1996) was never observed following single action potentials in st. radiatum interneurons at room temperature (n = 50). In only 8% of interneurons (4 of 50), a sAHP was identified after trains of action potentials.

**Repetitive firing and inward rectification**

Figure 2B shows representative samples of the firing discharge of a st. radiatum interneuron on injection of increasing depolarizing current pulses starting from the resting potential. The firing pattern was found to be regular for all suprathreshold stimulus intensities (n = 20). Injection of the same depolarizing current pulses when the membrane was hyperpolarized (−20 mV from rest) preserved the regularity of firing but slowed down the firing rate and delayed the onset of the first spike (n = 5, not shown). The maximum steady-state firing rate was 31 ± 4 Hz (n = 20), as reported for neocortical regularly
spiking interneurons (Erisir et al. 1999). The presence of spike frequency accommodation was investigated in 20 interneurons by injecting 500-ns-long depolarizing current pulses (40–120 pA). In Fig. 2C a plot of the spike sequence versus the reciprocal of the inter-spike interval (instantaneous frequency) for various injected currents shows that the number of spikes increased with the current strength, and that there was relatively weak accommodation. The average of early accommodation was 33 ± 9.8% and occurred mainly over the first few spikes. Late accommodation was minimal (9.4 ± 3.6%, n = 20). Figure 2D shows the voltage response elicited by depolarizing and hyperpolarizing pulses. Negative current injection reveals an inward rectification manifested as a large “sag” typically generated by the hyperpolarization-activated cation conductance (Ih) (Halliwell and Adams 1982). A depolarizing rebound that triggered action potentials followed the turning off of the hyperpolarizing pulse. These phenomena were typically observed in all st. radiatum interneurons investigated in the present report and were both abolished by CsCl (2 mM, data not shown). A lower rectification ratio was found in pyramids, in which the depolarizing rebound never triggered a spike.

Potassium conductances underlying mAHP

Several studies indicate that potassium conductances underlying the AHP play a fundamental role in shaping the discharge pattern of various neurons (for a review, see Sah 1996). In the present study the ionic conductances involved in generating the pattern of various neurons (for a review, see Sah 1996). In the present report the ionic conductances involved in generating the potassium tail currents under voltage-clamp mode following depolarizing steps of increasing voltage steps to threshold for activation of tail currents was 20 ms, 10-mV increments) starting from a holding potential of −50 mV. Representative tail currents recorded in TTX (1 μM) and TEA (0.5 mM) are shown in Fig. 3, A and Ca (control). Figure 3Ba shows the progressive activation of tail currents following 100-ms-long depolarizing steps of increasing amplitude (from −40 to +20 mV, 10-mV increments) starting from a holding potential of −50 mV. The apparent threshold for activation of tail currents was −30 mV (n = 4). Depolarizing voltage steps to +10 mV followed by a final voltage step varying between −75 and −45 mV evoked tail currents with a reversal potential around −75 mV. As the external K+ concentration was raised to 6.5 mM, the reversal potential shifted by approximately 10 mV (Fig. 3Bb).

A slow outward current (lasting more than 1 s), reminiscent of sAHP, appeared in 5 of 47 neurons (11%) and was completely and reversibly blocked by Ca2+-free solution (n = 2, not shown) or carbachol (40 μM, n = 4, Fig. 3Cb). We cannot exclude that sAHP might have been underestimated in this study due to the EGTA-containing pipette solution (see, for example, Madison and Nicoll 1984; but also Zhang et al. 1995) and the low temperature (Lancaster and Adams 1986; Sah 1996).

Apamin-sensitive I\(_{AHP}\)

To investigate whether a calcium-sensitive component takes part in generating the potassium tail currents elicited by short depolarizing pulses, we applied Ca\(_{2+}\)-free medium containing 1 mM EGTA. After 5 min superfusion of Ca\(_{2+}\)-free solution, a reversible decrease (58 ± 6%) in the peak amplitude of the tail potassium current was observed (n = 5, Fig. 3Aa). Superfusion of Cd\(^{2+}\) (200 μM) + Ni\(^{2+}\) (50 μM) similarly reduced the tail currents (49 ± 6%, n = 6; not shown).

Apamin is known to block specifically Ca\(_{2+}\)-activated potassium channels of the SK type in different brain regions (Sah 1996). Sensitivity to apamin was tested by recording tail currents in the absence and in the presence of apamin. The proportional contribution of apamin-sensitive tail currents varied appreciably from cell to cell. On average, the effect of apamin was dose dependent, with small reductions in the peak of tail currents observed already at subnanomolar concentrations (see the example in Fig. 4A at 500 μM), and stronger reductions observed at the saturating concentration of 100 nM (Fig. 4Ba). In control conditions, tail currents had a peak amplitude of 206 ± 84 pA and could be fitted by two exponentials with decay time constants (τ) of 15 ± 1 ms and 56 ± 16 ms, respectively (n = 15). As shown in Fig. 4Ba, in the presence of apamin (100 nM) the peak amplitude of the tail currents was depressed by 38.5 ± 6% (range in different cells: from 17 to 79%, n = 15). After digital current subtraction (Fig. 4Bb), the apamin-sensitive tail currents were shown to have a monoexponential decay (τ = 50 ± 20 ms, n = 15), corresponding to the slower component of the tail current deactivation. By delivering voltage steps of fixed amplitude (±10 mV) and increasing duration (from 3 to 100 ms) in the absence and presence of apamin (100 nM), interestingly we observed that even voltage steps as short as 3 ms were able to generate a substantial fraction of apamin-sensitive current (data not shown).

Expression of SK channel subunit transcripts in st. radiatum interneurons

Three members of the SK family of Ca\(_{2+}\)-activated K+ channels have been so far cloned and characterized. They give origin to channels with different sensitivity to apamin, the most sensitive being SK2 homomultimers (IC\(_{50}\) ~ 63 pM) (Ishii et al. 1997; Köhler et al. 1996), while SK3 homomultimeric channels present an intermediate sensitivity (IC\(_{50}\) ~ 2 nM: Ishii et al. 1997), and SK1 channels a low sensitivity (IC\(_{50}\) > 100 nM: Ishii et al. 1997; Köhler et al. 1996; IC\(_{50}\) ~ 3.3–12 nM: Shah and Haylett 2000; Strobaek et al. 2000).
To identify molecularly SK channels in CA3 st. radiatum interneurons, we studied the expression of SK subunit mRNAs by in situ hybridization with oligonucleotide probes. Analysis at cellular resolution revealed the presence of only one SK transcript, namely SK2, at high levels in scattered CA3 st. radiatum interneurons (Fig. 5B), whereas SK1 and SK3 mRNAs were below the threshold limit of detection (Fig. 5, A and C). In the adjacent CA3 pyramidal layer, SK subunits presented a different expression pattern, with SK2 being the most abundant transcript (Fig. 5B) but SK1 and SK3 mRNAs displaying also moderate to high expression levels (Fig. 5, A and C) (see also Stocker et al. 1999). The high expression level of SK2 transcript is additionally illustrated in the high power photomicrograph showing clusters of silver grains on single CA3 interneurons (Fig. 5D). These results are in agreement with the presence of an apamin-sensitive Ca\(^{2+}\)-dependent K\(^+\) current in CA3 st. radiatum interneurons. Based on the exclusive high expression of SK2 mRNA, our results suggest that homomultimeric SK2 channels might mediate the apamin-sensitive I\(_{\text{AHP}}\) in these neurons.

**Ca\(^{2+}\)-dependent apamin-sensitive mAHP**

The functional role of the apamin-sensitive Ca\(^{2+}\)-dependent potassium current was studied in current-clamp mode in Ca\(^{2+}\)-free solutions as well as in the presence of apamin. A change in action potential waveform was observed after 5-min superfusion with Ca\(^{2+}\)-free solution, and recovered when standard external solution was reapplied (n = 5). The action potential firing threshold decreased (from \(-38 \pm 2.2\) mV to \(-40 \pm 2.5\) mV), and a broadening of the action potential at the late stage of repolarization was observed (Fig. 6Aa). Action potential amplitude and repolarizing rate decreased in Ca\(^{2+}\)-free solutions by 5.3 ± 3.6% and by 25 ± 5.0%, respectively. Action potential duration was prolonged by 30 ± 10%. In three of five cells the mAHP was completely blocked; in the remaining two
FIG. 3. Tail currents are Ca\textsuperscript{2+} dependent, and sI\textsubscript{AHP} is present in a small number of cells. A: superimposed tail currents following depolarizing voltage step (100 ms to +10 mV) recorded from a holding potential of −50 mV before and after superfusion of Ca\textsuperscript{2+}-free solution. A partial recovery is obtained. B: average current-voltage (I-V) relation (n = 4) of tail currents. Tail currents were normalized with respect to that obtained by stepping the voltage from −50 to +20 mV. Bars are SE. Bb: plot of the average of tail currents evoked in 5 cells either in 3 or 6.5 mM K\textsubscript{1} medium by applying 100-ms voltage steps to +10 mV and varying the subsequent voltage step from −75 to −45 mV. Tail current peak amplitudes were measured in respect to the steady-state value reached on decay to baseline and were normalized with respect to the current obtained by stepping the final voltage step from +10 to −45 mV in 3 mM K\textsuperscript{+} medium. Bars are SE. Ca: superimposed tail currents recorded in the absence and presence of carbachol (40 μM). Cb: same traces as in Ca, but expanded on the amplitude axis: note the presence of a slow outward current completely abolished after superfusion of carbachol.
cells it was reduced by 62%. Reduction or block of mAHP always increased the firing rate (34 ± 14% change, n = 5; see Fig. 6Ab).

Apamin (100 nM) specifically and irreversibly reduced the mAHP amplitude (40 ± 16%) and duration (42 ± 9.2%), without affecting the action potential (Fig. 6Ba, n = 6). In four of six cells the effect on the mAHP was sufficient to significantly increase the rate of action potential firing (17 ± 10% change, Fig. 6Bb), while in the remaining two no significant change in spike frequency was observed.

**Carbachol-sensitive potassium conductances**

The hippocampus is innervated by cholinergic fibers (Freund and Buzsáki 1996; Miettinen and Freund 1992), and the acetylcholine receptor agonist carbachol is known to affect the mAHP of CA1 pyramidal cells by blocking the potassium current $I_{M}$ (Storm 1989). In CA3 st. radiatum interneurons the proportional contribute of carbachol-sensitive tail currents in the presence of TTX (1 mM) and TEA (0.5 mM) varied from 14 to 24% of the total tail current (18 ± 4%, n = 6; data not shown). Apamin-insensitive potassium tail currents, recorded in the presence of apamin (100 nM), were further reduced by carbachol (40 μM). A representative example is shown in Fig. 7A. Carbachol reduced the peak amplitude of apamin-resistant tail currents by 25.8 ± 21% (from 128 ± 23 to 92 ± 33 pA; n = 5; Fig. 7B). The effect was suppressed by atropine (1 μM), indicating that the response was mediated by muscarinic receptors (Fig. 7B). In three of five cells the suppression...
of mAHP induced an increase in firing rate (16 ± 4% change; 
\( n = 3, P = 0.036 \)), an effect attenuated by atropine (Fig. 7C).

**DISCUSSION**

To date, there is very little detailed characterization of the morphological and functional properties of the hippocampal interneurons located in st. radiatum. Moreover, very few studies have been performed on nonpyramidal neurons in the CA3 hippocampal subfield (Arancio et al. 1994; Gulyás et al. 1993; McBain and Dingledine 1993; Miles et al. 1996; Poncer et al. 1995). In the present study, we used intracellular staining, in situ hybridization, and whole cell recordings to provide a first detailed description of the intrinsic membrane properties and firing behavior of CA3 st. radiatum interneurons and outlined the role and the molecular nature of potassium conductances underlying the mAHP and regulating their discharge pattern.

**Morphological features of CA3 st. radiatum interneurons**

As observed by others (Gulyás et al. 1993; McMahon and Kauer 1997; Woodson et al. 1989), CA3 interneurons represent a heterogeneous population of cells with variable patterns of dendritic arborization, mostly confined to st. radiatum. As for their functional connectivity, CA3 st. radiatum interneurons are supposed to be contacted by afferents to pyramidal cell apical dendrites such as the commissural-associational fibers, which might provide a feed-forward drive (Gulyás et al. 1993), while input from recurrent collaterals of pyramidal cells to st. radiatum interneurons would mediate feedback inhibition (Freund and Buzsáki 1996).

In our study, in 3 of 15 slices from neonatal rats, labeled interneurons appeared to be dye-coupled, suggesting the presence of gap junctions. Gap junctions (Fukuda and Kosaka 2000; Katsumaru et al. 1988; Kosaka and Hama 1985) and dye-coupling (Michelson and Wong 1994; Strata et al. 1997) have been previously described in hippocampal interneurons.

**Physiological properties of st. radiatum interneurons**

Despite their heterogeneous morphological features, CA3 st. radiatum interneurons studied in the postnatal period P10–21 exhibited similar basic membrane properties, compatible with a complete postnatal development at P10. This is in line with the fact that interneurons appear early in the hippocampal formation, many of them before the principal cells (Altman and Das 1965; Amaral and Kurz 1985; Bayer 1980), in which developmental changes, mainly related to cell excitability and action potential properties (Costa et al. 1991; Spigelman et al. 1992), still occur up to P20.

Membrane properties of st. radiatum interneurons were found unambiguously different from those of pyramidal cells. In particular, a higher input resistance and shorter membrane time constant characterized these cells, in accordance with the data obtained from other hippocampal regions (Lacaille et al.
mediated by the hyperpolarization-activated current $I_h$ (Lacaille and Williams 1988; Morin et al. 1996). Higher input resistances and shorter membrane time constant should be responsible for the generation of larger and faster synaptic potentials. Indeed, excitatory postsynaptic potentials (EPSPs) recorded from guinea pig st. radiatum interneurons were larger and faster than EPSPs of CA3 pyramidal cells (Miles 1990). Time constant and input resistance values found in the present study are higher than those reported for other interneurons (Morin et al. 1996; Williams et al. 1994), most likely due to the different recording conditions (patch-clamp vs. intracellular recording), temperature, and animal age. Anyway, it was reported that interneurons located in CA3 area have larger time constant and input resistance values than those reported in other hippocampal regions (Chitwood et al. 1999).

Spontaneous firing was found in st. oriens neurons (Maccalferri and McBain 1996; McBain 1994) but was absent in st. L-M interneurons (Lacaille and Schwartzkroin 1988) and axo-axonic cells (Buhl et al. 1994), as well as in the interneurons characterized in the present study.

In CA3 st. radiatum interneurons the maximum firing rate at the steady state was 31 Hz, comparable with that observed in neocortical “regular firing” nonpyramidal neurons (Erisir et al. 1999). The firing rate slightly decelerated over the first few spikes only. Little accommodation has also been found in CA1 st. L-M interneurons, which showed a voltage-dependent mode of firing (Lacaille and Schwartzkroin 1988), never observed in st. radiatum interneurons.

Inward rectification with anodal brake excitation was observed in CA3 st. radiatum interneurons and was abolished by external Cs$.^+$ In this respect, these cells resemble more closely nonpyramidal cells in st. oriens, in which both effects are mediated by the hyperpolarization-activated current $I_h$ (Lacaille and Williams 1990; Maccalferri and McBain 1996).

Potassium conductances underlying the mAHP

Several types of K$^+$ currents underlie the AHP in nerve cells. A Ca$^{2+}$- and voltage-dependent, TEA-sensitive potassium current ($I_{AHP}$) (Adams et al. 1982) contributes to action potential repolarization and the fast and medium AHP (Storm 1987, 1989). At least two other potassium conductances contribute to the mAHP: $I_{M}$, due to the activation of small-conductance, Ca$^{2+}$-activated, and voltage-insensitive SK channels of the apamin-sensitive type (Aoki and Baraban 2000; Sah 1996; Stocker et al. 1999); and $I_{K}$, a voltage-gated K$^+$ current suppressed by muscarinic agonists (Halliwell and Adams 1982; Storm 1989). The slow AHP is instead mediated by $I_{AHP}$- (Lancaster and Adams 1986; Sah 1996), an apamin-insensitive Ca$^{2+}$-activated K$^+$ current lasting more than 1 s and sensitive to modulation by many neurotransmitters.

As observed in many nonpyramidal cells (Aoki and Baraban 2000; for a review see Freund and Buzsáki 1996), CA3 st. radiatum interneurons fired action potentials followed by a medium-duration (~200 ms) mAHP, which may account for the slight accommodation observed in response to sustained current injection. Tail potassium currents underlying the mAHP were characterized in CA3 st. radiatum interneurons and did not show substantial differences in kinetics from those observed in pyramidal cells (Stocker et al. 1999), st. L-M interneurons (Aoki and Baraban 2000), as well as in hypoglossal motoneurons (Lape and Nistri 2000). As observed in other cells (see, for example, Hoshi and Aldrich 1988; Johansson et al. 1996), the reversal potential ($E_{rev}$) for the tail currents slightly deviated from the predicted value for K$^+$. Anyway, a positive shift in $E_{rev}$ was observed by raising the external K$^+$ concentration. This suggested that the tail currents were mainly mediated by an increased membrane permeability to K$^+$. The
systematic difference between the measured $E_{K}$ and the predicted $E_{K}$ could be due to incomplete equilibration of the pipette $K^+$ with all cytosolic compartments, accumulation of $K^+$ near the membrane, or interference by inward currents of other origin.

**Apamin-sensitive potassium conductances**

Apamin-sensitive potassium currents have been detected in the hippocampus in st. oriens (Zhang and McBain 1995) and in st. L-M (Aoki and Baraban 2000) interneurons as well as in CA1 pyramidal cells (Stocker et al. 1999).

Our results reveal that the mAHP of CA3 st. radiatum interneurons, as that observed in st. L-M interneurons of CA1 region, is mostly due to the activation of apamin-sensitive $Ca^{2+}$-activated $K^+$ channels, and that its reduction increases their firing discharge. The observed variability in the effect of apamin (range in different cells: from 17 to 79% reduction of $I_{AHP}$, $n = 15$) might suggest a certain variability in the level of expression of apamin-sensitive channels in subsets of CA3 str. radiatum interneurons. Nevertheless, the presence in most cells of a significant apamin-sensitive current component is supported by a high expression of mRNA coding for the highly apamin-sensitive SK2 subunit, as detected by in situ hybridization in CA3 str. radiatum nonpyramidal cells. As a note of caution, from the in situ experiments we cannot exclude that some of the labeled cells in the CA3 st. radiatum might correspond to cell types that were not characterized electrophysiologically in this study. The apamin-sensitive $I_{AHP}$ does not contribute to action potential repolarization (Lape and Nistri 2000; Sah and McLachlan 1992; Stocker et al. 1999) but is activated during the mAHP following single action potentials. The apamin-sensitive tail current was characterized by a monoexponential deactivation with a time constant ($\sim 50$ ms) in the same range as found in CA1 st. L-M hippocampal interneurons ($\sim 70$ ms) (Aoki and Baraban 2000).

**Carbachol-sensitive potassium conductances**

Cholinergic innervation from the medial septum (Freund and Buzsáki 1996; Miettinen and Freund 1992) synaptically contacts hippocampal pyramidal cells and inhibitory neurons that contribute to theta rhythm generation by rhythmically inhibiting pyramidal cells (Tóth et al. 1997). Agonists of muscarinic receptors and stimulation of cholinergic afferents were found to increase the rate of occurrence of spontaneous inhibitory postsynaptic potentials (IPSPs) recorded in hippocampal pyramidal cells (Ptíler and Alger 1992), suggesting their positive modulation of interneuron excitability. In all hippocampal regions, muscarine mostly excited interneurons (McQuiston and Madison 1999a; Parra et al. 1998), for example, by suppressing the AHP and enhancing the ADP (McQuiston and Madison 1999b).

In CA1 st. oriens (Zhang and McBain 1995), muscarinic agonists reduced the mAHP amplitude, increasing the firing discharge rate. It is interesting to note that, in st. oriens interneurons, carbachol did not alter the AHP in the presence of $Ca^{2+}$-free solution (Zhang and McBain 1995). Our data reveal that CA3 st. radiatum interneurons possess functionally active muscarinic receptors that, on activation, induce a reduction of mAHP with a consequent increase in firing discharge. Apamin-insensitive tail potassium currents were reduced by carbachol, revealing that more than one type of potassium current contributes to the mAHP generation. A possible candidate to mediate the apamin-insensitive, muscarine-sensitive mAHP component is $I_{M}$, which has been shown to contribute to the mAHP for example in CA1 pyramidal neurons (Storm 1989).

In CA1 st. L-M interneurons, outward tail currents are dominated by a $Ca^{2+}$-dependent current, blocked by apamin and TEA, largely insensitive to carbachol (Aoki and Baraban 2000). Different expression of potassium channels with distinct pharmacological sensitivity seems therefore to characterize the interneurons in various hippocampal fields, contributing to determine different firing pattern and conferring them subtype-specific properties.

In conclusion, our data show that st. radiatum interneurons of the CA3 hippocampal region represent a morphologically heterogeneous class of cells with similar membrane properties. High-input resistance, short lasting action potentials, and AHPs of medium duration discriminate them from pyramidal cells. Sustained firing allows them to be classified as “regular firing” cells, in which the apamin- and carbachol-sensitive potassium conductances underlying the mAHP are mainly shaping the firing pattern. Neurotransmitters and neuromodulators affecting mAHP can therefore differentially regulate the firing properties of these interneurons, mediating a control of hippocampal circuits.

The authors are grateful to Prof. E. Cherubini for useful suggestions and comments on the manuscript and to Dr. M. Stocker for the in situ hybridization work.

This work was supported by grants from Istituto Nazionale Fisica della Materia and Ministero dell’ Università e della Ricerca Scientifica e Tecnologica (co-finanziamento ricerca) to M. Sciancalepore, and from Deutsche Forschungsgemeinschaft (SFB-406, Project C8) and Human Frontier Science Program Organization to P. Pedarzani.

**REFERENCES**


Potential-dependent block of $\mathrm{K}\text{Ca}^{2+}$ channels.


Pedrazzini P and Storm JF. PKA mediates the effects of monoamine transmitters on the K’ current underlying the slow spike frequency adaptation in hippocampal neurons. Neuron 11: 1023–1035, 1993.


