Properties of a Calcium-Activated K+ Current on Interneurons in the Developing Rat Hippocampus

TAKUYA AOKI AND SCOTT C. BARABAN
Departments of Pediatrics and Neuroscience, Case Western Reserve University, Cleveland, Ohio 44106

Aoki, Takuya and Scott C. Baraban. Properties of a calcium-activated K+ current on interneurons in the developing rat hippocampus. J Neurophysiol 83: 3453–3461, 2000. Calcium-activated potassium currents have an essential role in regulating excitability in a variety of neurons. Although it is well established that mature CA1 pyramidal neurons possess a Ca2+-activated K+ conductance ($I_{K(Ca)}$) with early and late components, modulation by various endogenous neurotransmitters, and sensitivity to K+ channel toxins, the properties of $I_{K(Ca)}$ on hippocampal interneurons (or immature CA1 pyramidal neurons) are relatively unknown. To address this problem, whole-cell voltage-clamp recordings were made from visually identified interneurons in stratum lacunosum-moleculare (L-M) and CA1 pyramidal cells in hippocampal slices from immature rats (P3–P25). A biphasic calcium-activated K+ tail current was elicited following a brief depolarization from the holding potential (−50 mV). Analysis of the kinetic properties of $I_{K(Ca)}$ suggests that an early current component differs between these two cell types. An early $I_{K(Ca)}$ with a large peak current amplitude (200.8 ± 13.2 pA, mean ± SE), slow time constant of decay (70.9 ± 3.3 ms), and relatively rapid time to peak (within 15 ms) was observed on L-M interneurons ($n = 88$), whereas an early $I_{K(Ca)}$ with a small peak current amplitude (112.5 ± 7.3 pA), a fast time constant of decay (39.4 ± 1.6 ms), and a slower time-to-peak (within 26 ms) was observed on CA1 pyramidal neurons ($n = 85$). Removal of extracellular calcium or addition of inorganic Ca2+ channel blockers (cadmium, nickel, or cobalt) was used to demonstrate the calcium dependence of these currents. Addition of norepinephrine, carbachol, and a variety of channel toxins (apamin, iberiotoxin, verruculogen, paxilline, penitrem A, and charybdotoxin) were used to further distinguish between $I_{K(Ca)}$ on these two hippocampal cell types. Verruculogen (100 nM), carbachol (100 µM), apamin (100 nM), TEA (1 mM), and iberiotoxin (50 mM) significantly reduced early $I_{K(Ca)}$ on CA1 pyramidal neurons; early $I_{K(Ca)}$ on L-M interneurons was inhibited by apamin and TEA. Combined with previous work showing that the firing properties of hippocampal interneurons and pyramidal cells differ, our kinetic and pharmacological data provide strong support for the hypothesis that different types of Ca2+-activated K+ current are present on these two cell types.

INTRODUCTION

Neuronal calcium-activated potassium current, $I_{K(Ca)}$, has been extensively characterized in both primary cultures and the in vitro slice preparation (Meis and Pape 1997; Morita et al. 1982; Sacchi et al. 1995; Schwindt et al. 1992). In hippocampal pyramidal cells, action potential firing frequency, the rate of burst occurrence (Chamberlin and Dingledine 1988; Madison and Nicoll 1984), termination of epileptiform discharges (Alger and Nicoll 1980), spike afterhyperpolarization (Storm 1987), and afterhyperpolarizations following a spike train (Lancaster and Adams 1986) are regulated by a complex sequence of Ca2+-activated K+ conductances. Three calcium-activated potassium current components have been described for CA1 pyramidal neurons: a fast $I_{K(Ca)}$ that activates rapidly (<5 ms) after calcium influx, a medium $I_{K(Ca)}$ lasting 20–100 ms, and a slow $I_{K(Ca)}$ that activates with a time constant >100 ms and decays over several seconds (Lancaster and Adams 1986; Lancaster and Nicoll 1987; Storm 1989; Velumian and Carlen 1999). In the adult hippocampus, a great deal of information is already available concerning these Ca2+-dependent K+ currents e.g., second-messenger pathways, receptor systems, biophysical properties, and pharmacological sensitivities (Lancaster and Nicoll 1987; Madison and Nicoll 1982, 1986a, 1987; Sah 1996; Storm 1990). For example, charybdotoxin or high concentrations of tetraethylammonium chloride (TEA) reduce fast $I_{K(Ca)}$ (Lancaster and Adams 1986; Lancaster and Nicoll 1987), whereas slow $I_{K(Ca)}$ is abolished by neurotransmitters (e.g., acetylcholine, norepinephrine, dopamine, and histamine) (Sah 1996; Sah and Isaacson 1995) or iberiotoxin (Blatz and Magleby 1987). At the same time, the properties of $I_{K(Ca)}$ on developing CA1 neurons have received relatively little attention (Costa et al. 1991) and virtually nothing is known about this current on hippocampal interneurons.

Interneurons of stratum lacunosum-moleculare (L-M) of the CA1 subfield are predominantly GABAergic and exert extensive inhibitory control of pyramidal cells (Freund and Buzsaki 1996). It is well established that the intrinsic firing properties of these inhibitory hippocampal neurons are markedly different from responses of excitatory pyramidal cells. For example, interneurons have short-duration action potentials with prominent spike afterhyperpolarizations (Kunkel et al. 1988; Lacaille and Schwartzkroin 1988). Although the majority of these cells are silent at resting membrane potential, depolarizing current injection reveals a firing pattern characterized by fast-spiking and little spike frequency adaptation (Williams et al. 1994). In contrast, CA1 pyramidal neurons are regularly spiking cells exhibiting a significant degree of spike frequency adaptation (Madison and Nicoll 1982). In voltage-clamp studies, it was determined that the macroscopic properties of voltage-gated K+ channels differ between pyramidal cells and interneurons. These differences between pyramidal cells and interneurons are associated with differential expression of Kv-type potassium channel subunits (Martina et al. 1998; Massengill et al. 1997), distinct pharmacological sensitivities (Zhang and McBain 1995a,b), and unique physiological properties for the voltage-gated K+ currents (Chikwendu...
and McBain 1996). Although evidence for a calcium-activated K⁺ current on interneurons has been suggested by current-clamp analysis (Lacaille and Schwartzkroin 1988; Williams et al. 1994; Zhang and McBain 1995a), direct characterization of this current using voltage-clamp techniques has not been performed.

In recent years, new electrophysiological techniques have been developed that facilitate the study of identified neurons in an acute slice preparation (Stuart et al. 1993). We utilized these infrared video microscopy techniques (in combination with voltage-clamp recordings) to examine the properties of Iᵣ(Ca) on interneurons and pyramidal cells in the developing hippocampus. In addition, novel K-Ca channel toxins have recently become available (e.g., verruculogen, penitrem A, and paxilline) (Knaus et al. 1994; Sanchez and McManus 1996), and we used these toxins to further characterize Iᵣ(Ca). Our results indicate that interneurons possess a class of calcium-activated K⁺ current distinct from that found on hippocampal pyramidal neurons. This current differs in both its kinetic properties and pharmacological profile and may contribute to the differences in firing patterns observed between these hippocampal cell types.

**METHODS**

**Hippocampal slice preparation**

Transverse hippocampal slices (300 μm) were prepared from 3- to 25-day-old Sprague-Dawley rats. Briefly, rats were anesthetized and decapitated, and the brain was rapidly removed in ice-cold, oxygenated (95%O₂-5%CO₂) sucrose artificial cerebrospinal fluid (sACSF) consisting of (in mM) 220 sucrose, 3 KCl, 1.25 NaH₂PO₄, 1.2 MgSO₄, 26 NaHCO₃, 2 CaCl₂, and 10 dextrose (295–305 mOsm). The brain was then blocked and glued to the stage of a Vibroslicer (WPI) and hippocampal slices cut in 4°C oxygenated sACSF. The resulting slices were then transferred to a holding chamber where they were submerged in oxygenated normal ACSF (nACSF) consisting of (in mM) 124 NaCl, 3 KCl, 1.25 NaH₂PO₄, 1.2 MgSO₄, 26 NaHCO₃, 2 CaCl₂, and 10 dextrose (295–305 mOsm). Slices were held at 37°C for 45 min and then at room temperature for 6–7 h; experiments were performed within 8 h of slice preparation. For each experiment, an individual slice was transferred to a submersion-type recording chamber (Warner Instrument), where it was continuously perfused with oxygenated nACSF at a rate of ~2.5 ml/min at (33.2 ± 0.1°C, except as noted).

**Whole cell recording**

Tight-seal (4–16 GΩ) whole cell voltage-clamp recordings were made with an Axopatch 1-D amplifier (Axon Instruments) with appropriate series and capacitance compensation (Hamill et al. 1981). Patch pipettes were pulled from 1.5-mm borosilicate filament-containing glass tubing (WPI) using a two-stage process, fire polished and coated with silicone elastomer (Sylgard, Dow-Corning). Patch pipettes were filled with internal recording solution consisting of (in mM) 135 K gluconate, 8 NaCl, 10 HEPES, 2 Na-ATP, and 0.2 Na-GTP (pH 7.4; 285–290 mOsm); solutions were filtered through a 2-μm filter (Millipore) prior to use. Extracellular recording solution consisted of nACSF supplemented with...
0.5–1 μM tetrodotoxin. The pipette was positioned under visual control using a Zeiss Axioskop (Carl Zeiss, Germany) equipped with a water-immersion (×40) objective, differential interference contrast optics, and an infrared-sensitive camera (C2400, Hamamatsu, Japan). Cells were voltage-clamped at –50 mV and depolarizing steps (25–400 ms) of sufficient amplitude (typically +50 to +60 mV) to elicit a robust, unclamped Ca\(^{2+}\) action current was applied (Fig. 1) (Pedarzani et al. 1998; Sah and Isaacson 1995; Zhang et al. 1995). Using this protocol, currents evoked during the command potential were not fully clamped owing to space-clamp limitations. However, the voltage control during the \(I_{K(Ca)}\) signal that develops after the end of the depolarizing step can be well maintained (Constanti and Sim 1987; Zhang et al. 1995). The magnitude (in pA) of \(I_{K(Ca)}\) was determined at the peak (Fig. 1A). Time to peak (in milliseconds) was measured as the difference between peak current and offset of the depolarizing voltage step (Fig. 1A). Because a large capacitive artifact often accompanies the voltage step offset, this measurement is regarded as an estimate of the actual time-to-peak current. The time-dependent decay (τc, in milliseconds) of calcium-activated K\(^+\) currents was fitted between 90 and 10% of peak using a single exponential equation and a Chebyshev fit (Fig. 1B); early and late current components were fitted by separate time constants. Significant current rundown was observed previously using a K\(^+\)-gluconate-based patch solution to record \(I_{K(Ca)}\) in the hippocampal slice preparation (Pedarzani et al. 1998; Zhang et al. 1994). As these studies were performed at room temperature, we initially tested the effects of temperature on \(I_{K(Ca)}\) (Fig. 1C). Outward tail currents did not rundown during the recording period and showed marked temperature dependence (n = 12; Fig. 1C). As such, all subsequent studies were performed at bath temperatures between 32 and 34°C. Voltage-clamp command potentials and post hoc current analysis was performed using pCLAMP software (Axon Instruments). Current records were low-pass filtered at 2 kHz (−3 dB, 8-pole Bessel), digitized at 4–10 kHz using a Digidata 1200 A/D interface, and stored on a Pentium II microcomputer (Dell Computer). Whole cell access resistance (6–23 MΩ) and holding current (0.00 to −0.2 pA) were continuously monitored, and cells were discarded if either value changed by more than 25% during the recording period (typically 20–45 min).

**Drugs**

Tetraethylammonium chloride (TEA), arnerol (norepinephrine), carbacol, cadmium chloride, cobalt chloride, manganese chloride, nickel chloride, and all salts were purchased from Sigma (St. Louis, MO). Tetrodotoxin, paxilline, penitrem A, verruculogen, apamin, charybdotoxin, and iberiotoxin were purchased from Alomone Labs (Israel). Toxins were dissolved in dH2O, stored as frozen aliquots and thawed just prior to use. All drugs were dissolved in nACSF and applied to the slice via rapid bath perfusion.

**Statistical analysis**

To avoid errors associated with oversampling (or inadequate drug washout), only one cell was recorded per slice and each cell was exposed to only one drug challenge. Results are presented as means ± SE. Data was analyzed using a Student’s t test on the SigmaStat program (Jandel Scientific). Significance level was taken as \(P < 0.01\) except as noted.

**RESULTS**

**Kinetic properties of calcium-activated potassium currents in the developing hippocampus**

Whole cell voltage-clamp recordings (n = 173) were obtained from visualized interneurons in st. lacunosum-moleculare (L-M) or pyramidal neurons in CA1 (Fig. 2, A and B) of the immature rat hippocampus (P10–P20). A protocol involving short depolarizing voltage steps (120-ms; Fig. 2A, inset) was used to elicit depolarization-activated (presumably, Ca\(^{2+}\)-activated) outward tail currents. Increasing the amplitude of the depolarizing command—thus increasing calcium influx (Jahromi et al. 1999; Lancaster and Adams 1986)—enhanced outward tail current amplitude (Fig. 2, A and B). The outward tail current on L-M interneurons (n = 88) could be described as having two distinct components: “early” and “late.” The early component had a relatively large peak current amplitude between peak current and offset of the depolarizing voltage step (Fig. 1 inset, bottom 50 mV (2); measurements were made following a...
A 120-ms depolarizing step to 0 mV, a slow decay $\tau_c$ (70.9 ± 3.3 ms), and an estimated time-to-peak of 14.2 ± 0.1 ms; the late current component exhibited a slow rising phase (70.9 ± 3.3 ms; $P < 0.001$ Student’s $t$ test), small peak amplitude (112.5 ± 7.3 pA; $P < 0.001$), and an estimated time-to-peak of 25.5 ± 1.2 ms ($P < 0.001$). In contrast, the late current component had kinetic properties that were not distinct from those measured for interneurons (Table 1).

At a holding potential of -50 mV, brief (25–400 ms) depolarizing voltage steps to 0 mV were used to further analyze and compare the early current component (Fig. 3A). On both L-M interneurons and CA1 pyramidal neurons, current amplitude increased with longer depolarizing pulses. At each step duration tested, peak amplitude for the tail current was greater for L-M interneurons in comparison with CA1 pyramidal cells (Fig. 3B). In contrast, the estimated time-to-peak was always later for CA1 pyramidal neurons in comparison with L-M interneurons (Fig. 3C). Because reliable measurements of the $\tau_c$ of decay could not be made during short depolarizing steps (<50 ms), these data were not included. Taken together, our results demonstrate that distinct types of Ca$^{2+}$-activated K$^+$ current are present on hippocampal interneurons and pyramidal cells.

We next used brief depolarization voltage steps (120-ms, 0 mV; 6 repetitions per cell; $t \geq 5$ min) to study the kinetic properties of $I_{K(Ca)}$ at various postnatal ages (P3–P25). A distinct early $I_{K(Ca)}$ was observed on pyramidal cells and interneurons at even the youngest ages studied (P3). The peak amplitude for this current significantly increased during development for both cell types (Fig. 4, A and B). Although the decay $\tau_c$ for early $I_{K(Ca)}$ on CA1 pyramidal cells was shorter in younger animals and increased slightly with age (Fig. 4C), no developmental change was observed for early $I_{K(Ca)}$ on L-M interneurons (Fig. 4D).

Calcium dependence of calcium-activated potassium currents in the developing hippocampus

To examine the calcium dependence of outward tail currents, we used a variety of manipulations designed to block Ca$^{2+}$ entry into the cell. First, slices were bathed in a nominally Ca$^{2+}$-free artificial cerebrospinal fluid solution (0 Ca$^{2+}$/2 mM EGTA). In whole-cell recordings from CA1 pyramidal neurons and L-M interneurons, peak current amplitude was significantly reduced during bath perfusion with 0 Ca$^{2+}$/2 mM EGTA solutions (Fig. 5, A and B). An example of the effect of 0 Ca$^{2+}$/2 mM EGTA on early $I_{K(Ca)}$ is illustrated in Fig. 5A1. Zero calcium solutions also abolished late $I_{K(Ca)}$ on pyramidal cells ($n = 3$) or interneurons ($n = 2$). Second, slices were bathed in nACSF in which Ca$^{2+}$ was replaced with saturating concentrations of inorganic calcium channel blockers (e.g., 200 µM cadmium, 2 mM cobalt, or 2 mM nickel). An example of the effect of 2 mM Co$^{2+}$/nACSF on early $I_{K(Ca)}$ is illustrated in Fig. 5B1. Inorganic Ca$^{2+}$ channel blockers, markedly reduced peak current amplitude on both CA1 pyramidal neurons and L-M interneurons (Fig. 5, A2 and B2). The late current component was completely abolished by

---

**TABLE 1. Biophysical properties of slow calcium-activated K$^+$ currents**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>$n$</th>
<th>Peak Current, pA</th>
<th>Time to Peak, ms</th>
<th>$\tau_c$ of Decay, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1 pyramidal</td>
<td>35</td>
<td>39.2 ± 4.3</td>
<td>419 ± 10.5</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>L-M interneuron</td>
<td>14</td>
<td>38.3 ± 6.3</td>
<td>455.3 ± 25.8</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. L-M, stratum lacunosum-moleculare.
these manipulations (data not shown). Third, slices were bathed in nACSF supplemented with the dihydropyridine L-type Ca\textsuperscript{2+}-channel blocker, nifedipine (Fox et al. 1987). Nifedipine (5 μM) reduced early \(I_{\text{K(Ca)}}\) on L-M interneurons but had little effect on early \(I_{\text{K(Ca)}}\) on CA1 pyramidal cells (compare Fig. 5, A2 and B2). These results suggest that L-type Ca\textsuperscript{2+} channels participate in the generation of interneuron, but not pyramidal cell, early \(I_{\text{K(Ca)}}\). Collectively, our data suggest that Ca\textsuperscript{2+} entry

---

**FIG. 4.** Age-dependent properties of early \(I_{\text{K(Ca)}}\). A: scatter plot of the peak current amplitude vs. postnatal age for all CA1 pyramidal neurons (\(n = 104\); ○). All measurements were made following a 120-ms depolarizing step to 0 mV (h.p., −50 mV; 6 repetitions per cell). A regression line was drawn using all data points. B: same for L-M interneurons (\(n = 112\); ●). Note the increase in peak current amplitude during development for both cell types. Scatter plot of the time constant of decay vs. postnatal age for all CA1 pyramidal neurons (C) or L-M interneurons (D).

---

**FIG. 5.** Calcium dependence of early \(I_{\text{K(Ca)}}\). A1: superimposed traces from a CA1 pyramidal neuron during perfusion with normal bathing medium (black trace, baseline) and ~4 min following perfusion with 0 Ca\textsuperscript{2+}/2 mM EGTA bathing medium (gray trace, 0 Ca\textsuperscript{2+}). Currents were evoked by a 120-ms depolarizing step to 0 mV from the holding potential (~50 mV; 6 repetitions per cell). A2: box plot illustrating population data for the percent of early \(I_{\text{K(Ca)}}\), blocked by bath perfusion with zero calcium (0 Ca; \(n = 4\)), 200 μM cadmium (Cd; \(n = 7\)), 2 mM cobalt (Co; \(n = 4\)), 2 mM nickel (Ni; \(n = 3\)), and 5 μM nifedipine (Nif; \(n = 6\)). In the box plot, the median value is illustrated as the vertical line within the box. Inner quartiles are shown as the edges of the box and the outer quartiles as lines extending from the box. B1: superimposed traces from an L-M interneuron during perfusion with normal bathing medium (black trace, baseline) and ~4 min following perfusion with 0 Ca\textsuperscript{2+}/2 mM cobalt bathing medium (gray trace, 2 mM cobalt). B2: box plot illustrating population data for the percent of early \(I_{\text{K(Ca)}}\), blocked by bath perfusion with zero calcium (\(n = 5\)), cadmium (\(n = 8\)), cobalt (\(n = 4\)), nickel (\(n = 3\)), and nifedipine (\(n = 6\)). The acquisition program has clipped depolarizing outward K\textsuperscript{+} currents and hyperpolarizing capacitive artifacts; the dashed line indicates baseline. Note the substantial reduction of depolarization-activated outward tail currents in both cell types.
Calcium-activated potassium currents of immature hippocampal neurons

RESPONSE TO NEUROTRANSMITTERS. The hippocampus receives a rich innervation by catecholaminergic and cholinergic fibers (Lewis et al. 1967; Loy et al. 1980), and it is well established that these systems modulate the firing activity of hippocampal neurons in adult hippocampus (Madison and Nicoll 1982, 1986; Madison et al. 1987). To further characterize \( I_{K(Ca)} \) in the immature hippocampus, we tested the effects of norepinephrine and carbachol on \( I_{K(Ca)} \). Early \( I_{K(Ca)} \) on CA1 pyramidal cells was markedly inhibited by bath application of 100 \( \mu \)M carbachol, whereas 10 \( \mu \)M norepinephrine had little effect on this current (Fig. 6A). An example of the effect of 100 \( \mu \)M carbachol on \( I_{K(Ca)} \) is illustrated in Fig. 6A. In contrast, early \( I_{K(Ca)} \) on L-M interneurons was not altered during bath perfusion with either norepinephrine or carbachol (Fig. 6A2). NE and carbachol abolished late \( I_{K(Ca)} \) observed on pyramidal cells (n = 5; Fig. 6A, B and C) or interneurons (n = 2) as expected (Lancaster and Nicoll 1987; Sah and Isaacscon 1995).

PHARMACOLOGY. To obtain a pharmacological profile for early \( I_{K(Ca)} \) on both L-M interneurons and CA1 pyramidal cells, we tested a combination of established and putative \( \text{Ca}^{2+} \)-activated \( K^+ \) channel blockers. Apamin and tetrachrylammonium chloride (TEA) have been used to block \( I_{K(Ca)} \) on mature hippocampal neurons (Lancaster and Nicoll 1987; Stocker et al. 1999; Williamson and Alger 1990). Bath application of 100 nM apamin produced a modest (20–30%) inhibition of early \( I_{K(Ca)} \) on immature CA1 pyramidal cells and a profound (more than 75%) inhibition of early \( I_{K(Ca)} \) on L-M interneurons (Fig. 7, A2 and B2). Bath application of a high concentration of TEA (1 mM) inhibited the early current component on both cell types (Fig. 7, A2 and B2). The effects of both drugs were reversible on washout (20–40 min). A significant inhibition of early \( I_{K(Ca)} \) on CA1 pyramidal cells was also observed during bath application of 100 nM verruculogen (Fig. 7A, I and 2). However, the same concentration of verruculogen did not significantly reduce early \( I_{K(Ca)} \) on L-M interneurons (Fig. 7B, I and 2). 50 nM ibiotoxin was similarly effective at reducing early \( I_{K(Ca)} \) on CA1 pyramidal cells but not early \( I_{K(Ca)} \) on L-M interneurons. Bath application of nACSF containing 100 nM paxilline, 100 nM penitrem A, or 100 nM charybdotoxin did not significantly inhibit \( I_{K(Ca)} \) (Fig. 7, A2 and B2). Taken together, these results indicate that potassium channel activity is responsible, at least in part, for generation of \( I_{K(Ca)} \).

DISCUSSION

Previous studies (Lancaster and Nicoll 1987; Sah 1996; Storm 1989, 1990) indicated that calcium-activated \( K^+ \) current on mature hippocampal CA1 pyramidal neurons is made up of at least two distinct components: early (e.g., “fast” and “medium”) and late (i.e., “slow”). Using whole-cell voltage-clamp analysis in immature tissue slices, we extend these findings and...
show (for the first time) that calcium-activated K⁺ current on hippocampal interneurons is also made up of at least two distinct components. The late, or slow, current component had similar kinetic and pharmacological properties for both cell types. In both L-M interneurons and CA1 pyramidal neurons, this current peaked between 400 and 500 ms, was abolished by application of inorganic Ca²⁺ channel blockers, and was inhibited by acetylcholine and norepinephrine. Sah and Clements (1999) reported a decay time constant of 1.5 ± 0.2 s for the slow calcium-activated K⁺ current on hippocampal CA1 pyramidal neurons—a value similar to that reported here (see Table 1). The slow kinetics of this current may be due to mobilization of Ca²⁺ from intracellular stores (Sah and McLachlan 1991), diffusion of Ca²⁺ to K-Ca channels from remote sites of entry (Lancaster and Zucker 1994; Zhang et al. 1995), or to delayed facilitation of L-type voltage-activated Ca²⁺ channels (Cloues et al. 1997). In contrast, the early current components displayed distinct neuron-specific kinetic and pharmacological properties.

Several lines of evidence suggest that the early component we recorded on immature CA1 pyramidal neurons is distinct from a fast, transient Ca²⁺-dependent K⁺ current previously identified on mature CA1 neurons (Lancaster and Adams 1986; Lancaster and Nicoll 1987). First, fast \( I_{K(Ca)} \) (or fast AHP) activates in less than 5 ms after calcium influx and decays with a time constant around 25 ms (Alger et al. 1990; Storm 1989; Williamson and Alger 1990). Second, \( I_{K(Ca)} \) has only been identified in adult hippocampus (Lancaster and Adams 1986; Lancaster and Nicoll 1987)—is a component of mature CA1 pyramidal neurons and was therefore not present in immature tissue examined in our studies.

The early current component we recorded on CA1 pyramidal neurons is probably analogous to previously identified medium \( I_{K(Ca)} \) (or medium AHP) on mature hippocampal CA1 pyramidal neurons (Alger and Nicoll 1980; Storm 1989; Williamson and Alger 1990). First, the kinetic properties of this current are in good agreement with those reported for mature CA1 pyramidal neurons, e.g., activates in ~25 ms following calcium influx and decays with a time constant around 39 ms (Alger et al. 1994; Storm 1989; Williamson and Alger 1990). Second, the pharmacological profile of neonatal early \( I_{K(Ca)} \) is similar to that reported for medium AHP on mature CA1 pyramidal neurons (Lancaster and Nicoll 1987; Williamson and Alger 1990). Both currents are significantly reduced by carbachol but largely insensitive to norepinephrine. Third, estimated time-to-peak for early \( I_{K(Ca)} \) current (range: 8–58 ms) closely parallels time-to-peak for the rise in free intracellular calcium (range: 2–50 ms) measured in CA1 pyramidal neurons using calcium fluorescence imaging (Sah and Clements 1999). Finally, an interesting characteristic of early \( I_{K(Ca)} \) on CA1 pyramidal neurons is that it is potently inhibited by verrruculogen—a novel indole alkaloid previously shown to inhibit K-Ca channels on myocytes (Knaus et al. 1994). Because the functional role for early \( I_{K(Ca)} \) appears to be termination of epileptiform burst discharges (Alger et al. 1980, 1994; Storm 1989; Williamson and Alger 1990), it is interesting to speculate that the “immature” properties of early \( I_{K(Ca)} \) in young animals contributes to the enhanced seizure susceptibility observed at these ages (Moshe et al. 1983).

Here we have also characterized two components of Ca²⁺-activated K⁺ currents on immature CA1 pyramidal neurons: early \( I_{K(Ca)} \) and late \( I_{K(Ca)} \).
activated K⁺ current present on st. lacunosum-moleculare interneurons of the immature hippocampus. Previous studies from the McBain laboratory have shown that st. oriens-aleveus interneurons (Zhang and McBain 1995a,b), parvalbumin-containing interneurons of st. pyramidalale (Du et al. 1996), and st. L-M interneurons (Chikwendu and McBain 1996) possess distinct repertoires of voltage-gated K⁺ currents. These currents are thought to play a role in defining the unique fast-spiking firing pattern characteristic of interneurons (Kunkel et al. 1988; Lacaille and Schwartzkroin 1988; Martina et al. 1998; Storm 1990). Our data demonstrate that st. lacunosum-moleculare interneurons also express an early calcium-dependent K⁺ current distinct from that observed on CA1 pyramidal cells. \( I_{K(Ca)} \) in L-M interneurons is dominated by a large, slowly decaying current with rapid onset. This early current component was blocked by apamin and TEA, but largely insensitive to carbachol, verrruculogen, and iberiotoxin (i.e., drugs which inhibit early \( I_{K(Ca)} \) on CA1 pyramidal cells). A functional role for this early current component has not yet been identified, though it is likely that the unique neuron-specific properties of this current contribute to defining the intrinsic firing properties of interneurons. Further, our data on the kinetic and pharmacological properties of \( I_{K(Ca)} \) should be of considerable use in establishing reliable models of interneuron physiology and lead to direct testing of the roles played by various K⁺ currents in shaping firing activities.

In conclusion, despite the heterogeneity of hippocampal interneurons, it is becoming clear that they possess distinct potassium channel subunits. For example, fast-spiking interneurons in the dentate gyrus express Kv3.1 and Kv3.2 mRNA for a voltage-activated K⁺ current that is highly sensitive to TEA and 4-aminopyridine (Martina et al. 1998) and interneurons in st. oriens-aleveus possess a K⁺ current with sensitivity to apamin and insensitivity to IbTX (Zhang and McBain 1995b). Our voltage-clamp data support the conclusion that distinct forms of ion channels are present on inhibitory interneurons and provide direct evidence that an early K⁺ current component on immature hippocampal interneurons is a Ca²⁺-dependent current with unique properties. A likely explanation for the differences between early \( I_{K(Ca)} \) on interneurons and pyramidal cells is that K-Ca channel subunit expression is neuron-specific. From molecular studies, we know that K-Ca channel subtypes are prominently expressed in the hippocampal formation. In particular, large-conductance BK (mslo), small-conductance SK (rsk2 and hsk1), and intermediate-conductance IK (Slack) channels are found in pyramidal and granule cell layers of hippocampus (Joiner et al. 1998; Knaus et al. 1996; Kohler et al. 1996). However, it is not clear at this time which K-Ca channel subunits are expressed by hippocampal interneurons. An alternative explanation for the differences between early \( I_{K(Ca)} \) on interneurons and pyramidal cells could be that mechanisms to buffer intracellular calcium or permit calcium entry into the cell via membrane-bound channels are different between these two cell types. Nonetheless we anticipate that interneurons, which represent an important population of inhibitory neurons in the hippocampus (Freund and Buzsaki 1996), express a unique group of K-Ca channel subunits and that our characterization of \( I_{K(Ca)} \) will aide in the eventual identification of these subunits as well as lead to a greater understanding of the functional role of Ca²⁺-activated K⁺ channels in the CNS.

The authors thank R. A. Nicoll and H. L. Fields for critical comments on an earlier version of this manuscript. We also thank P. Castro for expert technical assistance.

This project was sponsored by a Junior Investigator grant from the Epilepsy Foundation of America (S. C. Baraban).

Present address and address for reprint requests: S. C. Baraban, Dept. of Neurological Surgery, Box 0520, University of California, San Francisco, 513 Parnassus Ave., San Francisco, CA 94143.

Received 15 December 1999; accepted in final form 17 February 2000.

REFERENCES


ZHANG L AND McBAIN CJ. Photolysis of [Ca$^{2+}$]-activated $K^+$ channels in stratum oriens-ala vein inhibitory neurones of the rat CA1 hippocampus. J Physiol (Lond) 488: 667–672, 1995a.

ZHANG L AND McBAIN CJ. Potassium conductances underlying repolarization and after-hyperpolarization in rat CA1 hippocampal interneurons. J Physiol (Lond) 488: 661–672, 1995b.