A Slowly Inactivating Potassium Current in CA3 Pyramidal Cells of Rat Hippocampus *In Vitro*

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The time- and voltage-dependent properties of a slowly inactivating K^+ current were investigated by using the single-electrode current- and voltage-clamp recording technique in CA3 hippocampal cells of organotypic slice cultures. After a period of prolonged hyperpolarization, the onset of action-potential discharge in response to depolarizing current injection was delayed by several seconds. The conductances underlying this delay were identified in voltage-clamp recordings. A biphasically decaying outward current was evoked when the membrane potential was stepped back to -60 mV after a 30 sec period of hyperpolarization. The fast component was identified as the previously described D-current and was blocked by $100 \mu\text{M}$ 4-aminopyridine (4-AP). The slow component, which we refer to as $I_{K(\text{slow})}$, appeared to be mediated by K^+ ions, because its reversal potential shifted in a Nernstian manner with changes in extracellular K^+ concentration. It decayed with a time constant of 7.5 sec and required a hyperpolarizing prepulse below -95 mV for 5.5 sec for

50% recovery from inactivation. $I_{K(\text{slow})}$ was found to be voltage-dependent, with 50% activation occurring at -65 mV and 50% steady-state inactivation occurring at -84 mV. It displayed minimal or no sensitivity to the K^+ -channel blockers 4-AP (0.1 – 5 mM), Cs^+ (1 mM), tetraethylammonium (10 – 50 mM), Ba^{2+} (1 mM), dendrotoxin- α (5 – $10 \mu\text{M}$), charybdotoxin (0.5 – $2.5 \mu\text{M}$), or glibenclamide (5 – $10 \mu\text{M}$) and was not affected by preventing increases in intracellular Ca^{2+} concentration with Ca^{2+} chelators. $I_{K(\text{slow})}$ was reduced by activation of metabotropic glutamatergic and cholinergic receptors. In summary, the biophysical characteristics of $I_{K(\text{slow})}$ suggest a role in determining discharge onset after a period of membrane hyperpolarization, and its modulation by G-protein-coupled receptors reveals an additional function for these receptors in the control of cellular excitability.

Key words: latency to discharge; transient K^+ currents; D-current; CA3 cells; metabotropic glutamate receptors; muscarinic receptors

Voltage-dependent K^+ conductances are involved critically in the patterning of neuronal discharge in response to depolarizing stimuli. They contribute in determining (1) the onset of discharge, (2) the rate of action-potential repolarization, and (3) the adaptation of discharge frequency (Hille, 1992). Whereas spike repolarization and adaptation of repetitive firing have been well characterized in terms of the underlying K^+ conductances (Brown et al., 1990; Storm, 1993), open questions remain concerning the mechanisms that control the latency to onset of spike discharge.

Action-potential discharge episodes frequently are preceded by a slow, depolarizing ramp potential, the duration of which is a function of the degree of neuronal membrane hyperpolarization before the depolarizing input. In mammalian neurons at resting potential, the onset of action-potential discharge in response to a depolarizing input is rapid and occurs in the range of one to three membrane time constants. In contrast, cells excited after a period of hyperpolarization exhibit a delay to the first action potential lasting between 0.4 and 10 sec (Byrne et al., 1979; Getting, 1983; Dekin et al., 1987; Storm, 1988; McCormick, 1991; Nisenbaum et al., 1994).

In hippocampal pyramidal cells, the control of the latency to discharge onset has been ascribed mainly to two K^+ currents that

recover from inactivation during a hyperpolarizing pulse. The A-current, first observed in molluscan neurons (Thompson, 1977) and later in guinea pig CA3 hippocampal cells (Gustafsson et al., 1982), is a rapidly inactivating K^+ current (τ between 15 and 50 msec) with an activation threshold of -60 mV. The D-current, first studied in CA1 hippocampal cells (Storm, 1988), inactivates more slowly (τ of several hundred milliseconds), with an activation threshold of -75 mV. The K^+ -channel blocker 4-aminopyridine (4-AP) selectively blocks the A-current (1 – 5 mM) and D-current (30 – $100 \mu\text{M}$) and reduces the time-to-onset of discharge (Gustafsson et al., 1982; Storm, 1988).

In many cases, however, the A- and D-currents cannot account completely for the latency to the initiation of the first action potential. Moreover, the fact that slow transient outward currents recorded in hippocampal pyramidal cells can be fitted only adequately by multiexponential functions, including components with time constants of several seconds (Storm, 1986; Numann et al., 1987; Ficker and Heinemann, 1992), suggests the involvement of additional currents that determine the time point for the initiation of action-potential discharge.

In this study, we report on a previously undescribed current in CA3 pyramidal cells that contributes to the control of discharge onset and characterizes its biophysical and pharmacological properties.

A preliminary version of these data has been presented previously in abstract form (Lüthi et al., 1994).

MATERIALS AND METHODS

Hippocampal slice cultures. Slices $400 \mu\text{m}$ thick were obtained from 4- to 6-d-old rat pups and cultured via the roller-tube technique for 3–6 weeks as described previously (Gähwiler, 1981).

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Electrophysiological recordings. The single-electrode voltage-clamp method was used to record from CA3 pyramidal cells (Axoclamp-2 amplifier, Axon Instruments, Foster City, CA). For this purpose, cultures were transferred to a temperature-controlled recording chamber that was mounted on the stage of an inverted microscope (Axiovert 35M, Zeiss, Jena, Germany) and superfused with a saline solution (33°C) containing (in mM): 149.2 Na^+ , 2.7 K^+ , 147.2 Cl^- , 2.8 Ca^{2+} , 0.5 Mg^{2+} , 11.6 (HCO_3^-), 0.4 ($H_2PO_4^-$), and 5.6 D-glucose. For current-clamp recordings, the concentration of Mg^{2+} was raised to 5 mM. Voltage-clamp recordings were done in the presence of tetrodotoxin (TTX; 0.5 μM). To eliminate contributions from the D-current, 0.1 mM 4-AP was added to the bath. When Cd^{2+} was used, ($H_2PO_4^-$) was omitted from the saline solution. Solutions were adjusted to pH 7.4 by bubbling with 95% O_2 /5% CO_2 and monitored with Phenol-red (10 mg/l).

CA3 pyramidal cells were impaled with thin-walled electrodes that were filled with a solution containing 2 M KCl, 2 M CsCl, or 1 M KMeSO₄, resulting in a tip resistance of 30–50 M Ω for KCl and CsCl and 60–80 M Ω for KMeSO₄. Before recording, electrodes were tested for polarization artifacts by using negative current injections of 1 nA for 30 sec. The average polarization was 0.7 ± 0.2 mV ($n = 8$). During voltage-clamp recordings, the switching rate ranged between 1.5 and 2.0 kHz, which provided sufficient resolution to analyze slow K^+ currents ($\tau = 7.5$ sec), and headstage output was monitored continuously to ensure adequate settling time between samples. Input resistance was assessed by applying 0.5 sec hyperpolarizing voltage commands of 10 mV. Only cells for which input resistance was sufficiently high (>50 M Ω) were studied.

Drugs were focally, iontophoretically, or bath-applied. For iontophoresis, patch electrodes filled with a solution containing 1-amino-cyclopentyl-*trans*-1S,3R-dicarboxylate (1S,3R-ACPD; 50 mM, pH 9.0), muscarine (10 mM, pH 7.0), or AMPA (10 mM, pH 9.0) were placed close to the recorded cell. The retaining current ranged from 30 to 200 nA, the ejection current ranged from 50 to 300 nA, and the appropriate polarity was chosen with respect to the charge of the ejected substance. The application time varied between 2 and 10 sec. Dendrotoxin- α (DTX) and charybdotoxin (CTX) were applied focally by application of small drops of solution (1–10 μ l) close to the recorded cell. Dilution factors were calculated by comparing the current responses to focally and bath-applied ($-$)- β -p-chlorophenyl-GABA (baclofen).

In some experiments, electrode tips were filled with 25 mM BAPTA and electrode shanks were back-filled with 2 M KCl. Suppression of the Ca^{2+} -dependent afterhyperpolarization current after 50 msec depolarizing voltage commands was used as an indicator for the effectiveness of BAPTA diffusion into the cell.

Data recording and analysis. Voltage records were digitized at 33 Hz (Digidata 1200 as analog-to-digital converter, Axon Instruments, Foster City, CA), which was sufficient to provide an undistorted record of $I_{K(slow)}$ ($\tau = 7.5$ sec). For current-clamp experiments, the digitization rate was 2 kHz. Data were recorded using a chart recorder (Gould, Cleveland, OH) and stored on-line with a PC. Acquisition and analysis were performed using pClamp version 6.0.1. (Axon) and FigP (Biosoft, Cambridge, UK) software. Quantitative data are given as mean $\pm$ SEM. Statistical analysis was performed using Student's *t* test.

Drugs and chemicals. 4-AP, tetraethylammonium (TEA), muscarine, acetyl- β -methylcholine chloride (methacholine, MCh), and strophanthidin were purchased from Sigma (St. Louis, MO). 1S,3R-ACPD and AMPA were purchased from Tocris Neuramin (Bristol, UK). DTX and CTX were from Affiniti Research Products (Nottingham, UK). Glibenclamide was from Research Biochemicals (Natick, MA). BAPTA was from Molecular Probes (Eugene, OR), and TTX was from Sankyo (Tokyo, Japan). Baclofen was donated by Ciba-Geigy (Basel, Switzerland).

RESULTS

Delayed onset of firing after hyperpolarization

Mechanisms underlying the delay in onset of action-potential discharge in response to excitatory inputs were investigated in CA3 pyramidal cells in organotypic hippocampal slice cultures. On the basis of previous studies, transient outward currents, inactivation of which is removed by hyperpolarizing prepulses, are important in determining the delay to spike onset (Storm, 1993). Therefore, experiments were designed to characterize the excitability of cells as a function of the amplitude and the duration of a hyperpolarizing prepulse. First, the threshold current required

Figure 1. The delay in onset of action-potential generation is dependent on the duration of the preceding hyperpolarizing current injection. This cell had a resting potential of -67 mV, and the threshold potential for eliciting action potentials was -44.8 ± 0.4 mV ($n = 15$ current injections). Current-injection protocols are displayed below each voltage recording. *A*, Depolarizing current injection at resting potential to threshold caused rapid onset in action-potential generation. Firing onset was delayed when hyperpolarizing current prepulses were applied. *B*, Hyperpolarizing pulse of 0.5 sec to -100 mV. *C*, Pulse of 30 sec to -100 mV. The threshold for action-potential generation was not changed after a hyperpolarizing prepulse (-44.4 ± 0.2 vs -44.8 ± 0.4 mV, $p > 0.05$; $n = 12$).

to evoke an action potential from resting potential was determined. Next, cells were hyperpolarized by current injection to potentials between -100 and -120 mV for various durations. Cells were then repolarized by injecting a current pulse corresponding to the absolute sum of the threshold and the hyperpolarizing current.

With this protocol, responses that depended on the parameters of the hyperpolarizing prepulse applied were elicited in CA3 pyramidal neurons. In Figure 1*A*, data are presented from a cell with a passive membrane time constant of 42 msec, as determined by monoexponential fitting of membrane voltage responses to negative current injections of 100 pA (data not shown). When depolarized from the resting potential of -67 mV, the latency to the first spike, measured from the onset of depolarization, was 110 ± 20 msec ($n = 3$). The latency increased to 870 ± 90 msec after current prepulses of 500 msec duration that hyperpolarized cells to -100 mV ($n = 3$; Fig. 1*B*). Prolonging these hyperpolarizing prepulses to 30 sec delayed onset of discharge to 4.1 ± 0.6 sec ($n = 3$, $p < 0.05$; Fig. 1*C*). Increasing the amplitude of the 30 sec hyperpolarization to -120 mV prolonged the delay to 7.1 ± 1.1 sec ($n = 3$, $p < 0.05$; data not shown). When the depolarizing current pulses exceeded threshold values, the delay was strongly reduced (data not shown). Thus, the latency to discharge onset at threshold potentials was dependent both on the amplitude and on the duration of the preceding hyperpolarization. Because maximal delays attributable to A- and D-currents are expected to be <1 sec (see below), additional currents appear to be recruited after a

Figure 2. $I_{K(\text{slow})}$ can be differentiated from I_D by pharmacological and kinetic criteria. **A**, The cell was held at -60 mV and stepped to -120 mV for 500 msec every 20 sec. This procedure evoked a small outward current relaxation that was mostly blocked by 4-AP ($100 \mu\text{M}$). **B**: **a**, When the hyperpolarizing voltage step was prolonged to 30 sec, the outward current decayed in a biexponential manner. **b**, Only the faster component (I_D) was sensitive to 4-AP, whereas the slower component ($I_{K(\text{slow})}$) persisted. **c**, Superposition of the two current relaxations. **d**, Biexponential fitting of the trace in **a** yielded time constants of 663 msec and 11.4 sec, respectively. **e**, Monoexponential fitting of the trace in **b** yielded a time constant of 9.5 sec. **C**: **a**, This cell was clamped at -60 mV and stepped to -120 mV for 500 msec to evoke D-current relaxations. **b**, Twenty seconds after focal application of DTX, the D-current was blocked. **c**, Partial recovery was obtained after 10 min of washout. **d**, Small relaxations were blocked by application of 4-AP (arrow marks onset of bath application). **e**, In the same cell, a 30 sec hyperpolarizing step evoked $I_{K(\text{slow})}$. **f**, **g**, $I_{K(\text{slow})}$ was insensitive to DTX.

prolonged hyperpolarization. Subsequent experiments to identify such putative currents were performed by using the single-electrode voltage-clamp technique.

Characterization of hyperpolarization-activated currents

Voltage-clamp commands were applied that corresponded to the protocols used in the current-clamp recordings described above. Brief voltage commands to -100 or -120 mV for 500 msec evoked an outward current of 144 ± 48 pA ($n = 14$) that decayed with a time constant of 754 ± 65 msec ($n = 14$) and was blocked by 50 – $100 \mu\text{M}$ 4-AP (Fig. 2*A*). Following the nomenclature of Storm (1988), we refer to this as the “delay” current (I_D). In some cases, especially with depolarization to above -60 mV, a fast

current relaxation persisting in the presence of 50 – $100 \mu\text{M}$ 4-AP (Fig. 2*Bb*) but blocked by 5 mM 4-AP was observed (data not shown). This transient, inactivating with a time constant of 63 ± 9 msec ($n = 4$), likely represents the A-current (Gustafsson et al., 1982; Storm, 1988). Therefore, all current-amplitude measurements were made 120 msec after stepping back to the resting potential, when I_A has inactivated.

In the absence of 4-AP, prolongation of the hyperpolarizing voltage step to -120 mV by 5 – 30 sec revealed an additional, slow outward current component after depolarization, leading to a biphasic decay of the total current measured from 120 msec after the voltage step. The faster component decayed with a time constant consistent with inactivation of I_D , whereas the remaining component displayed markedly slower decay kinetics ($\tau = 11.4$ sec, Fig. 2*B*; averaged data in Fig. 7*B*). After application of 4-AP ($100 \mu\text{M}$), the faster component corresponding to I_D was abolished, leaving only the slower component. Because of its long duration and its selectivity for K⁺ ions (see next section), we termed this current $I_{K(\text{slow})}$.

Three criteria enabled us to differentiate this slow current from I_D . (1) $I_{K(\text{slow})}$ is insensitive to 4-AP at concentrations from $100 \mu\text{M}$ (Fig. 2*B*) to 5 mM ($p > 0.05$, $n = 3$; Fig. 8*A*). Hence, all subsequent experiments to characterize the slow current were performed in the continuous presence of $100 \mu\text{M}$ 4-AP to prevent contamination by I_D . (2) $I_{K(\text{slow})}$ is insensitive to DTX (5 – $10 \mu\text{M}$; $107.5 \pm 21.6\%$ of control, $p > 0.05$, $n = 3$). DTX blocks the A-current (Halliwell et al., 1986) and the D-current ($n = 3$; Fig. 2*C*) (Wu and Barish, 1992). (3) $I_{K(\text{slow})}$ exhibits 10-fold slower decay kinetics (Figs. 2*B*, 7*A*).

The dependence of $I_{K(\text{slow})}$ amplitude on both the duration and the magnitude of the hyperpolarizing test pulse could be fitted monoexponentially: the time constant was 9.0 sec for recovery from inactivation ($n = 3$; Fig. 3*A*). Similar time constants were obtained with smaller step sizes in the range between -110 and -70 mV. This is consistent with a monoexponential process underlying recovery from inactivation. The voltage for half-activation lay between -95 and -100 mV and was independent of the duration of the hyperpolarizing step ($n = 5$; Fig. 3*B*).

Ionic nature of the current

Transient outward currents identified in a variety of neuronal cell types are carried by K⁺ ions (Rudy, 1988; Storm, 1993) or Cl[−] ions (Madison et al., 1986). Because $I_{K(\text{slow})}$ was observed to have similar amplitude when recorded with electrodes filled with 1 M KMeSO₄ instead of 2 M KCl, to shift the reversal potential for Cl[−] ions in the hyperpolarizing direction (Streit et al., 1989), it was possible to rule out an involvement of a Cl[−] conductance. The reversal potential for $I_{K(\text{slow})}$, obtained by constructing instantaneous current–voltage relationships, was -93.7 ± 1.9 mV ($n = 3$; Fig. 4*A*), which is close to the experimentally determined reversal potential for K⁺ ions (see Fig. 5*A*). Furthermore, the reversal potential for the current shifted in a Nernstian manner to -69.6 ± 1.0 mV when the external K⁺ concentration was increased from 2.7 to 8.0 mM ($n = 4$; Fig. 4*B*). The ratio of K⁺ to Na⁺ permeability, calculated from the Goldman–Hodgkin–Katz equation, was found to be 217 using a value of 119 mM for the intracellular K⁺ concentration (calculated from the experimentally determined E_K using baclofen iontophoresis; see below). These three results demonstrate that K⁺ ions are the principal charge carriers for $I_{K(\text{slow})}$.

Figure 3. Dependence of $I_{K(slow)}$ amplitude on the duration and the amplitude of the hyperpolarizing prepulse. *A*, Representative example of current responses is shown from a cell clamped at -60 mV and stepped to -120 mV for various durations (marked at the top of each example). In the graph, averaged data were plotted and normalized to the maximal current amplitude at 30 sec ($n = 3$). Monoexponential fitting revealed a time constant of 9.0 sec for recovery from inactivation. *B*, Similar analysis as in *A* shows the dependence of $I_{K(slow)}$ on the amplitude of the hyperpolarizing step (duration 15 sec) and gave a voltage for 50% of recovery from inactivation of -95 mV.

$I_{K(slow)}$ is associated with an increase in membrane conductance

The slow decay kinetics of $I_{K(slow)}$ and the long duration of the required hyperpolarizing prepulse raise the possibility that slow ionic transport processes, secondary K⁺ ion shifts, or the activation of an electrogenic ion exchanger pump is responsible for this current. First, changes in the distribution of K⁺ ions attributable to a sustained change in driving force can generate currents after repolarizing the cellular membrane (Noble, 1976). In the present case, however, the reversal potential for K⁺ ions before and after the hyperpolarizing pulse needed to evoke $I_{K(slow)}$ remained the same. Ionophoretic application of the GABA_A-receptor agonist baclofen activated an inwardly rectifying K⁺ conductance with a reversal potential of -99.7 ± 1.8 mV ($n = 4$; Fig. 5*A*), as reported previously (Newberry and Nicoll, 1984; Gähwiler and Brown, 1985a). When baclofen was applied immediately after a prolonged

Figure 4. Determination of the reversal potential for $I_{K(slow)}$. Averaged data are shown on the left, and representative raw data are shown on the right. Instantaneous current–voltage relationships (insets) with (solid circles) and without (open circles) a hyperpolarizing prepulse were constructed. *A*, The intersection between the two curves, fitted by linear regression (correlation coefficient $r = 0.99$ for both curves), yielded the reversal potential for $I_{K(slow)}$ (averaged data -93.7 ± 1.7 mV; $n = 3$). *B*, The reversal potential was -69.6 ± 1.0 mV ($n = 4$) when the extracellular K⁺ concentration was raised to 8.0 mM (correlation coefficient $r = 0.99$ for both curves). Under these conditions, voltage steps to a maximum of -95 mV were applied. Calibration bars apply to all raw data.

hyperpolarizing prepulse, identical to those used for evoking $I_{K(slow)}$, no change in the reversal potential was observed (-97.7 ± 2.0 mV, $n = 4$, $p > 0.05$; Fig. 5*A*). Thus, a current relaxation attributable to ionic redistribution can be ruled out.

Second, activation of an electrogenic ion exchanger pump could induce a slow current. An ouabain-sensitive pump current with a time course similar to $I_{K(slow)}$ has been described previously in the locus ceruleus (Johnson et al., 1992). Theoretical considerations predict that a current generated by a metabolically driven pump will be associated with negligible changes in input conductance (De Weer, 1984). Therefore, we measured the input conductance of cells by applying 500 msec hyperpolarizing voltage commands of 10 mV at 3 sec intervals during the experimental protocol to elicit $I_{K(slow)}$. Figure 5*B* illustrates a representative trace, demonstrating a significant increase in input conductance ($219 \pm 39\%$ of control, $p < 0.001$; $n = 9$) during $I_{K(slow)}$. Moreover, application of strophanthidin ($2\text{--}7$ μ M), an inhibitor of ATPase activity, was without effect ($p > 0.05$, $n = 4$; data not shown). Taken together, these findings exclude the involvement of an electrogenic pump.

Figure 5. *A*, $I_{K(\text{slow})}$ is not caused by a hyperpolarization-induced shift in E_K . Responses to iontophoretically applied baclofen at various potentials were not modified by a hyperpolarizing prepulse. *B*, $I_{K(\text{slow})}$ is associated with an increase in membrane conductance (left). Voltage steps from -60 to -120 mV were applied for 30 sec. Input conductance was measured by applying 10 mV hyperpolarizing pulses of 500 msec every 2 sec. The increased conductance during the hyperpolarizing pulse is attributable to inward rectification and was blocked by bath application of 1 mM Cs^+ (right).

In summary, we conclude that $I_{K(\text{slow})}$ likely is attributable to an increase in a K⁺ conductance with the following properties: inactivation with a time constant of 7.5 sec, and recovery from inactivation after a prolonged period of hyperpolarization.

Voltage dependence of activation and steady-state inactivation

The voltage dependence for activation and inactivation of $I_{K(\text{slow})}$ was characterized by measuring the current elicited at different holding potentials after a 30 sec hyperpolarizing voltage step (Fig. 6). Current amplitudes were normalized to the maximal amplitude and plotted against the membrane potential to which the neuron was stepped during activation of the current. Averaged results from seven cells were fitted by using the Boltzmann equation:

$$I/I_{\text{max}} = [1 + \exp(-k(V - V_{0.5}))]^{-1},$$

where V is the membrane potential, $V_{0.5}$ is the membrane potential at which the conductance is half-activated, I is the amplitude of the tail current at the beginning of activation, and k is the slope factor that determines the steepness of the fitted curve. The activation range of the current lay between -90 and -55 mV, with half-activation occurring at -65.1 ± 2.6 mV and a slope factor $k = 0.20/\text{mV}$. The maximal conductance (I_{max}) was calcu-

Figure 6. Voltage dependence of activation and inactivation of $I_{K(\text{slow})}$. Inset shows the voltage protocol used to obtain current responses for the activation (open circles) and inactivation curves (solid circles). All data were leak-subtracted. Activation was not studied at more depolarized potentials because contaminating currents were activated above -50 mV that could not be blocked without interfering with $I_{K(\text{slow})}$. Boltzmann fitting of the activation curve, therefore, should be considered to be tentative (see Results). The window current for $I_{K(\text{slow})}$ lay between -85 and -65 mV.

lated to be 7.3 ± 1 nS at -55 mV ($n = 7$) and 4.2 ± 1.5 nS at -75 mV ($n = 4$).

The voltage dependence of steady-state inactivation was determined by clamping the cell to incrementing voltages for 30 sec after the hyperpolarizing pulse. Over this time period, the current decayed completely. The remaining current evoked by stepping back to -60 mV was measured, and a similar analysis to that for the voltage dependence of activation was performed, yielding values of $V_{0.5} = -84.3 \pm 1.0$ mV and $k = -0.12/\text{mV}$ ($n = 7$).

Kinetics of inactivation

In the presence of 0.1 mM 4-AP, $I_{K(\text{slow})}$ was well fitted by a monoexponential function, with an inactivation time constant of 7.5 ± 0.5 sec at -60 mV ($n = 35$; Fig. 7A). The time constants for all cells ranged between 2 and 15 sec and were distributed in a Gaussian manner around the mean (Fig. 7B). Thus, $I_{K(\text{slow})}$ inactivated 3- to 10-fold slower than I_L . The time constant of inactivation decreased with hyperpolarization, as determined from the instantaneous current-voltage relationships (Fig. 7C). These data, however, are influenced by the kinetics of $I_{K(\text{slow})}$ deactivation, which cannot be isolated from inactivation because there is no situation in which $I_{K(\text{slow})}$ does not inactivate. The fitting of $I_{K(\text{slow})}$ was not improved by using a multiexponential function, indicating that this current reflects the decay of a single conductance.

K⁺-channel blockers

We determined the sensitivity of $I_{K(\text{slow})}$ to a variety of K⁺-channel blockers (Fig. 8B). Hyperpolarization-activated, inwardly rectifying currents in various cell types are reduced by low concentrations (1–2 mM) of extracellular Cs^+ (Halliwell and Adams, 1982; Mayer and Westbrook, 1983; McCormick and Pape, 1990). In CA3 neurons, extracellular application of Cs^+ (1 mM) reduced the amplitude of $I_{K(\text{slow})}$ by $19.6 \pm 4.5\%$ ($p < 0.005$; $n = 8$) without changing the time constant of inactivation ($91.9 \pm 7.4\%$

Figure 7. Kinetic analysis of $I_{K(slow)}$. *A*, Representative current relaxation (left) and a monoexponential fit (right) with an amplitude of 314 pA and an inactivation time constant of 8.07 sec are shown. *B*, Gaussian fitting of the distribution of time constants gave an average of 7.5 ± 0.5 sec. *C*, Voltage dependence of inactivation of $I_{K(slow)}$ ($n = 4$). Decay time constants were not analyzed above -55 mV because other currents activated at depolarized potentials contaminated any further analysis.

of control, $p > 0.05$; $n = 8$). In cells intracellularly perfused with Cs⁺, $I_{K(slow)}$ could not be evoked ($n = 5$; data not shown).

Slow transient K⁺ currents in the hippocampus are differentially sensitive to TEA (Segal and Barker, 1984; Numann et al., 1987; Ficker and Heinemann, 1992). In the case of $I_{K(slow)}$, we obtained a maximal suppression of $56.7 \pm 7.4\%$ ($p < 0.01$; $n = 8$) by using very high concentrations of TEA (50 mM). TEA at a concentration of 10 mM suppressed $I_{K(slow)}$ by $20.9 \pm 9.9\%$ ($p < 0.05$; $n = 6$). Extracellular application of Ba²⁺ (1 mM) reduced the amplitude of $I_{K(slow)}$ by $37.6 \pm 7.3\%$ ($p < 0.05$; $n = 7$). As mentioned above, DTX (5–10 μ M; Fig. 2*B*) blocked I_D but not $I_{K(slow)}$ ($107.5 \pm 21.6\%$ of control, $p > 0.05$). Focal application of CTX (0.5–2.5 μ M), known to block Ca²⁺-activated K⁺ conductances (Miller et al., 1985), had no effect on $I_{K(slow)}$ ($93.7 \pm 3.9\%$ of control, $p > 0.05$; $n = 8$), but it did prolong the decay time of the action potential ($p < 0.05$; $n = 3$), as shown previously (Storm, 1987). Another class of substances, the sulfonylureas, blocks ATP-dependent K⁺ conductances (Rorsman and Trube, 1990). Glibenclamide, a member of this class, had no effect on $I_{K(slow)}$ ($99.7 \pm 11.1\%$ of control, $p > 0.05$; $n = 7$).

One would expect that the large voltage steps required to elicit $I_{K(slow)}$ activate voltage-gated Ca²⁺ currents and, in turn, Ca²⁺-dependent conductances (Brown et al., 1990). $I_{K(slow)}$, however, was not modified by changes in intracellular Ca²⁺ levels. The current was not affected by preventing Ca²⁺ influx with extracellular application of Cd²⁺ (50 μ M, $100.4 \pm 35.6\%$ of control, $p > 0.05$; $n = 8$) or low extracellular Ca²⁺ concentrations (0.5 mM Ca²⁺, 10 mM Mg²⁺, $93.8 \pm 8.1\%$ of control, $p > 0.05$, $n = 11$; data not shown). In recordings from cells intracellularly perfused with BAPTA (25 mM), $I_{K(slow)}$ was not altered ($n = 4$; data not shown), ruling out a dependence on a change in intracellular Ca²⁺.

Figure 8. Effects of K⁺-channel blockers on $I_{K(slow)}$. *A*, 4-AP does not suppress $I_{K(slow)}$. Left, Currents evoked after a 30 sec hyperpolarizing step to -120 mV for 30 sec. Middle, The faster current component was blocked after application of 100 μ M 4-AP. Right, Raising the concentration of 4-AP to 5 mM still had no effect on $I_{K(slow)}$. *B*, Histogram summarizing the effects of K⁺-channel blockers on $I_{K(slow)}$. Asterisk denotes significant difference from control; Glib, glibenclamide.

Metabotropic agonists

$I_{K(slow)}$ was reduced by application of *IS,3R*-ACPD (100 μ M), a specific agonist for metabotropic glutamate receptors, and by MCh (5 μ M) or muscarine (10 μ M), agonists at muscarinic cholinergic receptors (Fig. 9*A*). It should be noted that these agonists did not alter significantly the peak amplitude of the current (271 ± 34 vs 266 ± 37 pA for *IS,3R*-ACPD at 100 μ M, $n = 15$; 163 ± 38 vs 205 ± 27 pA for MCh at 5 μ M, $p > 0.05$, $n = 3$). Rather, they induced an apparent, concentration-dependent increase in the rate of decay of $I_{K(slow)}$, as assessed by monoexponential fitting (Fig. 9*B*). Compared with the effects on previously studied K⁺ currents, higher concentrations of these metabotropic agonists were required for suppression (Gerber and Gähwiler, 1994). The actions of MCh were antagonized fully by 1 μ M atropine. Metabotropic agonists did not block I_D or change its time course (741 ± 45 vs 729 ± 32 msec for *IS,3R*-ACPD at 100 μ M, $n = 3$; 675 ± 96 vs 673 ± 42 msec for MCh at 5 μ M, $n = 6$; Fig. 9*C*).

The apparent suppression of $I_{K(slow)}$ after stimulation of metabotropic receptors could arise either via a direct inhibition of the underlying conductance or via activation of an inward current that superimposes on $I_{K(slow)}$. To address this question, experiments were designed to examine the action of agonists at restricted time points during $I_{K(slow)}$ activation using the iontophoretic technique of drug application (Fig. 10). If $I_{K(slow)}$ is suppressed by activation of metabotropic receptors, the pharmacological response should be greater at the peak amplitude of

Figure 9. Reduction of $I_{K(slow)}$ by metabotropic glutamatergic and cholinergic agonists. *A*, Effects of 1S,3R-ACPD (*top*) and MCh (*bottom*) are shown at two different concentrations. Cells were clamped at -120 mV for 30 sec and then stepped back to -60 mV. Control and test responses at maximal agonist concentration are shown superimposed and enlarged by a factor of two on the *right*, aligned at their peak amplitudes. Responses to agonists were characterized by measuring the initial amplitude and the apparent decrease in inactivation time constant of $I_{K(slow)}$. *B*, Responses were concentration-dependent, with an IC_{50} for 1S,3R-ACPD of $13 \mu M$ and an IC_{50} for MCh of $0.06 \mu M$. Each concentration was tested 3–15 times. *C*, Metabotropic agonists at their maximal concentrations for $I_{K(slow)}$ suppression had no effect on inactivation of I_D , evoked at -60 mV after a 500 msec hyperpolarizing step from -60 to -120 mV.

$I_{K(slow)}$. Indeed, the amplitude of the response to agonists when applied during $I_{K(slow)}$ was significantly greater than in control (1S,3R-ACPD: 179 ± 48 vs 108 ± 34 pA, $p < 0.05$, $n = 3$; MCh: 228 ± 25 vs 124 ± 13 pA, $p < 0.001$, $n = 6$; Fig. 10*A,B*). On the other hand, iontophoretic application of AMPA caused current responses that remained unchanged in amplitude in the presence of $I_{K(slow)}$ (293 ± 36 vs 292 ± 36 pA, $p > 0.05$, $n = 4$; Fig. 10*C*). Thus, these experiments suggest that $I_{K(slow)}$ can be modulated by activation of metabotropic receptors.

DISCUSSION

A slow, voltage-dependent K⁺ current is evoked when the membrane potential is stepped back to resting potential after a period of prolonged membrane hyperpolarization in hippocampal CA3 pyramidal cells. The purpose of this study was to characterize this current, to classify it within the family of known hippocampal K⁺ currents, and to describe the physiological context in which it may be of importance. To reflect the slow kinetics of this current, we propose that it be named $I_{K(slow)}$.

Prolonged hyperpolarization (tens of seconds) preceding a depolarizing stimulus induces a delay in onset of action-potential generation consistent with the activation of $I_{K(slow)}$. A maximal delay in depolarization-induced discharge of 4–5 sec is observed after a 30 sec hyperpolarizing period at -100 mV, which corresponds to the voltage protocol needed to activate $I_{K(slow)}$ strongly. The duration of the delay lies in the same range as the time required for $I_{K(slow)}$ inactivation. In contrast, hyperpolarizing commands with a duration of 0.5–1 sec cause a much briefer delay to the first action potential. Although these observations strongly suggest that $I_{K(slow)}$ contributes to the control of discharge onset,

specific blockers of the ionic channel underlying $I_{K(slow)}$ will be required to prove this hypothesis definitively.

For the voltage-clamp characterization, prepulses to -120 mV were used to activate $I_{K(slow)}$ maximally, because this facilitated analysis. Under partially disinhibited conditions leading to epileptiform activity, inhibitory synaptic potentials can be evoked with a peak amplitude up to 30 mV below resting potential and a duration of 2 sec (Scanziani et al., 1991). Thus, it is likely that under these physiological conditions $I_{K(slow)}$ can be activated at least partially (Figs. 3, 6).

Compared with previously described hippocampal ionic currents, the slow current presented in this study exhibits unique kinetic features. $I_{K(slow)}$ decays with a slower time course than previously identified transient K⁺ currents (Storm, 1986; Ficker and Heinemann, 1992; Wu and Barish, 1992) and requires a much longer hyperpolarizing conditioning pulse to recover from inactivation. In one study, however, complete recovery from inactivation of I_D was observed after only 5 sec (Storm, 1988). This may reflect a contribution of a current corresponding to $I_{K(slow)}$ (Storm, 1986).

The slow kinetics prompted us to consider ionic mechanisms other than a conductance change that could be evoked by prolonged hyperpolarization. The experimental data, however, argue against a current attributable to ionic redistribution or electrogenic pump activity. Thus, the properties of $I_{K(slow)}$ are consistent with activation of a slow transient voltage-dependent K⁺ conductance. Ion channels with kinetics that could generate a macroscopic current similar to $I_{K(slow)}$ have been cloned and characterized. The Shaker-channel variant ShB from *Drosophila* displays

Figure 10. Responses to metabotropic agonists are enhanced when applied during $I_{K(slow)}$. *A*, Iontophoretic application of 1S,3R-ACPD under control conditions induced small inward currents (arrows) caused by suppression of K⁺ conductance (left). When the same metabotropic response was evoked during $I_{K(slow)}$ (middle), the response was greater, suggesting that an additional current, namely $I_{K(slow)}$, is blocked. $I_{K(slow)}$ during control is displayed for comparison on the right. *B*, Similar enhancement was observed when muscarine was applied iontophoretically. *C*, In contrast, responses to AMPA, an ionotropic agonist, were not increased during $I_{K(slow)}$. Dashed lines indicate peak of iontophoretic responses in control.

inactivation kinetics in the range of seconds (Hoshi et al., 1991), giving rise to a slowly inactivating current. Heteromultimeric K⁺ channels composed of two different RCK proteins (RCK1 and RCK4, corresponding to K_v 1.1 and 1.4; for review, see Pongs, 1992) expressed in HeLa cells cause rapidly activating (~10 msec) and slowly inactivating (~1 sec) currents that require a time period of 5–6 sec for 50% recovery from inactivation (Ruppersberg et al., 1990). Thus, structural substrates for channels causing prolonged K⁺ currents have been identified, and these substrates may share similarities with the channel underlying $I_{K(slow)}$.

A number of transient K⁺ currents have been characterized in hippocampal neurons (Segal and Barker, 1984; Numann et al., 1987; Ficker and Heinemann, 1992). A current activating in <100 msec and decaying biexponentially with time constants of 500 msec and 3.4 sec has been identified in cultured pyramidal cells (Ficker and Heinemann, 1992), thus suggesting similarities to I_D and $I_{K(slow)}$. 4-AP, however, suppressed the fast and slow components of this current to the same extent, and the current was mostly blocked with 20 mM TEA. The activation-inactivation properties of the slow current component revealed a window

current around –30 mV, whereas the window current of $I_{K(slow)}$ is close to resting potential. An additional 4-AP-resistant, slowly inactivating current (time constant of ~10 sec) with similar voltage dependence to $I_{K(slow)}$ has been described in CA1 pyramidal cells, but further characterization will be required to identify it as $I_{K(slow)}$ (Storm, 1986). These results taken together suggest that $I_{K(slow)}$ is a novel member of the class of transient K⁺ currents. Alternatively, $I_{K(slow)}$ may represent a deactivating tail current of a rapidly activating, K⁺-selective inward rectifier. The fact that Cs⁺ blocks $I_{K(slow)}$ only weakly does not rule out this possibility, because the blockade of inward-rectifier currents by Cs⁺ is voltage-dependent, being relieved after depolarization (Hagiwara et al., 1976). On the other hand, the linear instantaneous current-voltage relationship exhibited by $I_{K(slow)}$ at both 2.7 and 8.0 mM extracellular K⁺ concentration argues against an inwardly rectifying current (Fig. 4).

Metabotropic glutamatergic (and cholinergic) receptor activation leads to a long-lasting increase in cellular excitability via G-protein-mediated suppression of numerous K⁺ conductances (Gerber and Gähwiler, 1994). In the present study, we found that stimulating these receptors with high concentrations of agonists decreased the apparent decay time constant of $I_{K(slow)}$. Because activation of metabotropic receptors improves the space-clamp because of the reduction of leak conductances, this effect may be attributable merely to changes in the electrotonic properties of the neurons. Three observations, however, argue against such an interpretation: (1) both the amplitude and time constant of another transient K⁺ current, I_D , were unchanged after activation of metabotropic receptors; (2) a reduction in passive membrane conductance in the presence of extracellular Cs⁺ had no effect on $I_{K(slow)}$ decay kinetics; and (3) results from iontophoretic application of metabotropic agonists show an increase in the magnitude of the iontophoretic response. Thus, the reduction of this current is not an indirect effect; rather, it reflects a coupling between metabotropic receptors and the conductance underlying $I_{K(slow)}$. A change in the kinetics, but not in the initial amplitude, of a current after receptor activation could reflect a voltage dependence in the signal-transduction mechanism (Bean, 1989). Because $I_{K(slow)}$ can be evoked only within a limited voltage range, this hypothesis could not be tested satisfactorily.

In conclusion, the modulation of $I_{K(slow)}$ by metabotropic glutamatergic and cholinergic receptors represents an additional mechanism by which these receptors can control neuronal function. Voltage-dependent K⁺ currents, such as I_{AHP} and I_M , that are known to be inhibited after activation of metabotropic receptors typically are evoked by depolarizing steps well above resting potential. In contrast, $I_{K(slow)}$ activates at potentials close to rest and displays a window current around –75 mV, that is, far below spike threshold. $I_{K(slow)}$, therefore, would modify cellular responsiveness to depolarizing input as a function of the prevailing membrane potential. The observation that metabotropic glutamatergic and cholinergic receptors can be activated with synaptic stimulation (Gähwiler and Brown, 1985b; Gerber et al., 1993) suggests that modulation of $I_{K(slow)}$ by neurotransmitter receptors contributes to synaptic processing.

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