Pharmacology of a Slowly Inactivating Outward Current in Hippocampal CA3 Pyramidal Neurons

Riccardo Bianchi, Shih-Chieh Chuang, and Robert K. S. Wong
Department of Physiology and Pharmacology, State University of New York Downstate Medical Center, Brooklyn, New York

Submitted 2 May 2006; accepted in final form 31 May 2006

INTRODUCTION

Studies in hippocampal pyramidal cells indicate that a prominent portion of the voltage-dependent potassium current shows near maximum inactivation at subthreshold membrane potentials. This phase of transient potassium current has been attributed to the activation of at least two types of potassium channels, mediating the A- and D-currents, the slowest decaying component of the transient potassium current. The different components of the transient potassium current are distinguished by the rate at which they inactivate and by their pharmacological properties. The transient potassium current activated near the resting membrane potential of the cell (~50 mV) typically decays with a multiexponential time course. The component with the fastest decay (time constant of 10–50 ms) is the A-current (I_A) and is blocked by 4-aminopyridine (4-AP, 1–5 mM). A second component, the D-current (I_D), decays with a slower time course and was first observed in the hippocampal cells by Storm (1988). Storm described that the decay time course of I_D consisted of multiple exponential components. I_D has been distinguished from I_A based on the higher sensitivity to 4-AP (30–100 μM 4-AP completely blocks I_D; Storm 1988, 1990; Wu and Barish 1992) and on the sensitivity of the current to dendrotoxin (1 μM dendrotoxin blocks I_D but not I_A; Storm 1990; Wu and Barish 1992). In CA3 pyramidal cells, Lüthi et al. (1996) showed that the I_D component of the transient potassium current decayed with a time constant of about 750 ms. In addition, they proposed that a distinct slowly inactivating potassium current, termed I_K(slow), accounted for the slowest decaying component of the transient potassium current. I_K(slow) inactivates with a time constant of several seconds (ranging from 2 to 15 s) and can be distinguished from I_D by its insensitivity to 0.1–5 mM 4-AP (Lüthi et al. 1996). At present, the properties of the slowly inactivating outward current have not been extensively studied. Because of this lack of characterization, its role in the control of hippocampal neuronal excitability is unclear and its status as a current sustained by a voltage-dependent conductance is not fully acknowledged. We now report that the slowest decaying component of the transient outward current is blocked by intracellular QX-314, providing additional evidence that the current is a distinct component of the transient outward current.

METHODS

Slice preparation

Transverse hippocampal slices (about 300 μm thick) were prepared from adult guinea pigs as previously described (Bianchi et al. 1999). Brains were rapidly removed from the skull of anesthetized animals according to approved procedure by the IACUC of SUNY Downstate Medical Center. One hippocampus was dissected out in ice-cold solution containing (in mM): NaCl 124.0, NaHCO3 26.0, KCl 2.5, MgCl2 8.0, CaCl2 0.5, and d-glucose 10.0. The middle portion of the hippocampus was glued to the stage of a Lancer Vibratome 1000 (Vibratome, St. Louis, MO) and sliced. Slices were stored on a mesh in a beaker containing artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl 124.0, NaHCO3 26.0, KCl 5.0, MgCl2 1.6, CaCl2 2.0, and d-glucose 10.0, pH 7.4. The solution was continuously gassed with a 95% O2-5% CO2 mixture and the temperature, after a 30-min period at 35°C, was kept around 24°C. After ≥1 h of recovery from the dissection, one slice at a time was placed submerged in a coverslip-bottomed recording chamber (Luigs and Neumann, Ratingen, Germany) and superfused with aCSF at 2–3 ml/min, at 31–32°C. The slice in the chamber was held down by nylon

Address for reprint requests and other correspondence: R. Bianchi, Department of Physiology and Pharmacology, SUNY Downstate Medical Center, 450 Clarkson Ave., Brooklyn, NY 11203 (E-mail: rbianchi@downstate.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

First published June 7, 2006; doi:10.1152/jn.00465.2006.
threads glued to a platinum ring. The chamber was on a stationary stage with mounted micromanipulators that were moved through electromechanical remote controls (Luigs and Neumann). The chamber was under an upright microscope equipped with water-immersed objectives and IR-DIC microscopy (BX50WI, Olympus, Middlebush, NJ). A solid-state camera (Cohu, Electronics Division, San Diego, CA) connected to a video monitor was used to image the slice and the neurons from the microscope.

**Patch-clamp recordings**

Visually identified CA3 pyramidal cells were recorded in whole cell voltage clamp using glass pipettes (World Precision Instruments, Sarasota, FL) with resistances in the range of 3–6 MΩ. Voltage commands and membrane current recordings were carried out with a patch-clamp amplifier (EPC-7, HEKA Instruments, Southboro, MA). The signals, which were stored on an Intel-based computer running pCLAMP software (Molecular Devices, Sunnyvale, CA), were simultaneously displayed on an oscilloscope (DSO 400, Gould Instruments, Valley View, OH) and recorded on a chart (TA240, Gould Instruments). Recordings were sampled at 3 kHz and digitized at 0.1–1 kHz.

VISUALLY IDENTIFIED CA3 PYRAMIDAL CELLS WERE RECORDED IN WHOLE CELL VOLTAGE CLAMP USING GLASS PIPETTES (WORLD PRECISION INSTRUMENTS, SARASOTA, FL) WITH RESISTANCES IN THE RANGE OF 3–6 MΩ. VOLTAGE COMMANDS AND MEMBRANE CURRENT RECORDINGS WERE CARRIED OUT WITH A PATCH-CLAMP AMPLIFIER (EPC-7, HEKA INSTRUMENTS, SOUTHBORO, MA). THE SIGNALS, WHICH WERE STORED ON AN INTEL-BASED COMPUTER RUNNING PCLAMP SOFTWARE (MOLECULAR DEVICES, SUNNYVALE, CA), WERE SIMULTANEOUSLY DISPLAYED ON AN OSCILLOSCOPE (DSO 400, GOULD INSTRUMENTS, VALLEY VIEW, OH) AND RECORDED ON A CHART (TA240, GOULD INSTRUMENTS). RECORDINGS WERE SAMPLED AT 3 KHZ AND DIGITIZED AT 0.1–1 KHZ.

**FIG. 1.** Activation and inactivation properties of a slowly inactivating outward current in CA3 pyramidal cells of hippocampal slices. Here and in all the following figures, whole cell patch-clamp recordings were carried out in the presence of tetrodotoxin (TTX; 1 µM). Aa: current responses (top) of a CA3 pyramidal cell to consecutive depolarizations from −100 mV to the range from −80 to −30 mV in 10-mV increments (bottom, voltage protocol). Cell was held at −100 mV for 4 s, before the depolarization. Current at the holding voltage of −50 mV was 98 pA. Dashed lines indicate the current levels reached at the end of the depolarizations to −50, −40, and −30 mV. Note that these depolarizations induced slowly decaying currents. Depolarizations to −70, −60, and −50 mV elicited an initial fast inward current that could result from activation of low threshold Ca2+ currents and/or from deactivation of Ica. Inward currents were much faster than the slowly decaying outward current. Outward current dominated at potentials > −40 mV. Top current response at −30 mV indicated by the asterisks is expanded in Ab. Ab: decaying current elicited by the depolarization to −30 mV was fitted (solid line) with the single exponential function y = A0 + a e−bx (R = 0.983), with the amplitude of the decaying current a = 136 pA, the decay time constant b = 3.454 s, and the current asymptote reached at the end of the depolarization (dashed line) y0 = 369 pA. Average decay time constants at −40 and −30 mV were 3.194 ± 0.460 and 3.082 ± 0.581 s, respectively (n = 8 cells). Ac: similar measures from a total of 8 neurons were averaged and plotted against the depolarization levels to build the activation curve for the slowly inactivating outward current. Mean amplitudes of the current at −50, −40, and −30 mV were 30.0 ± 7.8, 75.1 ± 25.6, and 162.7 ± 38.3 pA, respectively. Ba: current responses (top records) of another CA3 pyramidal cell that was held at −45 mV, hyperpolarized for 3 s to voltages ranging from −60 to −105 mV in 15-mV increments, and then depolarized back to −45 mV (bottom, voltage protocol). Holding current at −45 mV was 120 pA. Larger hyperpolarizing prepulses elicited larger-amplitude transient outward currents. Currents indicated between the asterisks are expanded in Bb and fitted with single exponential functions (solid lines). Bc: average inactivation curve for the transient outward current elicited at −45 mV in 3 cells was obtained by plotting the current amplitudes measured from the single exponential fits as shown in Bb vs. the prepulse voltage.
Drug application

Pharmacological agents were added to the perfusing solution at the indicated final concentrations. The Mn$^{2+}$/low Ca$^{2+}$-containing solution had the same composition as the aCSF except for 0.2 mM CaCl$_2$ and added 0.5 or 1 mM MnCl$_2$. All chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

Data analysis and statistics

Usually, to elicit slowly inactivating outward currents, cells were first hyperpolarized to about \(-100\) mV for 3–5 s and then depolarized to voltages up to \(-30\) mV and kept at the depolarized levels for \(\geq 16\) s. The slowly decaying currents recorded at the depolarized potentials were fitted with single exponential functions starting at 2 s after the beginning of the depolarization to minimize the contribution of the capacitive current, of fast inward currents (e.g., activation of Ca$^{2+}$ currents and deactivation of I$_\text{Na}$), and of inactivating outward currents (I$_\text{Na}$ and I$_\text{To}$) with decay faster than that of I$_\text{K(slow)}$. When the tail of the slowly decaying current was recorded (Figs. 3 and 4), the fitting monoeponential function started 2 s after stepping from 0 mV to the voltage at which the current was measured. The best-fitting function \((R > 0.9)\) for each current record was selected. The term \(a\) in the single exponential function \(y = y_0 + ae^{-bx}\) is the value of the fit at the beginning of the depolarization minus the baseline (dashed line in Fig. 1Ab) and was taken as the amplitude of the slowly decaying outward current (Amplitude in Fig. 1Ac). The time constant of decay was calculated as \(\tau = 1/b\). SigmaPlot 8 (Systat Software, Point Richmond, CA) was used for fits and plots. Statistical comparisons of data were carried out with the indicated tests and the level of significance was set at \(P = 0.05\) (GB STAT, Dynamic Microsystems, Silver Spring, MD).

RESULTS

Whole cell voltage-clamp recordings were carried out in 71 CA3 pyramidal cells of adult guinea pig hippocampal slices. In all experiments tetrodotoxin (TTX; 1 \(\mu\)M) was present in the perfusing solution to block voltage-activated sodium currents. Holding current at \(-50\) mV was 74.3 \pm 15.6 pA \((n = 30)\).

Activation and inactivation properties of the slowly inactivating outward current in CA3 pyramidal cells

Figure 1Aa shows typical current responses to a series of depolarizations from a conditioning potential of \(-100\) mV obtained in the presence of TTX (1 \(\mu\)M). Step depolarizations to \(-60\) mV and beyond elicited a transient outward current. The current decayed to the baseline at the holding potential of \(-50\) mV. The time course of decay of the slowest component of the transient outward current (the slowly inactivating outward current) could be well described by a single exponential fit for the data starting at 2 s after the onset of the depolarization. This fitting procedure should minimize the contributions of the faster inactivating components, including I$_\text{Na}$ and I$_\text{To}$, to the time constant of the slowly decaying current. On average, the transient outward current elicited at the holding potential of \(-50\) mV had a mean decay time constant of 3.439 \pm 0.507 s \((n = 8)\). The amplitude of the slowly inactivating outward current was determined by extrapolating the exponential curve fitted for the decay of the current to the onset of the depolarization. The current–voltage \((I-V)\) relationship shows that the slowly inactivating outward current had a threshold of about \(-60\) mV (Fig. 1Ac). Additional depolarizations beyond \(-60\) mV elicited a steep rise in the amplitude of the current. Depolarizations were not made to exceed \(-30\) mV because K$^+$ currents other than the transient ones were activated beyond this level (Numann et al. 1987; Storm 1990).

The voltage dependency of the inactivation of the slowly inactivating outward current was examined. Cells were held at...
−45 mV. Hyperpolarizing prepulses to −105 mV in 15 mV increments were used to activate the current at the holding potential (Fig. 1, Ba and Bb). The I−V plot (Fig. 1Bc) shows that the current amplitude increased with increases in the level of the hyperpolarizing prepulses. The plot indicates that the slowly inactivating current was completely inactivated at about −45 mV and that the inactivation was fully removed at about −105 mV.

**Pharmacological and ionic properties of the slowly inactivating outward current**

The transient outward current was elicited at a holding potential of −30 mV after a hyperpolarization to −70 mV (Fig. 2Aa). The amplitudes of the slowly inactivating outward currents activated in TTX (Fig. 2, Aa, Ba, and Ca) were compared with those obtained after addition of Cs⁺ (1 mM; Fig. 2Ab), or 4-AP (1 mM; Fig. 2Bb) to the perfusate, or after switching the perfusion to a solution containing Cs⁺ (1 mM), Mn²⁺ (0.5 mM), and low Ca²⁺ (0.2 mM; Fig. 2Cb). Summatory data provided in Fig. 2D show that these broad-spectrum potassium channel blockers did not significantly affect the amplitude of the slowly decaying outward current.

To examine the ionic properties of the slowly inactivating outward current, we determined its reversal potential by recording the tail of the current at membrane potentials between −90 and −30 mV (Fig. 3). The current was activated by a depolarization to 0 mV that followed a 5-s hyperpolarization (Fig. 3A, inset). In the presence of extracellular TTX, Cs⁺, Mn²⁺, and low Ca²⁺, inward currents and Ca²⁺-activated outward currents were suppressed. After 2 s at 0 mV, which faster decaying outward currents inactivated, the voltage was stepped to the hyperpolarized levels (−90 to −30 mV) to record the slowly inactivating outward current (Fig. 3A). Figure 3Aa shows that with extracellular K⁺ concentration ([K⁺]₀) of 5 mM the decaying current reversed polarity between −90 and −75 mV (Fig. 3Aa, bottom two traces). In the same cell, increasing [K⁺]₀ to 8.5 mM (Fig. 3Ab) and to 12 mM (Fig. 3Ac) shifted the reversal potential to more depolarized levels. Linear regressions of the I−V plots obtained from monoexponential fits of the current records in Fig. 3A show that the reversal potential of the current was −81.4, −71.6, and −64.3 mV at [K⁺]₀ values of 5, 8.5, and 12 mM, respectively (Fig. 3B). Average values of the reversal potential from four cells (−83.1 ± 1.2 mV at 5 mM; −73.5 ± 1.5 mV at 8.5 mM; and −65.0 ± 2.1 mV at 12 mM; Fig. 3C) were close to the equilibrium potentials for K⁺ estimated with the Nernst equation at 31.5°C (−86.0, −72.0, and −63.0 mV at [K⁺]₀ values of 5, 8.5, and 12 mM, respectively, and with [K⁺]₀ = 132 mM). The depolarizing shift of the reversal potential caused by increasing [K⁺]₀ was statistically significant (Fig. 3C). These results indicate that the slowly inactivating current is mainly mediated by K⁺.

The sensitivity of the slowly inactivating outward current to K⁺ channel blockers was further tested at different membrane potentials with a voltage protocol similar to that used for the

---

**FIG. 3.** Changes in extracellular K⁺ concentration shifted the reversal potential of the slowly inactivating outward current. A: in the presence of TTX (1 μM), Cs⁺ (1 mM), Mn²⁺ (0.5 mM), and low Ca²⁺ (0.2 mM), a CA3 pyramidal cell was first hyperpolarized to −100 mV for 5 s and then depolarized to 0 mV for maximal activation of the slowly inactivating outward current (Aa, asterisk, voltage protocol). After 2 s at 0 mV, the voltage was stepped to levels ranging from −30 to −90 mV in 15-mV intervals (Aa, asterisks) and the decaying current responses were recorded at different extracellular concentrations of K⁺ ([K⁺]₀; Aa, 5 mM; Ab, 8.5 mM; Ac, 12 mM) in the same cell. Solid lines through the responses are monoexponential fits of the decaying currents. Arrows indicate the current levels at the holding voltage of −50 mV (Aa: 198 pA; Ab: −158 pA; Ac: −305 pA). B: plots of the current amplitudes measured from the current traces shown in A at different values of [K⁺]₀, (filled circles, 5 mM; hollow circles, 8.5 mM; gray squares, 12 mM). Reversal potentials obtained from intersections of branches of the x-axis by the linear regressions of the plots were: −81.4, −71.6, and −64.3 mV at [K⁺]₀, of 5 mM (solid line), 8.5 mM (long dashed line), and 12 mM (short dashed line), respectively. C: mean ± SE of the reversal potentials for the slowly inactivating current measured in 4 cells at the 3 different [K⁺]₀ (at 5 mM, filled histogram: −83.1 ± 1.2 mV; at 8.5 mM, hollow histogram: −73.5 ± 1.5 mV; at 12 mM, gray histogram: −65.0 ± 2.1 mV). Note that increasing [K⁺]₀ significantly shifted the reversal potential of the current to more positive values (one-way ANOVA, P < 0.05; post hoc Newman–Keuls test: 8.5 vs. 5 mM, **P < 0.05; 12 vs. 5 mM, ***P < 0.01) as expected for a current carried by K⁺.
FIG. 4. Effects of 4-AP and tetraethylammonium (TEA) on the slowly inactivating outward current elicited at various membrane potentials. *Ac: recording conditions as in Fig. 3Aa. Slowly inactivating outward current was recorded in a CA3 pyramidal cell at −45, −60, −75, and −90 mV (between asterisks in inset, voltage protocol) after a 5-s hyperpolarization to −100 mV followed by a 2-s depolarization to 0 mV. Current responses to the same voltage protocol were recorded in the same cell 10 min after addition of 4-AP (5 mM; Ab) and 12 min after subsequent addition of TEA (50 mM; Ac) to the perfusate. Solid lines through the responses are monoexponential fits of the decaying currents. Arrows indicate the current levels at the holding voltage of −50 mV (Az: 148 pA; Ab: 96 pA; Ac: 95 pA). B: plots of the current amplitudes and regression lines obtained from the current traces shown in A before (Control, hollow circles, solid line) and after addition of the K+ channel blockers (4-AP, filled circles, long dashed line; and 4-AP + TEA, gray squares, short dashed line). Note that 4-AP did not affect the amplitude of the slowly inactivating current at the tested voltages, whereas TEA partially reduced the current amplitude. Reversal potentials obtained from intersections of the x-axis by the linear regressions of the plots were unaffected (Control, −82.6 mV; 4-AP, −81.9 mV; 4-AP + TEA, −81.8 mV). C: mean ± SE of the slowly inactivating current amplitude measured at −45 mV in the presence of the K+ channel blockers and normalized to the control values in 4 different CA3 cells. 4-AP did not significantly change the current amplitude (filled histogram, 103.3 ± 7.7%), whereas addition of TEA reduced the current amplitude to 75.8 ± 6.6% of control (gray histogram, *P < 0.05; one-way ANOVA and post hoc Newman–Keuls test).
containing pipettes (Fig. 6, A and C). In contrast, when recordings were made with Cs⁺-substituted, K⁺-free pipettes, transient outward currents were no longer activated by the depolarizing pulses (Fig. 6, B and C).

Figure 7 shows that the slowly inactivating outward current was suppressed by the inclusion of QX-314 (5 mM), a quaternary derivative of the local anesthetic lidocaine, to the intracellular recording K⁺ gluconate solution (QX-314 solution). A depolarization from −100 to −30 mV delivered soon after the break-in of a CA3 neuron in the whole cell configuration using the QX-314 solution elicited a transient outward current (Fig. 7A, 2 min). With time, the amplitude of the transient outward current decreased (Fig. 7, A and B). Suppression was most obvious for the slowest decaying component, i.e., the slowly inactivating outward current, with the longest decaying time constant. The gradual suppression of the current probably reflected the progressive diffusion of the recording pipette solution into the intracellular compartment because recordings of the slowly inactivating outward current were stable in K⁺ gluconate solution (e.g., Figs. 1−5, and 8A). The suppression of the slowly inactivating outward current by the QX-314 solution was seen at all membrane potentials examined (Fig. 8, A and C, filled circles). The QX-314 solution also blocked the slowly inactivating outward current in the presence of extracellular TTX (1 μM), Cs⁺ (1 mM), Mn²⁺ (0.5 mM), low Ca²⁺ (0.2 mM), 4-AP (5 mM), and TEA (50 mM; Fig. 8, B and C, filled squares).

**DISCUSSION**

Our results show that a transient outward current exhibiting near maximal inactivation at subthreshold voltages of about −50 mV was elicited in CA3 pyramidal cells of hippocampal slices. The slowest decaying component of the transient current persisted when 4-AP (1−5 mM) was added to the extracellular solution. This property distinguishes the slowest decaying component from the two other faster decaying currents, Iₛ and I₉, both of which are suppressed by 4-AP. The properties of the slowest decaying outward current component are similar to those of the slowly inactivating K⁺ current [Iₛ(slow)] recorded in the cultured slice preparation (Lüthi et al. 1996). The slowly inactivating outward current had a reversal potential close to the equilibrium potential for K⁺, was resistant to extracellular Cs⁺, was partially suppressed by TEA, and was blocked by intracellular Cs⁺. Furthermore, the current was not suppressed by the extracellular Mn²⁺/low Ca²⁺ solution, distinguishing it from the Ca²⁺−dependent, slow decaying outward current observed in dissociated CA1 pyramidal cells (Numann et al. 1987). The lack of effect of the Mn²⁺/low Ca²⁺ perfusing solution on the amplitude of the slowly inactivating K⁺ current suggests that the current is not gated by intracellular calcium. Although the Mn²⁺/low Ca²⁺ solution may not have blocked all transmembrane Ca²⁺ entry by the voltage-gated Ca²⁺ channels, it should have suppressed a significant portion of such Ca²⁺ entry. The finding that the time course of the slowly inactivating current decreased (Fig. 7, A and B).

---

**Fig. 6.** Intracellular Cs⁺ blocked the slowly inactivating outward current. To facilitate voltage clamping, extracellular solution containing Mn²⁺ (0.5 mM) and low Ca²⁺ (0.2 mM) was used to suppress Ca²⁺-mediated spiking. A: currents (top records) elicited by depolarizations to −50 and −35 mV from −90 mV (bottom, voltage protocol) in a CA3 pyramidal neuron recorded with a K⁺-containing pipette (132 mM). Current levels reached at the end of the depolarizations (dashed lines) are indicated. Current at the holding voltage of −50 mV (arrow) was 57 pA. C: summary data show that the slowly inactivating currents recorded with K⁺-containing pipettes (filled histograms; n = 10) at the 2 indicated voltages were blocked in cells recorded with Cs⁺-containing pipettes (hollow histograms; n = 8; ***P < 0.001. Student’s-test for unpaired data).

**Fig. 7.** Intracellular QX-314 suppressed the slowly inactivating outward current. A: current responses (top traces) of a CA3 pyramidal cell to the voltage protocol shown in the bottom at the indicated times subsequent to breaking into whole cell recording with a K⁺ gluconate−filled pipette containing QX-314 (5 mM). Dashed line: current level (101 pA) reached at the end of the depolarization for the 16-min trace. B: current resulting from subtraction of the last record in A (16 min) from the first record in A (2 min) and likely representing the current suppressed by diffusion of QX-314 into the cell. Dashed line: current level reached at the end of the depolarization.
inactivating K⁺ current was not altered by the Mn²⁺/low Ca²⁺ solution makes it unlikely that the current is dependent on Ca²⁺ entry because a commonly noted property of Ca²⁺-dependent K⁺ currents is their sensitivity to suppression of voltage-gated Ca²⁺ currents (Storm 1990). The results also indicate that intracellular QX-314 (5 mM) is an effective blocker of the slowly inactivating outward current (Figs. 7 and 8).

QX-314 is known to be an effective blocker of the voltage-dependent Na⁺ current (Connors and Prince 1982; Isaac and Wheal 1993). In addition, the agent is effective against the hyperpolarization-activated cation current Iₚ (Perkins and Wong 1995), the Ca²⁺-activated K⁺ current (Oda et al. 1992), the K⁺ leak current (Perkins and Wong 1995; Segal 1988; for review see Kindler and Yost 2005), the G-protein–coupled K⁺ current (Andrade 1991; Nathan et al. 1990), and Ca²⁺ currents (Talbot and Sayer 1996) in hippocampal cells. Thus the QX-314 action on the slowly inactivating outward current is not unique and its use as a pharmacological tool against this current is limited. Further studies on the dose–response properties of QX-314 action against each of the above-mentioned ionic channels will be required to facilitate the use of the agent as a selective blocker.

There are at least two known mechanisms whereby QX-314 suppresses the currents mentioned above. The agent directly interacts with the channel protein and blocks ion flow (e.g., Na⁺ channel; Ragsdale et al. 1994) or, for G-protein–gated channels, QX-314 interferes with the G-protein function (e.g., G-protein–coupled inward rectifier K⁺ channel; Hollmann et al. 2001). Our results do not provide indications on how QX-314 blocked the slowly inactivating potassium current. Furthermore, the data do not eliminate the possibility that the current is perpetually gated by G-protein activation by transmitters such as adenosine (Alzheimer and ten Bruggencate 1991; Ikeuchi et al. 1996). However, this seems unlikely in view of the finding that the Mn²⁺/low Ca²⁺ perfusing solution, which suppresses Ca²⁺-dependent transmitter release, did not affect the amplitude of the slowly inactivating K⁺ current (Fig. 2, C and D).

The identification of QX-314 as an effective blocker of the slowly inactivating outward current is useful in two ways. First, it adds to the pharmacological identity of the relatively unexplored current. Second, it provides support for a voltage-dependent conductance as a mechanism underlying the current. The slowly inactivating outward current was observed only after prolonged hyperpolarizing prepsules. Such voltage perturbation can cause shifts in transmembrane ionic concentration gradients resulting in voltage-independent current flow. This issue has been raised with respect to the generation mechanisms of Iₖ(slow) (Lüthi et al. 1996). The blockade of the slowly inactivating outward current by QX-314 suggests that...
ionic shifts during the prolonged hyperpolarizing prepulse per se cannot account for the generation of the current.

Recent studies show that the $I_A$ and $I_D$ components of the transient outward current are targets for synaptic and pharmacological modulation. Modulation of $I_A$ has been emphasized as a mechanism for synaptic plasticity in CA1 pyramidal cells (Ramakers and Storm 2002; Watanabe et al. 2002; Yuan et al. 2002). Activation of cannabinoid receptors reduces both $I_A$ and $I_D$ (Mu et al. 1999). In addition, Wu and Barish (1999) showed that metabotropic glutamate receptor (mGluR) activation suppressed $I_D$ by increasing the rate of inactivation. A similar effect of mGluR activation on $I_{K(slow)}$ has also been described (Lüthi et al. 1996). However, the data regarding a specific action of mGluR stimulation on $I_{K(slow)}$ may be complicated by the fact that mGluR agonists have multiple effects on hippocampal cells. In particular, the activation of a slow inward current (Chuang et al. 2001) can emerge to overlap with the time course of $I_{K(slow)}$, thereby causing an apparent accelerated decay of $I_{K(slow)}$. QX-314 may be applied in this case to separate the overlapping actions of mGluR activation and to evaluate its specific action on the slowly inactivating outward current.

**GRANTS**

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-35481.

**REFERENCES**


