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Three Kinetically Distinct Ca^{2+} -Independent Depolarization-Activated K^+ Currents in Callosal-Projecting Rat Visual Cortical Neurons

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Abstract

Three kinetically distinct Ca^{2+} -independent depolarization-activated K^+ currents in callosal-projecting rat visual cortical neurons. *J. Neurophysiol.* 78: 2309–2320, 1997. Whole cell, Ca^{2+} -independent, depolarization-activated K^+ currents were characterized in identified callosal-projecting (CP) neurons isolated from postnatal day 7–16 rat primary visual cortex. CP neurons were identified in vitro after in vivo retrograde labeling with fluorescently tagged latex microbeads. During brief (160-ms) depolarizing voltage steps to potentials between -50 and $+60$ mV, outward K^+ currents in these cells activate rapidly and inactivate to varying degrees. Three distinct K^+ currents were separated based on differential sensitivity to 4-aminopyridine (4-AP); these are referred to here as I_A , I_D , and I_K , because their properties are similar (but not identical) K^+ currents termed I_A , I_D , and I_K in other cells. The current sensitive to high ($> 100 \mu\text{M}$) concentrations of 4-AP (I_A) activates and inactivates rapidly; the current blocked completely by low ($< 50 \mu\text{M}$) 4-AP (I_D) activates rapidly and inactivates slowly. A slowly activating, slowly inactivating current (I_K) remains in the presence of 5 mM 4-AP. I_A , I_D , and I_K also were separated and characterized in experiments that did not rely on the use of 4-AP. All CP cells express all three K^+ current types, although the relative densities of I_A , I_D , and I_K vary among cells. The experiments here also have revealed that I_A , I_D , and I_K display similar voltage dependences of activation and steady state inactivation, whereas the kinetic properties of the currents are distinct. At $+30$ mV, for example, mean $\pm$ SD activation τ s are 0.83 ± 0.24 ms for I_A , 1.74 ± 0.49 ms for I_D , and 14.7 ± 4.0 ms for I_K . Mean $\pm$ SD inactivation τ s for I_A and I_D are 26 ± 7 ms and 569 ± 143 ms, respectively. Inactivation of I_K is biexponential with mean $\pm$ SD inactivation time constants of 475 ± 232 ms and $3,128 \pm 1,328$ ms; $\sim 20\%$ of the 4-AP-insensitive current is noninactivating. For all three components, activation is voltage dependent, increasing with increasing depolarization, whereas inactivation is voltage independent. Both I_A and I_K recover rapidly from steady state inactivation with mean $\pm$ SD recovery time constants of 38 ± 7 ms and 79 ± 26 ms, respectively; I_D recovers an order of magnitude more slowly (588 ± 274 ms). The properties of I_A , I_D , and I_K in CP neurons are compared with those of similar currents described previously in other mammalian central neurons and, in the accompanying paper, the roles of these conductances in regulating the firing properties of CP neurons are explored.

Introduction

A variety of K^+ currents with differing kinetic, pharmacological and voltage-dependent properties have been described in neurons in the mammalian CNS (for reviews, see: Rudy 1988; Storm 1990). In addition, in most CNS neurons, multiple types of K^+ currents have been shown to be coexpressed. As in other excitable cells (Barry and Nerbonne 1996; Rudy 1988), K^+ currents in mammalian CNS neurons appear to be more numerous and more diverse than the other types (i.e., Na^+ , Ca^{2+}) of currents expressed. This multiplicity and diversity has a physiological significance in that the various K^+ currents function to control resting potentials, the threshold for action potential generation, the rate of action potential repolarization, and the rate of repetitive firing. In addition, differences in the types, densities, and/or the detailed properties of the K^+ currents expressed are expected to contribute importantly to determining the regular-spiking, fast-spiking, and intrinsically bursting firing patterns in central neurons (Connors and Gutnick 1990; McCormick et al. 1985).

Depolarization-activated, Ca^{2+} -independent K^+ currents are expected to influence action potential repolarization and the frequency of repetitive firing. Two types of K^+ currents that are likely to subserve these functions have been described in a number of mammalian CNS neurons: a rapidly activating and inactivating current that is blocked by 4-aminopyridine and usually is referred to as I_A (Beck et al. 1992; Budde et al. 1992; Ficker and Heinemann 1992; Huguenard et al. 1991; Numann et al. 1987; Segal and Barker 1984; Storm 1988; Surmeier et al. 1989; Wu and Barish 1992); and a 4-aminopyridine-insensitive, delayed, outwardly rectifying K^+ current, usually referred to as I_K (Beck et al. 1992; Budde et al. 1992; Huguenard and Prince 1991; Numann et al. 1987; Rudy 1988; Segal and Barker 1984; Storm 1990). These are broad classifications, however, and the detailed time- and voltage-dependent properties of the various currents termed I_A and I_K do vary among cells (Rudy 1988; Storm 1990). In addition, in some CNS neurons, an additional, rapidly activating, slowly inactivating Ca^{2+} -independent K^+ current has been identified based on sensitivity to micromolar concentrations of 4-aminopyridine and, in some cases, also to the dendrotoxins (Ficker and Heinemann 1992; Hammond and Crepel 1992; McCormick 1991; Stefani et al. 1995; Surmeier et al. 1991; Wu and Barish 1992). These currents have been referred to variably as I_D or “slow transient” currents to distinguish them from I_A and I_K . It is unclear if I_D is expressed in all central neurons because, in many cases, this possibility has not been explored directly.

Although there have been many studies characterizing the repolarizing K^+ currents in CNS neurons, few have been completed on neocortical neurons (but see Albert and Nerbonne 1995; Foehring and Surmeier 1993; Hamill et al. 1991; Spain et al. 1991). This largely reflects the heterogeneity of cortical neurons and the difficulties associated with reliably identifying specific cell types. In previous studies on neocortical neurons, for example, the cells either were not identified directly (Foehring and Surmeier 1993; Spain et al. 1991) or were identified based on in vitro morphology (Hamill et al. 1991), which, as we have shown previously, is not a reliable method for identifying cortical neurons (Giffin et al. 1991). To circumvent these difficulties, we have exploited in vivo retrograde labeling to permit the in vitro identification of cortical neurons (Giffin et al. 1991; Katz and Iarovici 1990; Katz et al.

1984; Solomon et al. 1993). In the experiments described here, we have characterized the detailed time- and voltage-dependent properties of the Ca^{2+} -independent, depolarization-activated K^+ currents in isolated, identified callosal-projecting (CP) neurons from postnatal rat primary visual cortex. CP cells were selected to correspond to the “regular-spiking” phenotype described previously in recordings from cortical neurons in vivo and in the in vitro slice preparation (Connors and Gutnick 1990; McCormick et al. 1985). Here, we show that there are three kinetically and pharmacologically distinct Ca^{2+} -independent, voltage-gated K^+ currents expressed in CP cells that we refer to as I_A , I_D , and I_K because their properties are similar (although not identical) to I_A , I_D , and I_K currents described in other mammalian central neurons. In the companion paper (Locke and Nerbonne 1997), we demonstrate that CP neurons are indeed regular spiking, and we explore the roles of I_A , I_D , and I_K in shaping the waveforms of action potentials and in regulating the firing properties of CP neurons.

Methods

Isolation and identification of callosal-projecting neurons

Whole cell voltage-clamp recordings were obtained from identified CP visual cortical neurons isolated from postnatal day 7–16 (P7-P16) Long Evans rat pups. Methods for identifying and isolating CP neurons have been previously described (Giffin et al. 1991; Solomon et al. 1993). Briefly, CP neurons were labeled in vivo by pressure injection of fluorescein tagged latex beads (Katz and Iarovici 1990) into the right visual cortex on postnatal day 4, 5, or 6. On postnatal day 7–16, animals were anesthetized with 5% halothane, and the left visual cortex was removed. Cells were dissociated, plated on a glial monolayer (Raff et al. 1979), and maintained in a 95% O_2 -5% CO_2 37°C incubator. CP neurons were identified in vitro before electrophysiological recordings under epifluorescence illumination by the presence of fluorescently labeled beads in the cell soma.

Electrophysiological recordings

Whole cell patch-clamp recordings were obtained at room temperature (23–25°C) from identified, CP neurons within 30 h of cell dissociation. Data were collected using a Dagan 8900 patch-clamp amplifier, and experimental parameters were controlled by an IBM-compatible 486 computer with TL-1 interface using *PClamp* (Axon Instruments). Neurons without extensive processes were selected for recording (see below). All voltage-clamp data were collected in the presence of 1 μM tetrodotoxin (TTX) and 100 μM Cd^{2+} to block voltage-gated Na^+ and Ca^{2+} currents, respectively. Electrodes were fabricated from soda lime glass (Kimble No. 73811) on a two-stage puller and the shanks (within $\sim 50 \mu\text{m}$ of the tips) were coated with silicone elastomer (Sylgard; Dow Corning). Pipette resistances were 1.2–2.5 $\text{M}\Omega$ after fire polishing. Series resistances, estimated from the decays of the uncompensated capacitive transients, were 2.5–5 $\text{M}\Omega$ and were compensated electronically by 80–90%. Because peak current amplitudes were $< 8 \text{ nA}$, voltage errors arising from the uncompensated series resistance were always $< 8 \text{ mV}$ and were not corrected. Depolarization-activated K^+ currents were evoked routinely during voltage steps to potentials between -50 and $+60 \text{ mV}$ from a holding potential (HP) of -70 mV . Other voltage-clamp protocols are described in the text. Data were sampled at various rates (1–70

kHz) depending on the particular experimental paradigm, and current signals were filtered at 5 kHz before digitization and storage.

Solutions

The standard bath solution contained (in mM) 140 NaCl, 2.5 CaCl₂, 4 KCl, 2 MgCl₂, 5 glucose, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.1 CdCl₂, and 1 μ M TTX (pH 7.4; 305 mOsm). Because millimolar concentrations of Cd²⁺ have been shown in some cells to alter the time- or voltage-dependent properties of K⁺ currents (Mayer and Sugiyama 1988), some experiments also were completed with the organic Ca²⁺ channel blocker nifedipine used in place of Cd²⁺ to block currents through voltage-gated Ca²⁺ channels. No differences in the time- or voltage-dependent properties of the K⁺ currents were observed. The pipette solution contained (in mM) 135 KCl, 10 ethylene glycol-bis (β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 10 HEPES, 5 glucose, 3 MgATP, and 0.5 NaGTP (pH 7.4; 305 mOsm). Stock solutions of 4-aminopyridine (4-AP) or dendrotoxin (DTX) were diluted into the standard bath solution immediately before experiments. Tetraethylammonium chloride (TEACl) was substituted for NaCl in the bath solution on an equimolar basis. All chemicals, except DTX, were obtained from Sigma. DTX was obtained from Alamone Laboratories, Israel. Drugs were applied using a perfusion pipette, which consisted of four pieces of PE10 tubing encased in a small glass tube. During superfusion, the pipette was placed within 200 μ m of the cell, and the bath volume was kept constant using a small glass tube, attached to a large reservoir, that controlled the solution level by capillary action.

Data analyses

Data were compiled and analyzed using Clampfit (Axon Instruments), Excel (Microsoft), CSS Statistica (Stat Soft), and Nfit (Island Products). All data are means $\pm$ SD. The plateau current was defined as the current remaining 150 ms after the onset of voltage steps, and the peak current was defined as the maximum value the current attained during the 160-ms pulse. For each cell, the spatial control of membrane voltage was assessed by directly analyzing the decay of the capacitative currents. Only cells with capacitative transients well described by single exponential functions were analyzed further. Leak currents were always <100 pA at -70 mV and were not corrected. The mean $\pm$ SD input resistance of analyzed CP cells was 1.7 ± 1.1 G Ω ($n = 70$), and the mean $\pm$ SD whole cell membrane capacitance of these cells was 14.4 ± 6.6 pF ($n = 70$). Equations used in fitting the data are presented in the text. Statistical significance was examined using analysis of variance (ANOVA) and Student's *t*-test; *P* values are presented in the text.

Results

Depolarization-activated currents in callosal-projecting neurons

In the experiments here, CP rat visual cortical neurons were labeled *in vivo* by pressure injection of fluorescein tagged latex beads (Katz and Iarovici 1990) into the right visual cortex on postnatal day 4, 5, or 6. After isolation at postnatal day 7–16, CP neurons were identified *in vitro* under epifluorescence illumination before electrophysiological recordings. With voltage-gated Ca²⁺ and Na⁺ currents blocked, whole cell, depolarization-activated

outward currents were recorded routinely from isolated, identified P7-P16 CP neurons ($n = 70$) during 160-ms voltage steps to potentials between -50 and $+60$ mV from an HP of -70 mV (Fig. 1). The absolute rates of rise and the amplitudes of the currents increase with increasing depolarization; the largest and most rapidly activating current in each panel in Fig. 1 was evoked at $+60$ mV. No depolarization-activated currents were evoked when the K^+ in the recording pipette was replaced by Cs^+ ($n = 4$). The currents recorded and analyzed here, therefore, are interpreted as reflecting only the activation of Ca^{2+} -independent, depolarization-activated K^+ channels. Interestingly, the waveforms of the currents evoked by small depolarizations inactivate more rapidly and to a larger extent than the currents evoked by large depolarizations. In addition, although the currents in all cells activate rapidly, the relative amplitudes of the peak and plateau currents vary markedly among cells (Fig. 1). For the cell in Fig. 1A, for example, the amplitude of the peak current is nearly twice that of the plateau current; the peak-to-plateau current ratios are ~ 1.5 and 1 for the cells in Fig. 1, B and C, respectively. Similar variability was observed among cells isolated from animals at postnatal ages (P7–P12), as well as in cells maintained for various times in vitro (3–30 h).

Separation of K^+ current components

The variability in peak-to-plateau current ratios, as well as the differences in the waveforms of the currents evoked by small and large depolarizations (Fig. 1), suggested that the total Ca^{2+} -independent, depolarization-activated K^+ currents recorded in CP visual cortical neurons reflected the simultaneous activation of two (or more) K^+ channel types and that these are distributed differentially among CP cells. In some cells, rapidly activating, rapidly inactivating currents (termed I_A) have been isolated by taking advantage of the sensitivity of I_A to holding potential. Preliminary experiments on CP cells, however, revealed that neither the amplitudes nor the waveforms of the currents are affected by changes in HP between -90 and -70 mV. The waveforms of the outward currents evoked from HPs of -30 and -70 mV are also not significantly different, although the amplitudes of the currents recorded from an HP of -30 mV are reduced substantially (not shown). Similar results were obtained in experiments conducted on 34 cells, suggesting either that CP neurons do not express multiple types of K^+ channels or that the voltage dependences of the different K^+ channels in these cells are not significantly different. To distinguish between these possibilities, subsequent experiments focused on examining the pharmacological sensitivities of the K^+ currents in CP neurons to the K^+ channel blocker 4-AP primarily because different types of K^+ currents in other cells have been shown to display differential sensitivities to 4-AP (Rudy 1988; Storm 1990).

In these experiments, the effects of varying concentrations ($50 \mu M$ to 10 mM) of 4-AP on the K^+ currents in CP neurons were examined. Control K^+ currents were recorded before superfusion of 4-AP-containing bath solutions, and when the effect of 4-AP had reached a steady state, outward currents were recorded again. To obtain the current(s) blocked by different concentrations of 4-AP, records obtained in the presence of (each concentration of) 4-AP were subtracted digitally from the controls (recorded in the absence of 4-AP). Typical examples of the subtracted current waveforms blocked by different concentrations of 4-AP are shown in Fig. 2. As is evident, the current blocked by $50 \mu M$ 4-AP (Fig. 2A1) activates

rapidly and decays only slightly during the course of 160-ms voltage steps. Increasing the concentration of 4-AP resulted in marked changes in the waveforms of the subtracted current records (Fig. 2A, 2–4). Specifically, the amplitudes of the currents at early times after the onset of depolarizing voltage steps in these subtracted records are increased (relative to the 50- μ M subtracted records) as a function of the concentration of 4-AP (Fig. 2A). In contrast, there appears to be little or no change in the amplitudes of the plateau currents in the subtracted records as a function of 4-AP concentration (Fig. 2A). To quantify the effects of varying concentrations of 4-AP, peak and plateau current amplitudes were measured at 3 and 150 ms, respectively, and the percent suppression of the peak and plateau currents (relative to the control peak and plateau current amplitudes in the same cell) was calculated. The percent suppression of the peak and plateau currents were determined in many cells, and mean normalized data are presented in Fig. 2B. As is evident, concentrations of 4-AP $>50 \mu$ M block increasing amounts of the peak, but not of the plateau, currents. Maximal block of the plateau currents is achieved at 50 μ M 4-AP, whereas maximal block of the peak currents is observed at 5 mM (Fig. 2B).

To isolate the currents blocked by the high concentrations of 4-AP, currents recorded in the presence of 5 mM 4-AP (Fig. 3C) were subtracted from those recorded in 50 μ M 4-AP (Fig. 3B). Comparison of the waveforms of these currents (Fig. 3E) and the 50- μ M 4-AP-sensitive currents (Fig. 3D) reveals that, although both currents activate rapidly, the decay phases of the currents are markedly different. The 50- μ M-sensitive currents (Fig. 3D) inactivate slowly and resemble currents referred to as I_D , or slow transient currents, in other preparations (Ficker and Heinemann 1992; Storm 1988; Wu and Barish 1992). The currents blocked by 5 mM (but not by 50 μ M) 4-AP, in contrast, inactivate rapidly (Fig. 3E) and are similar to I_A currents in other cells (for reviews, see Rudy 1988; Storm 1990). The currents remaining in the presence of 5 mM 4-AP (Fig. 3C) are kinetically distinct from both of the 4-AP-sensitive currents (Fig. 3, D and E). Specifically, the 5-mM 4-AP-insensitive currents activate slowly and do not inactivate during 160-ms depolarizations at all test potentials, much like delayed rectifier, or I_K , currents described in other cell types (for reviews, see Rudy 1988; Storm 1990). Taken together, these data suggest the presence of at least three Ca^{2+} -independent K^+ currents in isolated, identified P7–P16 CP neurons. We refer to these currents as I_A , I_D , and I_K primarily because the properties of the currents in CP cells are qualitatively similar to those of currents referred to by these names in other cells (see DISCUSSION).

To test the validity of the separation of the currents (I_A , I_D , and I_K) based on differential sensitivities to 4-AP described above (Fig. 3), several types of experiments were performed to determine if these current components could be isolated by other methods. The current blocked by high (100 μ M) concentrations of 4-AP, which we refer to as I_A , inactivates rapidly on membrane depolarization (Fig. 3E), whereas the current component that is blocked completely by 50 μ M 4-AP, I_D (Fig. 3D), and the 4-AP-insensitive current, I_K , inactivate slowly. This finding suggested that it should be possible to isolate I_A based on its inactivation kinetics. To explore this possibility, outward K^+ currents were recorded during depolarizing voltage steps presented directly from the holding potential of -70 mV (Fig. 4A) and after a 50-ms conditioning prepulse to 0 mV (Fig. 4B). Subtraction of the currents

recorded with and without the prepulse revealed rapidly activating and inactivating current waveforms (Fig. 4C) indistinguishable from those obtained on subtraction (Fig. 3E) of currents recorded in the presence of 5 mM (Fig. 3C) from those in 50 μ M (Fig. 3B) 4-AP, i.e., I_A . Similar results were obtained on eight cells. These results suggest that I_A waveforms isolated using 4-AP-sensitive currents reflect the activation of a unique current and not voltage-dependent effects of 4-AP (Thompson 1982).

In several other mammalian neurons, the currents displaying sensitivity to low concentrations (micromolar) of 4-AP also are blocked selectively by nanomolar concentrations of the mamba snake toxins, the DTXs (Penner et al. 1986; Stansfeld et al. 1986; Surmeier et al. 1991). To determine the effects of DTX on CP neurons, outward K^+ currents were recorded before and after bath application of α -DTX. Although no attempts were made to examine dose-response curves, similar effects were observed at all concentrations of α -DTX (20–100 nM) examined ($n = 10$). A typical example of the effect of α -DTX on K^+ currents in CP neurons is illustrated in Fig. 5. The α -DTX-sensitive currents (Fig. 5C), obtained by subtraction of current recorded in the presence of α -DTX (Fig. 5B) from the controls (Fig. 5A), appear indistinguishable from the 50- μ M 4-AP-sensitive currents (Fig. 3D). Similar to the findings with 50 μ M 4-AP, α -DTX blocks a similar percent of the peak ($18 \pm 9\%$) and the plateau ($17 \pm 8\%$) currents. Thus the pharmacological properties of the current in CP neurons we refer to as I_D are similar to those of rapidly activating, slowly inactivating currents described in other cells (Penner et al. 1986; Stansfeld et al. 1986; Surmeier et al. 1991).

As illustrated in Fig. 3C, the currents (I_K) remaining in the presence of 5 mM 4-AP are kinetically distinct from the 4-AP-sensitive currents, I_D (Fig. 3D) and I_A (Fig. 3E). Because I_K in other preparations is blocked selectively by millimolar concentrations of TEA (for recent reviews, see Rudy 1988; Storm 1990), the effects of TEA on the Ca^{2+} -independent, depolarization-activated K^+ currents in CP neurons also were examined (Fig. 6). The TEA-sensitive currents (Fig. 6C), obtained by subtraction of records in the presence of 30 mM TEA (Fig. 6B) from controls (Fig. 6A), activate slowly and undergo little or no inactivation during 160-ms voltage steps; the waveforms of the TEA-sensitive currents, therefore, appear indistinguishable from the currents remaining in 5 mM 4-AP (Fig. 3C). Similar results were obtained in experiments completed on seven other cells. In addition, currents with identical waveforms (but smaller amplitudes) were obtained in 15 other cells using lower concentrations (3–10 mM) of TEA. Taken together, these experiments have provided independent means of isolating I_A (voltage-clamp protocol), I_D (DTX sensitivity), and I_K (TEA sensitivity), and, in each case, the properties of the currents appear indistinguishable from those separated using 4-AP (see below and DISCUSSION). For all subsequent analyses, therefore, the properties of the currents (I_A , I_D , and I_K) separated using 4-AP were examined. In several cases, the properties of the currents separated using these alternative methods also were analyzed.

It is of interest to note that although I_A , I_D , and I_K were identified readily in all CP cells studied ($n = 40$), the absolute amplitudes of the individual current components were found to vary markedly among cells. Normalizing current amplitudes for differences in cell sizes revealed similar variability in current densities among cells. I_A density, for example, varied

between 94 and 489 pA/pF, and the mean I_A density was 215 ± 83 pA/pF ($n = 40$). Similar variations in I_K densities were observed; I_K density varied over the range of 65 to 465 pA/pF with a mean of 173 ± 94 pA/pF ($n = 40$). In all cells, the density of I_D was substantially less than the density of either I_A or I_K ; I_D density ranged from 11 to 127 pA/pF with a mean of 54 ± 31 pA/pF. On average, therefore, the densities of both I_A and I_K are approximately four times the density of I_D . More importantly, these analyses revealed that the relative density of I_A , I_D , and I_K (i.e., the ratio of $I_A:I_D:I_K$) varies markedly among cells. The variability in the relative densities of I_A , I_D , and I_K likely underlies the differences (Fig. 1) in the waveforms of the total Ca^{2+} -independent outward currents observed among CP neurons (see DISCUSSION).

Kinetic analyses of I_A , I_D , and I_K

Time constants (τ s) of activation for I_A , I_D , and I_K were determined from single exponential fits to the rising phases of the separated current components. For these analyses, I_A and I_D were obtained using the subtraction procedures described above and illustrated in Fig. 3, D and E; the current remaining in 5 mM 4-AP (Fig. 3C) was analyzed as I_K . For all three components, the rising phases of the currents at each test potential were well described by single exponentials (Fig. 7B). Mean activation time constants for I_A , I_D , and I_K are plotted as a function of test potential in Fig. 7 A. As is evident, I_A and I_D activate rapidly, although at all test potentials, I_A activates approximately twofold faster than I_D . At +30 mV, for example, mean activation τ s are 0.83 ± 0.24 ms ($n = 14$) and 1.74 ± 0.49 ms ($n = 18$) for I_A and I_D , respectively. The observed differences between I_A and I_D activation τ s are statistically significant at all test potentials ($P < 0.002$ at +60 mV; $P < 0.001$ at all other voltages). Analyses of the rising phases of I_A isolated using the conditioning prepulse protocol (Fig. 4C) and of I_D isolated using α -DTX (Fig. 5C) yielded results indistinguishable from those obtained for currents isolated using 4-AP. I_A activation time constants at +60 mV, for example, are 0.61 ± 0.22 ms ($n = 9$) using the conditioning prepulse protocol and 0.55 ± 0.16 ms ($n = 14$) for the currents separated using 4-AP. Activation τ s of the α -DTX-sensitive currents (Fig. 5C) and the 50- μ M 4-AP-sensitive currents (Fig. 3D) are also not significantly different. At +60 mV, for example, the 50- μ M 4-AP- and 10–100 nM α -DTX-sensitive currents activate with time constants of 0.93 ± 0.33 ms ($n = 18$) and 1.29 ± 0.46 ms ($n = 11$), respectively, consistent with the suggestion that α -DTX and 50 μ M 4-AP both block I_D in CP neurons.

I_K activates ~ 10 -fold more slowly than I_A or I_D at all test potentials; the mean activation time constant for I_K at +30 mV is 14.7 ± 4.0 ms ($n = 19$). Activation τ s of the TEA-sensitive currents ($n = 8$) also were determined from single exponential fits to the rising phases of subtracted current waveforms such as those in Fig. 6C. These analyses revealed that the time constants of activation of the TEA-sensitive currents and the currents remaining in the presence of 5 mM 4-AP are indistinguishable. The activation time constants of the 4-AP – insensitive currents (Fig. 3C) and the TEA-sensitive currents (Fig. 6C) at +60 mV, for example, are 8.2 ± 2.2 ms ($n = 19$) and 6.9 ± 2.8 ms ($n = 8$), respectively. For all three currents, activation time constants are voltage dependent, decreasing with increasing depolarization. The variations in τ with respect to voltage are well described by single exponential functions of the form

$$\tau(V) = a + b [\exp(-V_m/c)]$$

where V_m is the test potential and c is a constant that defines the steepness of the voltage dependence. The best fits (continuous lines, Fig. 7 A) to the experimental data yielded $c = 32.1$ ($a = 0.38$, $b = 1.1$) for I_A ; $c = 38.4$ ($a = 0.2$, $b = 3.4$) for I_D , and $c = 34.8$ ($a = 3.7$, $b = 25.5$) for I_K . Comparison of the values for c indicates that the activation rates of all three currents vary similarly as a function of voltage.

Time constants of inactivation for I_A , I_D , and I_K were determined from exponential fits to the decay phases of current recorded during 12-s voltage steps to 0, 10, 20, and 30 mV in control bath solution and in the presence of 8 mM 4-AP (to block I_A and I_D). The decay phases of the currents blocked by 8 mM 4-AP were well described by the sum of two exponentials (Fig. 7D) with mean time constants of 19 ± 7 ms and 569 ± 143 ms ($n = 7$). Neither time constant varied as a function of voltage (Fig. 7C). The faster time constant is assumed to reflect the inactivation of I_A and the slower time constant the inactivation of I_D . To test this hypothesis, inactivation time constants for I_A were determined from single exponential fits to the decay phases of currents such as those in Figs. 3E and 4C. Inactivation of I_A at all test potentials was well described by a single exponential; mean inactivation time constants for I_A separated using 4-AP (Fig. 3E) and the conditioning prepulse (Fig. 4C) were found to equal 19 ± 5 ms ($n = 14$) and 17 ± 7 ms ($n = 9$), respectively. These values are not significantly different from the fast time constant obtained from the double-exponential fits to the 8 mM 4-AP-sensitive currents (19 ± 7 ms); this is consistent with the suggestion (above) that the fast component of the 8 mM 4-AP-sensitive currents reflects inactivation of I_A .

The decay phases of the 4-AP-insensitive currents (I_K) were well described in all cells and at all test potentials by the sum of two exponentials (Fig. 7D) with mean time constants of 475 ± 232 and $3,128 \pm 1,328$ ms ($n = 7$; Fig. 7C). In addition, $\sim 20\%$ of the current remaining in 8 mM 4-AP is noninactivating. Similar to the inactivation time constants for I_A and I_D , neither component of inactivation of the 8 mM 4-AP-insensitive current varied substantially with voltage (Fig. 7C). The relative amplitudes of the fast and slow components of I_K inactivation, however, did vary appreciably among cells, ranging from ≈ 1 to ≈ 4 . The finding of multiple components of inactivation of the 4-AP-insensitive currents suggests either that the properties of the underlying I_K channels are complex or that the 4-AP-insensitive currents comprise distinct conductance pathways (see below and DISCUSSION).

Reversal potentials of I_A , I_D , and I_K

Reversal potentials of I_A , I_D , and I_K , were determined from analyses of tail currents of the individual K^+ current components. For these analyses, I_A and I_D were obtained using the subtraction procedures illustrated in Fig. 3, and the plateau current remaining in the presence of 5 mM 4-AP was analyzed as I_K . From a holding potential of -70 mV, cells were depolarized to $+30$ mV for 6 ms (for I_A and I_D) or 150 ms (for I_K) to activate the outward currents and subsequently repolarized to potentials between -30 and -120 mV. Tail current

amplitudes at each potential were measured 1.5 ms after the onset of the hyperpolarizing voltage steps and plotted as a function of voltage. Reversal potentials were obtained by linear interpolation. Mean reversal potentials for I_A , I_D , and I_K in 3 mM $[K^+]_o$ were -73 ± 4 mV ($n = 4$), -82 ± 6 mV ($n = 7$), and -68 ± 4 mV ($n = 7$), respectively. The calculated equilibrium potential (E_K) for K^+ under the recording conditions employed here is -90 mV, suggesting that the channels underlying I_A , I_D , and I_K are K^+ selective. Because the experimentally determined reversal potentials, particularly for I_A and I_K , are positive to E_K , however, the underlying channels also may have finite permeabilities for other ions.

Voltage dependences of activation and steady state inactivation of I_A , I_D , and I_K

The voltage dependences of activation of I_A , I_D , and I_K were determined from analyses of current records such as those in Fig. 3. I_A and I_D amplitudes were measured at the peak of subtracted current records such as those in Fig. 3, $E(I_A)$ and $D(I_D)$, and I_K amplitudes were measured at the plateau (150 ms) of the current remaining in 5 mM 4-AP (Fig. 3C). I_A , I_D , and I_K conductances at each test potential were calculated in each cell using the experimentally determined reversal potentials and subsequently normalized to their respective conductance values at +60 mV (measured in the same cell). Data for each cell were fitted to a Boltzmann relation of the form

$$G = G_{\max} / [1 + \exp\{(V - V_{1/2})/k\}]$$

where G is the conductance at each potential, V ; $G_{\max}$ is the maximal conductance; $V_{1/2}$ is the voltage of half-maximal activation; and k is the slope factor. The $V_{1/2}$ and k values from individual cells then were averaged. Mean normalized conductances for I_A ($n = 14$), I_D ($n = 18$), and I_K ($n = 29$) are plotted as a function of test potential in Fig. 8A; the solid lines reflect the best Boltzmann fits to the averaged data. Mean $V_{1/2}$ (and k) values for I_A , I_D , and I_K are 6 ± 6 mV ($k = 16 \pm 1$ mV), 12 ± 8 mV ($k = 11 \pm 1$ mV), and 12 ± 5 mV ($k = 14 \pm 2$ mV), respectively. The activation threshold of I_A (approximately -50 mV) is slightly more hyperpolarized than that of either I_D or I_K ; both I_D and I_K begin to activate around -40 mV. In addition, although all three currents activate over similar voltage ranges, the $V_{1/2}$ of I_A (6 ± 6 mV) is significantly more hyperpolarized than the $V_{1/2}$ values of either I_D (12 ± 8 mV; $P < 0.05$) or I_K (12 ± 4 mV; $P < 0.002$).

The voltage dependences of steady state inactivation of I_A , I_D , and I_K were examined during voltage steps to +30 mV presented after 15- to 20-s conditioning prepulses to potentials between -90 and $+10$ mV (protocol shown in Fig. 8B, *inset*). Protocols were repeated under control conditions and in the presence of 4-AP or DTX. Subtracted current records (such as those described above and illustrated in Figs. 3 and 5) were obtained to isolate I_A and I_D . Current amplitudes recorded during the +30-mV steps were measured at the peak of subtracted records for I_A ($n = 9$) and I_D ($n = 5$) and at the plateau (150 ms) of the current remaining in 5 mM 4-AP for I_K ($n = 5$). I_A , I_D , and I_K conductances then were calculated for each conditioning potential and normalized to their respective conductance values after the conditioning step to -90 mV. Mean normalized I_A , I_D , and I_K conductances are plotted with respect to conditioning potential in Fig. 8B; the continuous lines represent the best

Boltzmann fits to the averaged data. Mean $V_{1/2}$ (and k) values for I_A , I_D , and I_K are -38 ± 3 mV ($k = -8 \pm 2$ mV), -21 ± 4 mV ($k = -9 \pm 3$ mV), and -26 ± 2 mV ($k = -13 \pm 4$ mV), respectively. All three currents are sensitive to steady state inactivation over similar voltage ranges, although the $V_{1/2}$ of I_A (-38 ± 3 mV) is significantly ($P < 0.005$) more hyperpolarized than the $V_{1/2}$ values of either I_D or I_K . In addition, these experiments revealed that $\sim 20\%$ of the plateau current (I_K) does not inactivate. The finding that steady state inactivation of the remaining 4-AP-insensitive current (i.e., the inactivating current) is well described by a single Boltzmann, suggests that the two inactivation time constants (475 and 3,128 ms; Fig. 8C) reflect inactivation of the same population of I_K channels.

Recovery from steady state inactivation of I_A , I_D , and I_K

To examine the time dependences of recovery from steady state inactivation of I_A , I_D , and I_K , cells first were depolarized to 0 mV for 12 s to inactivate the currents (longer depolarizations did not lead to further inactivation), subsequently hyperpolarized to -70 mV for varying times ranging from 0 to 5,000 ms to allow recovery, and, finally, stepped to $+30$ mV for 160 ms to activate the currents; the protocol is displayed below the current records in Fig. 9A. Peak (3 ms) and plateau (150 ms) control current amplitudes after each recovery time were measured, normalized to their respective current amplitudes after the 5-s recovery, and plotted as a function of recovery time (Fig. 9B). The normalized recovery data for both the peak and plateau currents were well fitted by the sum of two exponentials (Fig. 9B, solid lines). For this cell, recovery taus for the peak current were 36 and 738 ms; recovery time constants for the plateau current were 73 and 618 ms. Similar results were obtained in eight cells; mean recovery time constants were 38 ± 7 and 670 ± 202 ms for the peak current and 79 ± 26 and 734 ± 203 ms for the plateau current.

The slower time constant for recovery of the peak (670 ± 202 ms) and plateau (734 ± 203 ms) currents are not significantly different (Student's *t*-test), suggesting that both time constants reflect recovery of the same current component. Because I_D is the only one of the three voltage-gated K^+ currents in CP cells that contributes to both the peak and plateau currents, it seemed quite likely that the slower time constants of recovery of both the peak and the plateau currents reflect recovery of I_D . To test this hypothesis, recovery of I_D was examined directly. The protocol outlined above was completed first in control bath solution and subsequently in the presence of $50 \mu\text{M}$ 4-AP. Analyses of the rates of recovery of the subtracted currents (I_D) revealed that recovery was well described by a single exponential in all cells examined. The mean recovery time constant for I_D was found to equal 588 ± 274 ms ($n = 8$), a value not significantly different (ANOVA) from the slower component of recovery of the peak (670 ± 202 ms) and plateau (734 ± 203 ms) components of the control currents. These results confirm the hypothesis (above) that the slow time constants of recovery of the peak and plateau currents reflect recovery of the same population of (I_D) channels. Because only I_D and I_K contribute to the plateau currents, the fast component of recovery of the plateau current (mean tau of 79 ± 26 ms) must reflect recovery of I_K . The fast time constant for recovery of the peak (38 ± 7 ms), therefore, reflects I_A recovery. The waveforms of the currents recorded after brief recovery times are consistent with this assertion. As illustrated in Fig. 9A, the current recorded after 10 ms recovery (arrow) activates and inactivates rapidly and is indistinguishable from I_A isolated using 4-AP or the conditioning prepulse

protocol (see Figs. 3E and 4C). Similar results were obtained in experiments completed on six other cells. The mean activation and inactivation time constants for the currents recorded (at +30 mV) after 10-ms recovery at -70 mV were determined to be 0.76 ± 0.20 ms and 21.6 ± 12 ms, respectively. For comparison, the activation and inactivation time constants (at +30 mV) for I_A isolated using 4-AP were 0.83 ± 0.24 ms and 19.2 ± 3.8 ms, respectively. These values are not significantly different; this is consistent with the interpretation that the fast component of recovery of the peak current reflects I_A .

Discussion

The results presented here demonstrate the presence of three kinetically and pharmacologically distinct Ca^{2+} -independent, voltage-gated K^+ currents in isolated, identified postnatal day 7–16 CP visual cortical neurons. These cells were identified after in vivo retrograde labeling at postnatal day 5 (MATERIALS AND METHODS). Because many more cells in immature, than in mature, rat visual cortex make projections across the corpus callosum (Olavarria and Van Sluyters 1985; J. M. Nerbonne and A. Burkhalter, unpublished observations), some of the cells studied here are not destined to be CP cells in the adult. It is important to emphasize, however, that CP cells were selected for the present studies to represent “regular-spiking” cortical neurons rather than because they project across the callosum. Because neurons that retract their callosal projections during postnatal development do not die or undergo changes in morphology or transmitter phenotype (Olavarria and Van Sluyters 1985; J. M. Nerbonne and A. Burkhalter, unpublished observations), both the cells that retain and the cells that retract their callosal projections were expected to display regular-spiking behavior. As demonstrated in the subsequent paper (Locke and Nerbonne 1997), current-clamp recordings have revealed that this hypothesis is correct, i.e., all of the cells labeled in early postnatal animals based on the callosal projection are regular-spiking cortical neurons.

The Ca^{2+} -independent, voltage-gated K^+ currents characterized here in CP neurons are referred to as I_A , I_D , and I_K because their properties are similar, although not identical, to depolarization-activated K^+ currents termed I_A , I_D (or slow transient), and I_K in other mammalian neurons (Rudy 1988; Storm 1990). We emphasize that the properties of I_A , I_D , and I_K vary in different cell types (see below for further discussion) and that these names were selected to emphasize qualitative similarities. Although I_A , I_D , and I_K were identified readily in all CP cells studied ($n = 40$), the absolute amplitudes and densities of the individual current components varied markedly among cells. Mean I_A , I_K , and I_D densities determined in the experiments here were 215 ± 83 , 173 ± 94 , and 54 ± 31 pA/pF, respectively. On average, therefore, the densities of both I_A and I_K are approximately four times the density of I_D . In addition, the relative $I_A:I_D:I_K$ density varies among cells; this variability underlies the observed differences in the waveforms of the total Ca^{2+} -independent outward currents among CP neurons (Fig. 1). It is important to note that, because the experiments here were performed on postnatal (day 7–16) CP neurons, it is certainly possible that the densities (and/or the detailed properties) of I_A , I_D , and I_K in adult CP cells are different. Further experiments will be necessary to explore this possibility directly.

Functionally, I_A and I_D underlie the peak outward currents in CP cells (Fig. 1); because activation is slow, I_K does not contribute appreciably to the peak. I_K , however, together with I_D , determines the plateau current amplitudes in CP cells (Fig. 1); I_A inactivates rapidly and, therefore, does not also contribute to the plateau currents. It is important to note that, because the goal of the experiments here was to define the detailed time- and voltage-dependent properties of the K^+ currents expressed in CP neurons, recordings were obtained only from neurons without extensive processes (to maintain adequate spatial control of the membrane voltage), and analyses were completed only on those cells that behaved as a single electrical compartment (i.e., cells with capacitive transients well described by single exponentials). The currents separated and characterized here, therefore, are assumed to reflect only the activation of voltage-gated K^+ channels expressed in CP cell soma. Depolarization-activated K^+ channels expressed predominantly or exclusively in dendrites are likely to be overlooked. It is certainly possible, therefore, that novel channel types are expressed in CP cell dendrites or that the relative densities of the K^+ channels in dendrites are different from those in cell bodies. Alternative experimental approaches will be needed to explore these possibilities directly.

Comparison of I_A , I_D , and I_K in CP neurons to currents in other mammalian neurons

Rapidly activating and inactivating currents, similar to I_A in CP neurons, have been characterized in neurons from rat hippocampus (Ficker and Heinemann 1992; Segal and Barker 1984; Storm 1988), dentate gyrus (Beck et al. 1992), neostriatum (Surmeier et al. 1989), lateral geniculate nucleus (Budde et al. 1992), thalamus (Huguenard et al. 1991), sensorimotor cortex (Foehring and Surmeier 1993; Hamill et al. 1991), and visual cortex (Albert and Nerbonne 1995; Foehring and Surmeier 1993); the guinea pig (Numann et al. 1987) and embryonic mouse (Wu and Barish 1992) hippocampus; and cat sensorimotor cortex (Spain et al. 1991). The time- and voltage-dependent properties and the pharmacological sensitivities of the currents termed I_A in these different preparations are similar. In general, for example, I_A activates rapidly at all test potentials, and activation is voltage dependent. Inactivation, although also rapid, is voltage independent (but see Beck et al. 1992; Budde et al. 1992; Spain et al. 1991), and I_A is blocked by millimolar concentrations of 4-AP (but see Spain et al. 1991). In most cells, the threshold for I_A activation lies between -65 and -50 mV, and steady state inactivation of I_A is characterized by a $V_{1/2}$ between -60 and -80 mV. In CP neurons, however, the activation threshold (approximately -50 mV) and the $V_{1/2}$ values (approximately -40 mV) for steady state inactivation of I_A are somewhat more depolarized than the values reported for many other cells. Similar voltage-dependent properties, however, have been described previously for I_A in rat cortical (Albert and Nerbonne 1995; Hamill et al. 1991) and neostriatal (Surmeier et al. 1989) neurons. The differences in the voltage-dependent properties of I_A in different cell types may reflect molecular heterogeneity in I_A channels or cell type-specific posttranslational regulation of I_A channel properties. Alternative experimental strategies will be needed to distinguish between these (and other) hypotheses.

Slow transient currents, which are distinct from I_A and I_K and often are referred to as I_D , have been identified in neurons from rat prefrontal cortex (Hammond and Crepel 1992), neostriatum (Surmeier et al. 1991), hippocampus (Ficker and Heinemann 1992), visual

cortex (Albert and Nerbonne 1995; Foehring and Surmeier 1993), globus pallidus (Stefani et al. 1995) and dorsal root ganglia (DRG) (Stansfeld et al. 1986) and from rat sensorimotor cortex (Foehring and Surmeier 1993), guinea pig lateral geniculate nucleus (McCormick 1991) and DRG (Penner et al. 1986), and mouse hippocampus (Wu and Barish 1992). In general, slow transient currents activate fast on membrane depolarization, inactivate slowly and are blocked by micromolar concentrations of 4-AP and often by DTX (Foehring and Surmeier 1993; Penner et al. 1986; Stansfeld et al. 1986; Surmeier et al. 1991). Inactivation is voltage independent, and best described by the sum of two exponentials with time constants of 100–500 ms and 2–7 s (Ficker and Heinemann 1992; McCormick 1991; Wu and Barish 1992). The $V_{1/2}$ of steady state inactivation of slow transient currents usually occurs between –95 and –60 mV; I_D channel availability, therefore, can be influenced by changes in the membrane potential around rest. In the experiments here, however, I_D in P7–P16 CP neurons is sensitive to steady state inactivation only at potentials positive to approximately –60 mV. It is certainly possible that the voltage-dependent properties of I_D vary during postnatal development and that the $V_{1/2}$ for steady state inactivation of I_D is more hyperpolarized in adult CP neurons. Further experiments will be necessary to test this hypothesis directly.

In some preparations, slow transient currents begin to activate between –75 and –60 mV, i.e., at potentials hyperpolarized to the action potential threshold (McCormick 1991; Storm 1988; Surmeier et al. 1991) and, as described originally in hippocampal neurons (Storm 1988), the activation of I_D in these cells controls the “delay” to firing in response to threshold current injections. In other preparations, however, slow transient currents begin to activate at more depolarized potentials: I_D in neonatal rat SCP neurons (Albert and Nerbonne 1995) and embryonic mouse hippocampal neurons (Wu and Barish 1992), I_{slow} in embryonic rat hippocampal neurons (Ficker and Heinemann 1992), I_{km} in rat LGN neurons (Budde et al. 1992) for example, begin to activate around –35 mV, similar to I_D in CP neurons (threshold approximately –40 mV; Fig. 8A). The depolarized activation thresholds of these currents suggest that they may contribute less to controlling delay to firing an action potential in response to depolarizing inputs than I_A . One factor underlying the differences in activation thresholds in different studies may be the age of the animals from which the cells were obtained: currents with depolarized activation thresholds were recorded from embryonic or neonatal neurons, whereas the currents with hyperpolarized activation thresholds were recorded from adult neurons. It is possible, therefore, that the activation threshold of I_D shifts toward more hyperpolarized potentials during development and that the functional role of I_D in adult CP (and other) neurons includes controlling the delay to firing in response to threshold depolarizing inputs. Further experiments aimed at examining the properties and densities of I_D in CP (and other) neurons isolated at later stages of development will be necessary to test this hypothesis directly.

The slowly activating, TEA-sensitive K^+ current, which we have termed I_K , in CP neurons closely resembles delayed rectifier type-currents described in neurons from rat thalamus (Huguenard and Prince 1991), sensorimotor cortex (Foehring and Surmeier 1993; Hamill et al. 1991), dentate gyrus (Beck et al. 1992), lateral geniculate nucleus (LGN) (Budde et al. 1992), hippocampus (Segal and Barker 1984), globus pallidus (Stefani et al. 1995), and

visual cortex (Albert and Nerbonne 1995; Foehring and Surmeier 1993) and from guinea pig hippocampus (Numann et al. 1987). As in CP neurons, the threshold of I_K activation in these cells is between -45 and -35 mV, i.e., positive to that of I_A described in the same cells. Activation ($\tau \sim 10$ – 30 ms at 0 mV) and inactivation (τ more than ~ 1 s) of I_K are slow, and, in some preparations, inactivation is best described by two exponentials (Beck et al. 1992; Huguenard and Prince 1991) or is incomplete even during prolonged depolarizing voltage steps (Beck et al. 1992; Foehring and Surmeier 1993; Hamill et al. 1991; Huguenard and Prince 1991; Numann et al. 1987). In CP neurons, inactivation of I_K is also slow and biexponential ($\tau_{\text{fast}} = 475$ and $3,128$ ms) and, in addition, $\sim 20\%$ of I_K is noninactivating. The finding that the voltage dependences of the inactivating components of the 4-AP-insensitive currents are the same (Fig. 9B) suggests that the biexponential kinetics reflects the gating properties of the underlying I_K channels. In many preparations, the rates of recovery of I_K from steady state inactivation are substantially slower than for I_A or I_D (in the same cells) and generally proceed during a time course of 100 – 500 ms (but see Hamill et al. 1991; Huguenard et al. 1991). In CP neurons, however, I_K recovers from inactivation somewhat more rapidly (79 ± 26 ms at -70 mV) and is similar therefore to I_K in rat LGN neurons (116 ms at -90 mV) (Budde et al. 1992). The voltage dependence of steady state inactivation of I_K varies widely among cell types with $V_{1/2}$ values ranging from -87 mV in guinea pig hippocampal cells (Numann et al. 1987) to -49 mV in rat LGN neurons (Budde et al. 1992). Additional experiments will be necessary to determine whether the differences in the time- and voltage-dependent properties of the delayed rectifier currents in various preparations reflect the expression of distinct types of I_K channels or cell type-specific regulation of the properties of a single type of I_K channel.

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Fig. 1. Waveforms of Ca^{2+} -independent, depolarization-activated K^+ currents in callosal-projecting (CP) visual cortical neurons vary among cells. Whole cell K^+ currents displayed in this and in subsequent figures were evoked as described in METHODS, unless stated otherwise. Currents plotted in A–C were recorded from 3 different CP cells isolated at postnatal day 9 ~10 h after plating; the cells in A and C were in the same preparation.

Fig. 2.

Increasing concentrations of 4-aminopyridine (4-AP) block increasing amounts of the peak, but not of the plateau, current. Currents were recorded in control bath solution and in the presence of varying concentrations of 4-AP. Current records displayed in *A* were obtained by digital subtraction of the current waveforms recorded in 4-AP from the control records in the same cell. Peak and plateau current amplitudes in individual cells were measured at 3 and 150 ms, respectively, after the onset of depolarizing voltages to +30 mV, and the percentages of the peak and plateau currents blocked by 4-AP were determined. *A*: representative waveforms of the currents blocked by 50 μ M (1), 100 μ M (2), 500 μ M (3), and 5 mM 4-AP (4). Note that the records in 1 and 4 are from the same cell; the records in 2 and 3 are from 2 other cells. *B*: mean $\pm$ SD percentages of the peak and plateau currents blocked by increasing concentrations of 4-AP. *n* values refer to the number of cells studied.

Fig. 3. Separation of 2 distinct 4-AP-sensitive current components from the total Ca^{2+} -independent, outward currents. After control currents (A) were recorded, the cell was 1st exposed to 50 μM and then to 5 mM 4-AP. Currents recorded in the presence of 50 μM and 5 mM 4-AP are displayed in B and C, respectively. Currents blocked by 50 μM 4-AP (D) were obtained by subtraction of records in B from those in A. The 5 mM, but not 50 μM , 4-AP-sensitive currents (E) were obtained by subtraction of records in C from those in B. Note that the currents blocked by low concentrations of 4-AP inactivate much more slowly than the currents sensitive only to the high concentration of 4-AP. Similar results were obtained on 13 cells.

Fig. 4.

I_A also can be isolated using a conditioning prepulse protocol. Currents were recorded during 160-ms voltage steps to potentials of 0, +20, +40, and +60 mV either directly from a holding potential of -70 mV (A) or after a 50-ms prepulse to 0 mV (B); the protocol is illustrated below the records. Current inactivated by the 50-ms prepulse to 0 mV (C) was obtained by subtraction of the records in B from those in A. Similar results were obtained in experiments conducted on 8 other cells.

Fig. 5. Rapidly activating, slowly inactivating K^+ current (I_D) also is blocked by α -dendrotoxin (α -DTX). Currents were recorded before (A) and after superfusion of 100 nM α -DTX (B). DTX-sensitive currents (C), obtained by subtraction of the records in B from those in A, activate rapidly and inactivate slowly, and are similar, therefore, to the currents blocked by 50 μ M 4-AP (Fig. 3D). Similar results were obtained on 10 other cells.

Fig. 6. Tetraethylammonium (TEA) blocks a slowly activating current (I_K) in CP neurons. Currents were recorded under control conditions (A) and after superfusion of 30 mM TEA (B). Current blocked by TEA (C) was obtained by subtraction of records in B from those in A. Similar results were obtained on 7 other cells.

Fig. 7.

Kinetics of activation (A and B) and inactivation (C and D) of I_A , I_D , and I_K are distinct. A and B: time constants (τ) of activation for I_A and I_D were determined from single exponential fits to the rising phases of the currents in subtracted records, such as those in Fig. 3, D and E, respectively. For I_K , activation τ were determined from fits to the rising phases of currents remaining in 5 mM 4-AP in records such as those in Fig. 3C. Typical fits for I_A , I_D , and I_K are illustrated in B. Mean $\pm$ SD activation τ s for I_A , I_D , and I_K are plotted as a function of test potential (note that the y-axis scale is different for the I_K data). Continuous lines are best fits to the averaged data using $\tau(V) = a [\exp(V_m/b)] + c$ (see text). C: time constants (τ s) of inactivation were determined from exponential fits to the decay phases of currents recorded during 12-s voltage steps to 0, 10, 20, and 30 mV from a holding potential of -70 mV in control bath solution and in the presence of 8 mM 4-AP (see text).

Mean $\pm$ SD inactivation τ_s for I_A ($n = 7$), I_D ($n = 7$), and I_K ($n = 7$) are plotted as a function of test potential. Typical fits are shown in *D*.

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Fig. 8.

I_A , I_D , and I_K display similar voltage dependences of activation (A) and steady state inactivation (B). To examine the voltage dependences of current activation, I_A , I_D , and I_K conductances at each test potential in individual cells were calculated from records such as those in Fig. 3, C–E, using the experimentally determined reversal potentials. Conductances at each test potential were normalized to their respective conductance values at +60 mV (in the same cell). Mean normalized conductances are plotted as a function of test potential and the Boltzmann fits to the averaged data are shown (—). B: to examine the voltage dependences of steady state inactivation, I_A , I_D , and I_K amplitudes, evoked at +30 mV after 15- to 20-s conditioning prepulses to potentials between -90 and +20 mV were measured; inset: protocol is illustrated. Using the experimentally determined reversal potentials, conductances then were calculated and normalized to their respective conductance values after the -90 mV conditioning prepulse. Mean normalized I_A ($n = 9$), I_D ($n = 5$), and I_K ($n =$

5) conductances are plotted as a function of conditioning potential, and the Boltzmann fits to the averaged data are shown (—).

Fig. 9.

Rates of recovery of I_A , I_D , and I_K are distinct. After inactivating the currents by a 12-s prepulse to 0 mV, the cell was hyperpolarized to -70 mV for times ranging from 0 to 5,000 ms before a test depolarization to +30 mV. Typical current waveforms recorded during the +30 mV depolarization after variable (0–5,000 ms) recovery periods are displayed in A; protocol is beneath the records. Note that the current recorded after a 10 ms recovery time at -70 mV (•) activates and inactivates rapidly (see text). B: amplitudes of the peak and plateau currents evoked at +30 mV after each recovery period were measured, normalized to their respective (peak and plateau; ■) amplitudes after a 5,000-ms recovery period and plotted as a function of time. Recovery of the peak and plateau currents was best described by the sum of 2 exponentials (—). Inset: initial phase of recovery of both the peak and the plateau currents is shown on an expanded time scale. Similar results were obtained on 7 cells.