Calcium-Activated Cation Nonselective Current Contributes to the Fast Afterdepolarization in Rat Prefrontal Cortex Neurons

SAMIR HAJ-DAHMANE AND RODRIGO ANDRADE
Department of Psychiatry and Behavioral Neurosciences and Cellular and Clinical Neurobiology Training Program, Wayne State University School of Medicine, Detroit, Michigan 48201

Haj-Dahmane, Samir and Rodrigo Andrade. Calcium-activated cation nonselective current contributes to the fast afterdepolarization in rat prefrontal cortex neurons. J. Neurophysiol. 78: 1983–1989, 1997. Pyramidal cells of layer V in rat prefrontal cortex display a prominent fast afterdepolarization (fADP) following an action potential. This ADP is blocked by replacing extracellular calcium with magnesium, by the application of the calcium-channel blocker cadmium, and by buffering intracellular calcium at near physiological levels. Thus this fast ADP appears mediated by a calcium-activated current. A prominent ADP is also observed following a calcium spike recorded in the presence of tetrodotoxin. The current underlying this ADP was recorded using a hybrid current-voltage protocol. A strong ADP could be observed in the presence of potassium channel blockers as well as at ECl. Furthermore, the current underlying the ADP increased with hyperpolarization in the subthreshold range and displayed an extrapolated reversal potential near +30 mV. Reducing the ratio of extracellular to intracellular sodium inhibited the current underlying the ADP and caused a hyperpolarizing shift in its reversal potential. We conclude that these cells express a calcium-activated cation nonselective current whose activation contributes to the generation of the fADP. This current could play an important role in determining the firing properties of pyramidal cells in cortex.

INTRODUCTION

Pyramidal cells of the cerebral cortex express a variety of ionic currents that shape their firing patterns in response to depolarizing stimuli. Pyramidal cells have been divided into two general classes based on their firing pattern in response to depolarizing stimuli; regular spiking and intrinsically bursting neurons (for review see Connors and Gutnick 1990). Since the initial classification (Connors et al. 1982; McCormick et al. 1985), it has become clear that regular spiking cells do not constitute an homogeneous functional class, and there have been several attempts to subdivide this class of cells into two or more subclasses (Amitai 1994; Chagnac-Amitai and Connors 1989; de la Peña and Geijo-Barrientos 1996; Foehring et al. 1991; Kang and Kayano 1994; Mason and Larkman 1990; Yang et al. 1996).

One prominent feature of a subgroup of regular spiking cells is their ability to fire an initial doublet or triplet of action potentials followed by slowly adapting tonic spiking when depolarized from rest (Foehring et al. 1991; Kang and Kayano 1994; Montoro et al. 1988; van Brederode and Snyder 1992; Yang et al. 1996). This firing pattern has been called phasic-tonic firing (Montoro et al. 1988; van Brederode and Snyder 1992), a nomenclature that will be used here. This firing pattern places these cells in an intermediate position between regular spiking and intrinsically bursting neurons, even as they are generally, but not always (Foehring and Waters 1991; Yang et al. 1996), classified in the first subtype. The ionic mechanism underlying this firing behavior is incompletely understood.

In layer V of the rat medial prefrontal cortex, a large proportion of our sharp microelectrode and tight cell recordings come from cells that exhibit this intermediate, phasic-tonic, firing pattern (Andrade 1991; Haj-Dahmane and Andrade 1996). During the course of unrelated experiments, we noticed the presence of a calcium-activated cation nonselective current in these cells. This current is likely to participate in the mechanism responsible for the fast afterdepolarization (fADP) and phasic firing seen in these cells. As such, this current is likely to contribute to their firing pattern displayed by these cells in response to depolarizing stimuli. Thus the expression of this current might be one of the defining characteristics of this subtype of regular spiking neuron. We now report on these findings.

METHODS

Whole cell recordings were obtained from pyramidal neurons of the rat medial prefrontal cortex as previously described (Haj-Dahmane and Andrade 1996). Briefly, adult male albino rats (200–250 g) were anesthetized with halothane and killed by decapitation. The brain was then removed, cooled, and blocked to isolate the anterior forebrain. Brain slices were cut at a thickness of 400 μm starting at the anterior pole of the brain and continuing to the genu of the corpus callosum. All the cutting procedure was conducted in ice-cold Ringer of standard composition (in mM: 119 NaCl, 2.5 KCl, 1.3 MgSO4, 2.5 CaCl2, 26.2 NaH2PO4, 11 glucose) bubbled to saturation with 95% O2–5% CO2. The freshly cut slices were transferred to a recovery chamber where they were maintained at room temperature on filter paper saturated with Ringer under a moist atmosphere of 95% O2–5% CO2. After at least 1 hr in this recovery chamber, slices were transferred, one at a time, to a recording chamber for the experiment. In the recording chamber the slice was continuously perfused with Ringer at 30°C while held suspended between two nylon nets (Nicoll and Alger 1981).

All recordings were obtained from layer V of the anterior cingulate subdivisions of the rat medial prefrontal cortex (Krettek and Price 1977) using the blind patch technique (Blanton et al. 1989). Electrodes were pulled from 1.2 mm OD glass (Glass Company of America) using a P97 puller (Sutter Instruments) to give resistances of 5–8 MΩ when filled with the appropriate intracellular solution. Recordings were obtained using either potassium- or cesium-based intracellular solutions. The composition for the potassium-based intracellular solution was (in mM) 120 KMeSO4, 5 KCl, 5 NaCl, 1 MgCl2, 10 N-2-hydroxyethylpiperazine- N’-2-eth-

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anesulfonic acid (HEPES), 0.02 ethylene glycol-bis(β-aminethyl ether) -N,N,N',N'-tetraacetic acid (EGTA), 2 ATP disodium salt, 0.5 guanosine triphosphate (GTP) sodium salt, pH 7.35. In some experiments where it was necessary to strongly buffer intracellular calcium, the EGTA was omitted and 10 mM bis-(o-aminophenoxy) -N,N',N'-tetraacetic acid (BAPTA) and 1 mM or 4 mM calcium were added to the intracellular solution. The free calcium concentration in these solutions, estimated using a standard algorithm (Schoenmakers et al., 1992), is ~10 and 100 nM, respectively. These values are slightly lower (10 nM) than and overlapping with (100 nM) the free calcium concentration of pyramidal cells at rest determined using calcium indicators (Yuste et al. 1994). The composition for the cesium-based intracellular solution was (in mM) 130 CsOH, 5 NaCl, 1 MgCl₂, 10 HEPES, 0.02 EGTA, 2 ATP disodium salt, 0.5 GTP sodium salt, and the pH was adjusted to 7.35 using gluconic acid. In some experiments the NaCl concentration was raised to 25 mM and the CsOH concentration reduced accordingly. All experiments were conducted using an Axoclamp 2A amplifier (Axon Instruments). Data were acquired using a 12-bit A/D converter under the control of an Intel processor-based computer running pClamp 5.5 (Axon Instruments). All voltage-clamp experiments were conducted using the discontinuous voltage-clamp mode of the amplifier. In these experiments the amplifier’s headstage output was monitored throughout the experiment to ascertain full decay of the transient between current injection cycles. The data were analyzed and plotted using Origin (versions 3.8 and 4.0, Microcal Software).

Several modified Ringer solutions were used in this study. To study the effect of calcium on the fADP, either extracellular cadmium (100 μM) was added to the Ringer or calcium was reduced by replacing it with magnesium on an equimolar basis. In some experiments it was desired to produce substantial reductions in extracellular calcium. For these experiments, cells were patched under control conditions but switched shortly thereafter from the Ringer to a modified control extracellular solution (composition in mM: 130 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 10–20 HEPES, 11 glucose, pH 7.4, bubbled to equilibrium with 100% O₂). This modified extracellular solution still contained high sodium but relied on HEPES rather than bicarbonate for pH buffering. The reduction in extracellular sodium was accomplished by changing this modified extracellular solution for one built along the same principle but where all but 10 mM sodium had been replaced by N-methyl-D-glucamine. The osmolarity of this solution was matched to that of the control solution using sucrose. This procedure allowed us to reduce sodium to a greater level than possible by simply replacing NaCl in the control Ringer. In some experiments the cells were directly switched from the Ringer to the low-sodium extracellular solution without affecting the results of the experiment. In those experiments aimed at examining the reversal potential of I_fADP, 2 mM cesium and 200 μM barium were included in the extracellular solution to minimize I_h and any residual inwardly rectifying potassium currents.

All drugs were administered in the bath dissolved at known concentrations. Most drugs and reagents were obtained from Sigma or Fisher. KMeSO₄ was purchased from ICN and tetrodotoxin (TTX) from Calbiochem.

**RESULTS**

As previously reported, most whole cell recordings obtained from layer V of medial prefrontal cortex are from cells that can be classified morphologically as pyramidal neurons (Haj-Dahmane and Andrade 1996). These cells exhibit the general electrophysiological characteristics of regular spiking neurons including spike firing adaptation in response to a long suprathreshold depolarizing stimulus (Fig. 1). A notable characteristic seen in most layer V pyramidal cells in this region is their ability to fire 2–3 spikes in short succession at the onset of the pulse when stimulated from rest (approximately −70 to −75 mV, Fig. 1). We refer to this firing pattern as phasic- tonic firing (Kang and Kayano 1994; Montoro et al. 1988; van Brederode and Snyder 1992). Similar observations have also been made by others in rat prefrontal cortex (Yang et al. 1996) as well as other areas of cortex (Amitai 1994; Foehring et al. 1991; Foehring and Wyler 1990; Mason and Larkman 1990; Montoro et al. 1988; van Brederode and Snyder 1992).

Clues as to the mechanism underlying this spiking behavior can be gathered by examining the spiking behavior of these cells around threshold (Fig. 2). Application of subthreshold current pulse from rest (generally −70 to −75 mV) results in an initial transient depolarization that sags over time. Previous studies have shown that this transient depolarization results primarily from the activation of an A-Type calcium current and I_h (Foehring and Waters 1991; Sutor and Zieglgansberger 1987). Increasing the stimulus strength triggers an action potential that takes off from the transient depolarization. The action potential is followed by a fast afterhyperpolarization, which is followed by an ADP. We call this ADP the fADP to distinguish it from the slow ADP (sADP) induced by muscarinic receptor activation in these cells (Andrade 1991). Under our recording conditions, fADPs are seen in the vast majority of layer V pyramidal cells of rat prefrontal cortex (30 of 30 cells recorded with a KMeSO₄-based intracellular solution in the present study).

Similar observations have been made for pyramidal cells in other regions of cortex (e.g., Connors et al. 1982; Foehring and Wyler 1990; Friedman and Gutnick 1987; Kang and Kayano 1994; Mason and Larkman 1990; Sutor and Ziegl-gansberger 1987; van Brederode and Snyder 1992; Yang et al. 1996).

Increasing the stimulus strength even further results in the triggering of the action potential earlier during the pulse. Eventually the fADP, riding on the current-induced depolarization, reaches threshold and triggers a second action potential. This doublet of action potentials is followed by an afterhyperpolarization that prevents further phasic firing. Previous studies have shown that this afterhyperpolarization is the result of several distinct potassium currents (Schwindt et al. 1988a,b). The importance of these potassium currents in curtailing regenerative firing is underscored by the observation that, if potassium channels are inhibited by intracellular application of cesium, a brief depolarizing pulse can trigger sustained spiking activity (Fig. 2B, n > 20 cells). The braking effect of the afterhyperpolarization, however, is limited. Increasing the stimulus intensity above that needed to trigger the first spike doublet or triplet results in the recruitment of additional action potentials to produce the characteristic phasic- tonic firing pattern illustrated in Fig. 1.

In the remainder of this paper, we examine the ionic mechanism responsible for the fADP. Previous studies on cortical pyramidal cells have shown that calcium currents are a major determinant of the fADP and the early spiking elicited by depolarizing stimuli in cortex (Foehring and Waters 1991; Friedman and Gutnick 1987; Yang et al. 1996) but not in hippocampus (Azouz et al. 1996). Therefore we examined the effect of lowering extracellular calcium on the fADP.
As illustrated in Fig. 3 (A and B), lowering extracellular calcium reversibly inhibited the fADP (n = 2 cells tested). A similar effect was observed when calcium influx into the cell was blocked by administration of the nonselective calcium channel blocker cadmium (100 μM, n = 3 cells tested). These results indicated that the fADP was calcium dependent.

The calcium dependence could reflect either of two mechanisms in the generation of I\textsubscript{fADP}. Calcium itself could be the charge carrier, in which case I\textsubscript{fADP} must be a calcium current. Alternatively, calcium influx, by increasing intracellular calcium, could activate a current to generate the fADP. In this case, I\textsubscript{fADP} would be a calcium-activated current. To distinguish between these two possibilities, we recorded with an intracellular solution containing 10 mM BAPTA and added calcium to buffer free intracellular calcium at either 10 or 100 nM. As illustrated in Fig. 4, buffering of intracellular calcium completely blocked the appearance of I\textsubscript{fADP} (n = 4 cells tested with 10 nM free calcium, n = 5 cells tested with 100 nM free calcium). This was not simply due to a nonspecific inhibition of calcium influx since the transient depolarization, which reflects in part T-type calcium current activation, was still observed under these conditions (n = 9 cells, Fig. 4, inset b). Buffering intracellular calcium at 100 nM had little effect on the passive membrane properties of the cell (Fig. 4, inset). However, buffering intracellular free calcium at 10 nM resulted in a reduction in membrane resistance and time constant.

Because the fADP was fast and relatively small under physiological conditions, we searched an alternative way to visualize the underlying current (I\textsubscript{fADP}). One procedure that proved helpful was to trigger the fADP using calcium spikes. As illustrated in Fig. 5 (left), when cells were recorded with a cesium-based intracellular solution in the presence of TTX (1 μM), depolarizing pulses could be used to trigger all or none of the calcium spikes that were followed by an ADP. As could be expected, this ADP was larger and longer lasting than the fADP following a single sodium spike. However, like the fADP following a single sodium spike, this ADP was blocked by buffering intracellular calcium (n = 6 cells), whereas bath administration of cadmium (100 μM) completely blocked the calcium spike and the ADP (n = 4 cells). We interpret the ADP following a calcium spike to reflect a magnified form of fADP seen after a single sodium spike.

By using a hybrid current/voltage clamp protocol, it was possible to directly measure the current underlying this ADP (I\textsubscript{fADP}). As illustrated in Fig. 5 (right), when the amplifier was switched from current to voltage clamp immediately after the calcium spike, a large inward current (I\textsubscript{ADP}) is recorded. This current was largest immediately after establishing the clamp and decayed (>90%) within ~100–200 ns at room temperature and thus was considerably faster than the sADP seen after muscarinic stimulation in these cells (Andrade 1991). Since the ADP seen in current clamp decayed more slowly than the underlying current (Fig. 5) or the time constant of the membrane, the ADP recorded in cesium would appear to correspond to a plateau potential made possible by a transient equilibrium between depolarizing and hyperpolarizing currents following the spike.

The ability to directly record I\textsubscript{fADP} allowed us to examine its ionic basis. Calcium-activated chloride currents have been previously reported to mediate depolarizing afterpotentials.
(reviewed in Higashi et al. 1993). Their involvement in the fADP, however, seemed unlikely since we routinely recorded $I_{\text{fADP}}$ at or near $E_{\text{Cl}}$ (approximately $-62 \text{ mV}$ for the potassium and $-75 \text{ mV}$ for the cesium-based intracellular solutions). Similarly, the involvement of a calcium-inactivated potassium current (Kramer and Levitan 1988; Kramer et al. 1988) also seemed unlikely since $I_{\text{fADP}}$ could be recorded when the potassium channel blocker cesium was used as the principal cation in the intracellular solution. This suggested that the most likely mechanism for $I_{\text{fADP}}$ was a calcium-activated sodium or cation current.

We first tested this possibility by replacing extracellular sodium with $N$-methyl-$d$-glucamine. As illustrated in Fig. 6, reducing extracellular sodium from 146 mM to 10 mM produced a large reduction in the amplitude of $I_{\text{fADP}}$. In a group of four cells tested using this protocol, $I_{\text{fADP}}$ was reduced from $-1.52 \pm 0.26 \text{ nA}$ to $-0.49 \pm 0.08 \text{ nA}$ ($P < 0.02$).

To further test this possibility, we examined the voltage dependence of $I_{\text{fADP}}$. As illustrated in Fig. 6, the amplitude of this current increased linearly with hyperpolarization in the $-40$ to $-80 \text{ mV}$ range. By extrapolating from this linear range, it was possible to estimate a reversal potential for $I_{\text{fADP}}$. In three cells tested in this way, $I_{\text{fADP}}$ reversed at $+34.6 \pm 13.6 \text{ mV}$ in control (130 mM) extracellular sodium. Reducing extracellular sodium to 10 mM resulted in parallel leftward displacement in the current-voltage ($I-V$) relationship for $I_{\text{fADP}}$ and a shift in its reversal potential to $-18 \pm 1 \text{ mV}$ ($n = 3$ cells). A similar hyperpolarizing shift in the reversal potential for $I_{\text{fADP}}$ was observed when intracellular sodium was increased by including 25 mM NaCl in the recording solution ($E_{\text{rev}} \approx -20 \text{ to } -25 \text{ mV}, n = 2$ cells tested). Reducing extracellular sodium to 10 mM with high internal sodium (25 mM) resulted in a further shift to the left of the $I-V$ relationship for $I_{\text{fADP}}$ ($E_{\text{rev}} \approx -40 \text{ to } -45 \text{ mV}, n = 2$ cells). For technical reasons it was not possible to explore a larger voltage range than that examined in these experiments. At potentials below $-80 \text{ mV}$, the linear relationship deteriorated, possibly because of the difficulty of clamping such a large current with the additional conductance loading it imposed. Steps to potentials greater than or equal to $-40 \text{ mV}$ themselves resulted in calcium influx and the development of an inward current that occluded $I_{\text{fADP}}$.

![Fig. 3. Effect of lowering extracellular calcium or blocking calcium channels on the fADP. A: voltage responses of a pyramidal neuron to a brief suprathreshold stimulus in control condition (left trace), and in calcium-free Ringer solution (middle trace). Bath application of calcium-free Ringer solution completely suppresses the fADP following an action potential. Right traces depict superimposed voltage responses obtained in control and in calcium-free Ringer solutions. Cell resting membrane potential: $-74 \text{ mV}. B$: bath application of a calcium channel blocker, cadmium (100 $\mu$M) strongly reduces the amplitude of the fADP. Cell resting membrane potential: $-72 \text{ mV}$.](http://jn.physiology.org/ by 10220:32 on July 7, 2017 http://jn.physiology.org/)

![Fig. 4. Buffering intracellular calcium suppresses the fADP. Left trace: voltage response of a pyramidal neuron to 30-ms-long suprathreshold stimuli 5 min after breaking into the cell. Inset depicts the voltage deflections elicited by a hyperpolarizing constant-current pulse at this time. Middle trace: response to a suprathreshold stimulus 15 min after breaking the cell. Note that the dialysis of the cell with 10 mM bis-(o-aminophenoxyl)-N,N,N’,N’-tetraacetic acid (BAPTA)/4 mM Ca$^{2+}$ (100 nM estimated free intracellular calcium concentration) abolishes the fADP. Inset a: voltage response to a hyperpolarizing constant-current pulse identical to that used at 5 min. Inset b: voltage response to a depolarizing constant-current pulse 30 min after breaking into the cell. Note the presence of a transient depolarization even at this late time. Traces on the right depict the superimposition of these voltage traces. Insets: 15 min after breaking into the cell. Notice that there is little change in the passive membrane properties of the cell following infusion of BAPTA sufficient to essentially abolish the fADP. Cell resting membrane potential: $-72 \text{ mV}$.](http://jn.physiology.org/ by 10220:32 on July 7, 2017 http://jn.physiology.org/)
was strongly inhibited by buffering intracellular calcium, suggesting that the most likely mechanism was a calcium-activated (or inactivated) current. Consistent with this possibility, calcium spikes triggered in the presence of intracellular cesium and extracellular TTX were found to be able to elicit strong ADP.

The properties of the current underlying the ADP triggered by a calcium spike, \( I_{\text{ADP}} \), were studied using a hybrid current-voltage protocol. A strong \( I_{\text{ADP}} \) could be observed at \( E_C \) under conditions that block potassium channels. This makes it unlikely that either chloride or potassium channels could mediate this current. Examination of \( I_{\text{ADP}} \) as a function of voltage indicated that it increased in amplitude with hyperpolarization and exhibited an extrapolated reversal potential near +30 mV. Similarly, reducing the ratio of extracellular to intracellular sodium reduced the amplitude of the current at rest and shifted its reversal potential in the hyperpolarizing direction. These results indicated that this inward current was most likely carried by sodium.

\( I_{\text{ADP}} \), however, is unlikely to be mediated by a pure sodium current. In these experiments we found that the reversal potential for the \( I_{\text{ADP}} \) was considerably more negative than expected for a pure sodium current both under control conditions (\( E_Na = +67 \) mV) and in reduced extracellular sodium (\( E_Na = 0 \) mV). Instead, the values observed under control conditions and in the presence of low extracellular sodium closely approximated those expected for a current carried by nonselective cation channels exhibiting equal permeabilities for sodium and potassium (assuming no permeability for cesium and a residual intracellular concentration of potassium of 15–20 mM following dialysis with the cesium-based intracellular solution). The reversal potential observed using 25 mM [Na\(^+\)], 10 mM [Na\(^+\)]\(_o\), was also in good agreement with that predicted for such a current. However, that estimated using 25 mM [Na\(^+\)], 130 mM [Na\(^+\)]\(_o\), was more

**Discussion**

Most pyramidal cells of the medial prefrontal cortex display a prominent FADP following an action potential. In the present study we have investigated the ionic mechanisms responsible for this phenomenon. The fADP in this region is reduced by calcium channel blockers as well as reductions in extracellular calcium, a finding that is in agreement with those of others in cortex (Foehring and Waters 1991; Yang et al. 1996). This calcium dependence suggests several possible mechanisms for the fADP including recurrent synaptic excitation (Higashi et al. 1993), a calcium current, or a calcium-activated current. In these experiments the fADP

**Figure 5.** \( I_{\text{ADP}} \) can be measured using a hybrid current-voltage clamp following a calcium spike. A: current-clamp recording of a pyramidal neuron recorded with cesium gluconate–based intracellular solution. A 30-ms suprathreshold stimulus induces a calcium spike followed by a large ADP. B: superimposed traces depicting hybrid current/voltage clamp recording from the same neuron. Calcium spikes were triggered and the amplifier was switched to voltage-clamp mode either 80 ms (●) or 300 ms (●) after the spike. The ADP is associated with a large inward aftercurrent that decays inward current was most likely carried by sodium.

**Figure 6.** \( I_{\text{ADP}} \) is due to the activation of a calcium-activated cation nonselective current. A: \( I_{\text{ADP}} \) recorded using a hybrid clamp protocol in control extracellular solution (130 mM sodium, left), in 10 mM sodium (middle), and following recovery (right). Lowering the extracellular concentration of sodium from 130 to 10 mM strongly reduces the amplitude of the \( I_{\text{ADP}} \) recorded near rest. Holding potential: −71 mV. Holding current: −200 pA. B: examples of current-voltage relationships for \( I_{\text{ADP}} \) recorded with varying intracellular and extracellular sodium concentrations. Filled square, control ([Na\(^+\)]\(_o\) = 130 mM, [Na\(^+\)] = 10 mM); open square, low extracellular sodium ([Na\(^+\)]\(_o\) = 10 mM, [Na\(^+\)] = 10 mM); open circle, high intracellular/low extracellular sodium ([Na\(^+\)]\(_o\) = 10 mM, [Na\(^+\)] = 25 mM). The I-V curves appeared linear between −90 to −40 mV and were extrapolated to estimate the reversal potentials. This figure depicts data obtained from 2 separate cells. I corresponds to the peak \( I_{\text{ADP}} \), whereas V corresponds to the voltage at which the membrane was held immediately after the spike.
hyperpolarized than predicted. We have no explanation for this deviation. All together we interpret these results to indicate that $I_{\text{fADP}}$ is mediated by the activation of a calcium-activated cation nonselective current.

A variety of mechanisms can and have been proposed to account for the fADPs that follow an action potential in central and peripheral neurons. These include recurrently activated GABAergic synapses (Higashi et al. 1993), somatic spread of dendritic excitation (Mainen and Sejnowski 1996), activation of pumps (Friedman et al. 1992), low-threshold calcium (White et al. 1989), and calcium-activated cation nonselective currents. In addition, plateau potentials sometimes similar to the ADP seen after a calcium spike have also been attributed calcium spikes at dendritic hot spots (Reuveni et al. 1993). In rat prefrontal cortex, a strong $I_{\text{fADP}}$ is observed in the presence of TTX while the fADP was strongly inhibited by buffering intracellular calcium. These observations suggest that neither a synaptic mechanism, nor passive current spread from sodium or calcium action potentials in the dendrites can account for the fADP. A role for a electrogenic sodium-calcium exchanger is also unlikely because such a mechanism would predict a voltage-independent current while $I_{\text{fADP}}$ increased with hyperpolarization.

From the results outlined above using calcium spikes, we conclude that pyramidal cells of layer V of rat prefrontal cortex express a calcium-activated cation nonselective current. Since the fADP expressed in these cells is mediated by a calcium-activated current, we propose that this calcium-activated cation nonselective current plays an important role in the generation of the fADP following a sodium spike. However, the present results do not allow us to completely exclude contributions by alternative mechanisms because it is difficult to assess the role of this cation nonselective current in the fADP triggered by a single action potential. As outlined above, there are alternative ionic mechanisms that can generate ADPs similar to those examined in this study. It is possible that some of these can also contribute the fADP seen after an action potential in prefrontal cortex.

Pyramidal cells of layer V of the medial prefrontal cortex often display a distinct firing pattern in response to constant current depolarizing stimuli. This firing pattern is characterized by an initial doublet or triplet of spikes followed by more slowly adapting firing. Cells with similar tonic-phasic firing patterns have been reported for other areas of cortex and have generally, but not always, been classified as a subtype of regular spiking cells. These cells exhibit two electrophysiological characteristics that could in principle account for this firing pattern. First, these cells can fire low-threshold calcium spikes that produce a transient depolarization at the onset of a depolarizing pulse. This transient depolarization could in principle drive the initial fast firing observed in these cells (Friedman and Gutnick 1987). Alternatively, these cells also display a prominent fADP, and summation of these fADPs could generate the initial fast spiking (Yang et al. 1996). Examination of the early portion of the initial phasic firing in the cell population examined in the current study shows that the second and third spikes appear to emerge from this afterpotential. Thus the initial doublet or triplet appears to be, at least in part, the result of self regenerative firing supported by the fADP. This process is self limiting as it is terminated by the activation of potassium current and leads to the tonic firing portion of the response. These observations suggest that the fADP is an important determinant of the firing pattern for these cells. As such, the expression of a calcium-activated cation nonselective current might be one of the defining elements responsible for the phasic-tonic firing pattern observed among pyramidal cells of the cerebral cortex.

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Address for reprint requests: R. Andrade, Dept. of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, 2309 Scott Hall, 540 East Canfield St., Detroit, MI 48201.

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