Voltage-Gated Sodium Channels Shape Subthreshold EPSPs in Layer 5 Pyramidal Neurons From Rat Prefrontal Cortex

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González-Burgos, Guillermo and German Barrionuevo. Voltage-gated sodium channels shape subthreshold EPSPs in layer 5 pyramidal neurons from rat prefrontal cortex. J Neurophysiol 86: 1671–1684, 2001. The role of voltage-dependent channels in shaping subthreshold excitatory postsynaptic potentials (EPSPs) in neocortical layer 5 pyramidal neurons from rat medial prefrontal cortex (PFC) was investigated using patch-clamp recordings from visually identified neurons in brain slices. Small-amplitude EPSPs evoked by stimulation of superficial layers were not affected by the N-methyl-D-aspartate receptor antagonist d-2-amino-5-phosphonopentanoic acid but were abolished by the AMPA receptor antagonist 6-cyano-7-nitroquinocyclene-2,3-dione, suggesting that they were primarily mediated by AMPA receptors. AMPA receptor-mediated EPSPs (AMPA-EPSPs) evoked in the apical dendrites were markedly enhanced, or increased in peak and duration, at depolarized holding potentials. Enhancement of AMPA-EPSPs was reduced by loading the cells with lidocaine and Ni^2+ to the soma or apical dendrite did not affect the AMPA-EPSPs. Like single EPSPs, EPSP trains were shaped by Na^+ but not Ca^2+ channels. EPSPs simulated by injecting synaptic-like current into proximal/middle apical dendrite were enhanced at depolarized holding potentials similarly to AMPA-EPSPs. Extensive blockade of Ca^2+ channels by co-application of Cd^2+ and Ni^2+ to the soma or apical dendrite did not affect the AMPA-EPSPs. Like single EPSPs, EPSP trains were shaped by Na^+ but not Ca^2+ channels. EPSPs simulated by injecting synaptic-like current into proximal/middle apical dendrite were enhanced at depolarized holding potentials similarly to AMPA-EPSPs. Extensive blockade of Ca^2+ channels by bath application of the Cd^2+ and Ni^2+ mixture had no effects on simEPSPs, whereas bath- applied TTX removed the depolarization-dependent EPSP amplification. Inhibition of K^+ currents by 4-aminopyridine (4-AP) and TEA increased the TTX-sensitive EPSP amplification. Moreover, strong inhibition of K^+ currents by high concentrations of 4-AP and TEA revealed a contribution of Ca^2+ channels to EPSPs that, however, seemed to be dependent on Na^+ channel activation. Our results indicate that in layer 5 pyramidal neurons from PFC, Na^+, and K^+ voltage-gated channels shape EPSPs within the voltage range that is subthreshold for somatic action potentials.

INTRODUCTION

In cortical pyramidal neurons, excitatory postsynaptic potentials (EPSPs) are generated in the dendrites, at places distant from the action potential initiation site, located in proximal segments of the axon (Colbert and Johnston 1996; Stuart et al. 1997, 1999). In a purely passive neuron, dendritic cable filtering attenuates strongly the excitatory postsynaptic potentials (EPSPs), reducing significantly the efficacy of most synapses to depolarize the axon (Henze et al. 1996; Spruston et al. 1994; Stuart and Spruston 1998). However, voltage-dependent ion channels are present not only in the axon but throughout the pyramidal cell membrane (Magee 1999). Therefore during propagation the EPSPs could generate subthreshold Na^+ and Ca^2+ currents to amplify the EPSPs, counteracting cable filtering (Crill 1999). Potassium- and hyperpolarization-activated conductances also act to shape EPSPs in ways different from the amplification by Na^+ and Ca^2+ inward currents. Interestingly, recent studies have shown that blockade of Na^+ and Ca^2+ currents affects significantly small subthreshold EPSPs only if the EPSPs are evoked at membrane potentials depolarized from rest, near firing threshold (Andreassen and Lambert 1999; Deisz et al. 1991; Fricker and Miles 2000; Gillessen and Alzheimer 1997; Hoffman et al. 1997; Lipowsky et al. 1996; Markram and Sakmann 1994; Schwindt and Crill 1995; Stuart and Sakmann 1995; Urban et al. 1998).

EPSP amplification remains largely unexplored in pyramidal cells from cortical regions other than CA1 hippocampus and sensorimotor cortex. Across cortical regions, expression of ion channel genes and sorting of channel proteins to specific membrane regions could be different in modes that may affect EPSP propagation. For example, the somatodendritic membrane distribution of K^+ channels differs significantly in CA1 hippocampal (Hoffman et al. 1997) compared with layer 5 somatosensory cortex pyramidal neurons (Bekkers 2000a; Korngreen and Sakmann 2000).

Here, we examined the role of voltage-dependent channels in shaping subthreshold EPSPs in layer 5 pyramidal neurons from rat medial prefrontal cortex (PFC). In vivo, PFC neurons are able to sustain firing at 20–50 Hz for seconds after a brief trigger stimulus ceases. This persistent, stimulus-outlasting firing is also found in other regions of cortex, but it is more robust and more frequently observed in PFC. Thus it is thought to be the cellular substrate of working memory (Fuster 1997; Goldman-Rakic 1995). Until now, it is not entirely clear whether sustained firing relates to unique electrophysiological properties of individual PFC neurons or to network interactions.

The intrinsic firing properties of PFC pyramidal neurons in vitro are consistently similar to those of neurons from other cortical regions (Ceci et al. 1999; Gejje-Barrientos 2000; Gejje-Barrientos and Pastore 1995; Gulledge and Jaffe 1998; 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.)
Henze et al. 2000; Yang and Seamans 1996; Yang et al. 1996; Zhou and Hablitz 1999). In contrast to firing properties, only a few studies investigated EPSP propagation in PFC neurons. Simulations in a model of layer 5 PFC cells have shown that the geometry of their dendritic tree indeed determines a substantial EPSP attenuation during passive propagation (Jaffe and Carnevale 1999). Seamans and colleagues focused on the role of Ca$^{2+}$ channels by simultaneously blocking Na$^+$ and K$^+$ conductances (Seamans et al. 1997). Their study showed that the proximal apical dendrite of layer 5 PFC neurons in vitro generates subthreshold Ca$^{2+}$-dependent potentials that can amplify distal EPSPs or initiate Ca$^{2+}$ spikes prior to the soma. Interestingly, in layer 5 neurons from somatosensory cortex, Ca$^{2+}$-dependent potentials are absent in proximal segments and restricted to the most distal portions of the apical dendrite (Larkum et al. 1999a,b; Schiller et al. 1997; Stuart et al. 1997; Zhu 2000).

We investigated whether Na$^+$ or Ca$^{2+}$ currents play a predominant role in shaping subthreshold EPSPs in neocortical layer 5 pyramidal neurons from rat PFC. We also tested if activation of K$^+$ channels participate in shaping EPSPs in these cells. PFC neurons were visually identified in brain slices using infrared differential interference contrast video microscopy (Stuart et al. 1993), which allowed localized application of channel blockers and also to perform dendritic injections of synaptic-like current (Stuart and Sakmann 1995) to test the effect of channel blockade on EPSP propagation independently of effects on transmitter release.

**Methods**

**Slice preparation**

Male Sprague-Dawley rats (22–33 days old; mean: 24.7) were deeply anesthetized and perfused intracardially with a solution of the following composition (in mM): 230.0 sucrose, 1.9 KCl, 1.2 NaH$_2$PO$_4$, 25.0 NaHCO$_3$, 20.0 glucose, 0.5 CaCl$_2$, and 6.0 MgCl$_2$, at 4°C. Coronal slices (300- to 400-m thick) were cut from the frontal cortex using cold sucrose-based solution.

**Electrophysiological recordings**

For recording, slices were transferred to a submersion recording chamber and superfused with the following solution (in mM): 125 NaCl, 2.5 KCl, 1.25 Na$_2$HPO$_4$, 25 NaHCO$_3$, 2.0 CaCl$_2$, 1.0 MgCl$_2$, and 20 glucose [with 2-amino-5-phosphonopentanoic acid (D,L-AP5) 50 μM added, when specified]. Bath solution temperature was set at 30–32°C when field-potential or whole cell voltage recordings were performed only from the soma. Experiments with simultaneous somatic and dendritic electrodes were made either at room temperature (20–24°C) or at 30–32°C. The effects of tetrodotoxin, Cd$^{2+}$, and Ni$^{2+}$ on simulated EPSPs (simEPSPs) were identical when tested at any of these temperatures. TEA and 4-aminoypyridine (4-AP) were tested only at 30–32°C. Field potentials were recorded with electrodes filled with 0.5 M NaCl (3–5 MΩ), using an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA). A field potential recording electrode was first placed on the surface of layer 1 and lowered into the slice at a depth at which maximal field potential amplitude was observed. Then, using low magnification and a calibrated video monitor, the electrode was placed at similar depth into the slice, to record the field potential every 50 μm from the initial position away from the pia.

For whole cell recordings, pyramidal neurons were identified visually with infrared illumination and differential interference contrast optics (Stuart et al. 1993). Somata of recorded neurons were located in the prelimbic or infralimbic regions of the rat medial PFC (Fig. 1A), at ~500 to ~900 μm from the pial surface, corresponding to layer 5 at the postnatal age range (van Eden and Uylings 1985). Patch pipettes were filled with (in mM): 120 K-methylsulphate, 10 KCl, 10 HEpes, 0.5 EGTA, 4.5 ATP 0.3 GTP, and 14 phosphocreatine. Simultaneous somatic and dendritic recordings were obtained with Axoclamp-2A amplifiers (Axon Instruments) operating in bridge mode. Somatic (3–6 MΩ) and dendritic (10–18 MΩ) patch electrodes were pulled from borosilicate glass. Membrane potential was not corrected for liquid junction potential (experimentally measured to be 6–7 mV). Dendritic voltage recordings were rejected for analysis if the series resistance exceeded 80 MΩ or if it changed rapidly, precluding adequate bridge balance control. Signals were low-pass filtered at 3 kHz, digitized at 10 or 20 kHz and stored on disk for off-line analysis. Data acquisition and analysis were performed using LabView (National Instruments).

**Synaptic stimulation and generation of simulated EPSPs**

For stimulation of distal inputs to layer 5 PFC neurons, a vertical cut was made in the slice from the border between layers 1 and 2 through the white matter (Fig. 1B), as described by Caulier and Connors (1994). Synaptic potentials were elicited applying extracellular current pulses (20–300 μs; 40–300 μA, 0.1 Hz) with bipolar stimulation electrodes (50-μm-diam Formvar wire) placed in layer 1, 250–400 μm away from the vertical cut (Fig. 1B). The recording parameters were kept constant. Extracellular recordings were made at different distances from the slice pial surface, indicated below each trace (see METHODS). Dotted lines indicate approximate limits between cortical layers. The arrows indicate the negative and positive inflections observed in the complex responses recorded in layer 3 and superficial layer 5, consistent with presence of both current sinks and sources at these locations.
electrodes were placed 200–300 \( \mu m \) lateral to the opposite side of the cut. In some experiments with simEPSPs, to verify the action of Na\(^+\) and Ca\(^{2+}\) channels blockers, synaptic responses were evoked by electrical stimulation applied to deep layer 3 or layer 5.

To elicit simulated EPSPs, dendritic current injections were generated using customized programs written in LabView as described previously (Urban and Barrionuevo 1998). In most experiments, EPSPs were simulated by injecting current with the shape of an alpha function: \( I(t) = I_0 g(t) \alpha e^{-(t - \tau)} \). In other experiments, EPSPs were generated by injecting current with a double-exponential time course of the form: \( I(t) = I_{max} [1 - e^{-(t - \tau_{on})}] + [e^{-(t - \tau_{off})}] \), with \( \tau_{on} = 1–5 \) ms and \( \tau_{off} = 5–20 \) ms. \( I_0 \) represents the maximal current. Both types of current waveforms reproduced accurately the EPSPs evoked by synaptic stimulation. Because drug effects using both current waveforms were identical, results were pooled.

In many cases, access resistance was high and unstable at the site of dendritic current injection. Therefore adequate dendritic voltage recording was not possible because series resistance and capacitance could not be adequately compensated by the bridge and capacitance compensation controls of the Axoclamp 2A amplifier. We report dendritic voltage recorded only for cases in which compensation was adequate. Fortunately, the access resistance at the dendritic site (even if large, not stable, and not well compensated) does not affect the voltage change recorded by the somatic electrode, unless it increases up to the point of reducing significantly the current-passing ability of the dendritic electrode. This was controlled by periodically monitoring the actual injected current or the somatic voltage change for a given set of parameters. Typically, data were excluded of analysis if a consistent decrease of 10% in the peak of the somatic simEPSP was observed for constant injection parameters that initially elicited a simEPSP with 2–3 mV of somatic peak amplitude.

**Statistical analysis**

Statistical significance of differences between group means were assessed by paired \( t \)-test or repeated-measures ANOVA followed by Dunnett’s \( t \)-test contrasts. In all cases differences were considered significant if \( P < 0.05 \).

**Drugs**

D-AP5 and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were purchased from RBI (Natick, MA). All other drugs were from Sigma Chemical (St. Louis, MO). Drugs were daily prepared as concentrated solutions in water and diluted in external recording solution shortly before application. When localized application was employed, pipettes with tip diameters of \( ~1–2 \mu m \) were filled with freshly oxygenated external solution containing the drugs as indicated. Flux of the external solution in the chamber was oriented so that the puffs did spread in a perpendicular direction away from the primary apical dendrite. When millimolar concentrations of 4-AP and TEA were employed, NaCl concentration was reduced to preserve osmolarity constant.

**RESULTS**

Isolation of synaptic responses evoked by layer 1 stimulation

In layer 5 pyramidal neurons, the most distal EPSPs are predicted to be the most strongly attenuated by dendritic cable filtering (Jaffe and Carnevale 1999; Stuart and Spruston 1998). Therefore if ion channels provide currents that amplify the EPSPs, removal of these currents should have its strongest effect on distal EPSPs. To determine whether voltage-dependent ion channels amplify EPSPs in layer 5 PFC neurons, we employed a slice preparation (Fig. 1A) in which a vertical cut sectioned all horizontal connections except those in layer 1, to restrict the synaptic responses to the distal apical dendrites (Cauller and Connors 1994; Zhu 2000). This preparation also facilitates to apply channel blockers locally without altering transmitter release (see following text).

We examined the spatial pattern of activation in slices prepared in this way by recording extracellular field potentials across layers 1–5 and evoked by stimulation of layer 1. In layers 1 and 2, field potentials were composed by a monophasic extracellular negativity, consistent with the presence of synaptic current sinks (Fig. 1B). Field potentials recorded in deeper layers (layer 3 and superficial layer 5) usually reversed in sign, consistent with the current sinks being predominantly in superficial layers (Fig. 1B). However, in layers 3 and 5, the field potentials often had complex waveforms, consistent with the presence of a mixture of current sinks and sources in these laminae (Fig. 1B). Therefore the spatial pattern of activation confirms that in this preparation the activated synapses are mainly restricted to layers 1 and 2 and thus to distal portions of the apical dendrite of layer 5 pyramidal neurons, although activation of some proximal synapses could not be completely ruled out.

Patch-clamp electrode voltage recordings were obtained from the soma of visually identified layer 5 PFC pyramidal neurons (\( n = 89 \)). Resting membrane potential (RMP) and input resistance (at RMP) measured at the soma were \(-67.8 \pm 3.5 \) mV and \(-58 \pm 7 \) M\( \Omega \) (means \pm SD). In response to somatic injection of depolarizing current, the majority of pyramidal cells (81/89) exhibited regular spiking firing mode; cells with weak bursting (5/89) or strong bursting firing modes (3/89) were found but were less common. No significant differences were observed in subthreshold EPSP amplification in cells with different firing modes, therefore results were pooled. In slices prepared as shown in Fig. 2A, only 57% of the layer 5 neurons yielded satisfactory responses to layer 1 stimulation (Fig. 2B). The remaining cells showed no responses or responded only to high stimulation intensities with potentials that were usually composed by multiple excitatory and inhibitory events, some of which had prolonged and highly variable latency and thus were likely polysynaptic. Synaptic inhibition was revealed as a fast hyperpolarization that truncated the EPSPs when recorded at depolarized potentials below spike threshold (50 to -45 mV). The GABA\(_A\) receptor antagonist bicuculline could not be employed to isolate EPSPs because, in its presence (5–10 \( \mu M \)), layer 1 stimulation caused epileptiform activity (\( n = 6 \) slices tested, data not shown). Responses with polysynaptic and inhibitory components were excluded from data analysis. We focused on monophasic responses with a latency that was short and exhibited very small trial-to-trial variability (Fig. 2B). Responses with these characteristics had small peak amplitudes (mean peak amplitude, <4 mV at RMP). In the absence of bicuculline, no inhibition could be detected in the majority of these responses, which therefore were considered as EPSPs. If an inhibitory component overlapping with the EPSP was detected, the cell was excluded from analysis.

The peak of EPSPs evoked by layer 1 stimulation and recorded at RMP was reduced to 11.7 ± 4% of control (Fig. 2C) 15 min after bath application of 10 \( \mu M \) of the AMPA/kainate glutamate receptor antagonist CNQX (\( n = 4 \) cells). In contrast, in five of five neurons recorded at RMP in our standard bath solution (external Mg\(^{2+}\) concentration: 1 mM),
In previous studies, it was shown that inhibition of voltage-dependent channels affects EPSPs more significantly, if not exclusively, when evoked at potentials depolarized from rest and close to action potential threshold. At these potentials, the peak and duration of the EPSPs is enhanced, or amplified, in control conditions and the increase is reduced by blockade of voltage-dependent channels. Therefore we first examined whether AMPA-EPSPs evoked by layer 1 stimulation in layer 5 PFC pyramidal neurons exhibited a similar voltage-dependent enhancement. Indeed, in contrast to what was expected if the AMPA-EPSPs propagated passively (EPSP reduction due to decrease in driving force or no change in the EPSP if the driving force at the distal synapse is not altered), the peak and area of the AMPA-EPSPs were markedly increased at depolarized potentials (Fig. 3A). EPSPs were enhanced maximally when evoked near spike threshold and, although the magnitude of maximal increase varied significantly across cells, it was consistently observed in all layer 5 PFC neurons (Fig. 3, A and B). Hyperpolarization below RMP reduced slightly, but not significantly, the EPSP peak and area (Fig. 3, A and B).

To test if, as in pyramidal neurons from other cortical areas, the voltage-dependent enhancement of EPSPs in PFC neurons...
is mediated by voltage-dependent channels, we first loaded the PFC cells with low concentrations of the Na$^+$ channel blocker lidocaine N-ethylbromide (QX-314) (2–5 mM). In this condition, neither EPSP peak nor area were enhanced when evoked at depolarized potentials (at $-75$ mV, peak: $6.3 \pm 0.3$, area: $607 \pm 62$ mV*ms; at $-45$ mV, peak: $1.7 \pm 0.2$ mV; area: $50 \pm 43$ mV*ms). The marked effects of QX-314 suggest that the voltage-dependent amplification requires activation of voltage-gated Na$^+$ channels. However, QX-314 also blocks low- and high-voltage-activated Ca$^{2+}$ currents (Talbot and Sayer 1996), K$^+$ currents (Pare and Lang 1998; Svoboda et al. 1997) and hyperpolarization-activated currents as well (Perkins and Wong 1995). Therefore the relative contribution of Na$^+$ channels versus other QX-314-sensitive channels in shaping the EPSPs is not clear.

To examine the effects of selective inhibition of Na$^+$ or Ca$^{2+}$ channels, we employed tetrodotoxin (TTX) or a mixture of Ni$^{2+}$ and Cd$^{2+}$, respectively. In response to layer 1 stimulation in our slice preparation, transmitter release presumably occurred at distal synapses (Fig. 1), therefore channel blockers could be applied locally to the apical dendrite and soma without altering glutamate release. Drugs were delivered by applying positive pressure to pipettes filled with oxygenated bath solution containing either 1 $\mu$M TTX or 2 mM Ni$^{2+}$ plus 200 $\mu$M Cd$^{2+}$ and placed near to the soma or the apical dendrite (Fig. 4A). In every tested neuron, application of TTX to the somatic region, abolished the initiation or strongly reduced the amplitude of action potentials elicited by somatic current steps. At the same time, the increase in EPSP peak and area at depolarized potentials was strongly and reversibly reduced by TTX applied to the soma (Fig. 4, B and C). In contrast, the layer 1-evoked small AMPA-EPSPs recorded at RMP or more hyperpolarized potentials ($-80$ to $-75$ mV) were not affected by TTX (Fig. 4, B and C). In marked contrast to TTX, somatic application of Cd$^{2+}$ and Ni$^{2+}$ had no detectable effects on the AMPA-EPSPs at any membrane potential tested (Fig. 4, B and C).

Since application of TTX to the somatic region strongly reduced the area of EPSPs recorded at depolarized potentials, activation of Na$^+$ channels located at or near the soma seems to account for a significant fraction of the voltage-dependent EPSP amplification, as reported previously. It has also been reported, however, that dendritic Na$^+$ and Ca$^{2+}$ channels contribute to EPSP amplification in addition to somatic Na$^+$ channels (Gillessen and Alzheimer 1997; Lipowsky et al. 1996). To examine the contribution of dendritic Na$^+$ and Ca$^{2+}$ channels to EPSP propagation in layer 5 PFC neurons, channel blockers were locally applied to the apical dendrite, 200–300 $\mu$m from the soma. In most cells (5 of 7 tested), application of TTX to the apical dendrite had no significant effects on the AMPA-EPSP at any membrane potential tested (Fig. 4C), although dendritic TTX attenuated strongly the AMPA-EPSPs regardless of membrane potential in two neurons, probably by acting on presynaptic Na$^+$ channels. As shown in Fig. 4C, application of Cd$^{2+}$ and Ni$^{2+}$ to the apical dendrite generally had no effects on the EPSPs in six of seven tested neurons (Cd$^{2+}$ and Ni$^{2+}$ attenuated the EPSP peak recorded at $-75$ mV to 25.3% of control in the remaining cell).

The kinetics of activation of some voltage-gated Ca$^{2+}$ channels is slow, relative to that of Na$^+$ channels (McCobb and Beam 1991; Meremstein et al. 2000). Thus, it is possible that single AMPA-EPSPs are too fast to gate Ca$^{2+}$ channels. If so, then a more prolonged EPSP-induced depolarization could reveal a Ca$^{2+}$ channel contribution. Indeed, it was previously shown that very prolonged depolarizations (0.5–1 s) in some cases activate Ca$^{2+}$ in addition to Na$^+$ conductances (Schwindt and Crill 1997). Synaptic input more prolonged than single EPSPs are too fast to gate Ca$^{2+}$ channels. As shown in Fig. 4C, application of Cd$^{2+}$ and Ni$^{2+}$ to the apical dendrite generally had no effects on the EPSPs in six of seven tested neurons (Cd$^{2+}$ and Ni$^{2+}$ attenuated the EPSP peak recorded at $-75$ mV to 25.3% of control in the remaining cell).

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To test if more prolonged EPSP-induced depolarizations, could reveal a contribution of Ca\(^{2+}\) channels, we applied layer 1 stimulation (Fig. 1) by delivering trains of five stimuli at inter-stimulus intervals of 25 or 50 ms. Also, to favor any possible contribution of NMDA receptor activation, the NMDA antagonist AP5 was omitted from the external solution. Figure 5A shows that the amplitude of subsequent EPSPs recorded at a somatic membrane potential of −75 mV displayed marked depression despite some degree of temporal summation. When evoked at depolarized potentials, the amplification of individual EPSPs lead to a pronounced summation and enhancement of the depolarization induced by the EPSP train (Fig. 5A). Simultaneous application of Cd\(^{2+}\) and Ni\(^{2+}\) (200 μM and 2 mM, respectively) to either the apical dendrite (140–230 μm from the soma) or the soma did not significantly alter the EPSP trains recorded at −75 mV nor at depolarized potentials (Fig. 5B). In contrast, localized application of TTX (1 μM) to the somatic region, removed almost completely the voltage-dependent amplification of the EPSP trains by membrane depolarization. During application of TTX to the soma, the overall depolarization induced by the EPSP trains recorded at −55 to −50 mV was not significantly different from that recorded at −75 mV (Fig. 5C). A similar voltage-dependent enhancement of EPSP trains by voltage-dependent Na\(^{+}\) channels was reported for layer 5 pyramidal cells in somatosensory cortex (Williams and Stuart 1999, 2000).

Overall, these results indicate a predominant contribution of somatic or proximal Na\(^{+}\) channels, but not of Ca\(^{2+}\) channels, in shaping the EPSPs. However, the role of Na\(^{+}\) or Ca\(^{2+}\) channels that may be located in the apical dendrite is not entirely clear. First, because responses were evoked without blockade of inhibitory transmission, we could not completely rule out that either evoked or spontaneous inhibitory postsynaptic potentials (IPSPs) prevented some form of Na\(^{+}\)- or Ca\(^{2+}\)-dependent dendritic electrogenesis (Kim et al. 1995; Larkum et al. 1999a,b; Miles et al. 1996; Tsubokawa and Ross 1996). Second, localized application of drugs to the dendrites may have been ineffective at producing the necessary level of blockade of the relevant dendritic channel types. To address these issues, we performed the experiments described in the next sections, using dual patch pipette recordings.

**Effects of Na\(^{+}\) and Ca\(^{2+}\) channel blockers on the EPSPs simulated by dendritic current injection**

To gain further insight into the mechanisms and types of voltage-dependent conductances involved in shaping EPSP waveforms in layer 5 PFC neurons, we obtained simultaneous current-clamp recordings from the soma and primary apical dendrite of these neurons (Fig. 6A). As described originally by Stuart and Sakmann (1995), one electrode was used to inject, into the apical dendrite, current waveforms similar to excitatory postsynaptic currents (see Methods) while simultaneously recording the somatic depolarization, referred to as simEPSPs. As shown in Fig. 6B, dendritic current injection could generate simEPSPs with time courses and amplitudes within the range observed for EPSPs evoked by extracellular stimulation of layer 1. This approach allowed us to test the effects of blockade of Na\(^{+}\) and Ca\(^{2+}\) channels throughout the membrane by bath applied drugs, independently of their effects on synaptic transmission. In addition, the simEPSPs were recorded during blockade of GABA\(\_A\) IPSPs by 10 μM bicuculline and thus propagated without interference of evoked or spontaneous IPSPs. In 56% of the experiments, AMPA and NMDA receptors were blocked by CNQX (10 μM) and AP5 (50 μM), also eliminating the potential effects of spontaneous excitatory synaptic transmission. Interestingly, no differences were observed in the effects of bath-applied Na\(^{+}\) or Ca\(^{2+}\) channel antagonists on the simEPSPs with or without CNQX and AP5. Dendritic current injections were performed into the primary apical dendrite of layer 5 PFC neurons at 30 to −280 μm from the soma, which corresponds to the proximal to mid segments of the primary apical dendrite (Fig. 6A). Within these compartments, the effects of Na\(^{+}\) and Ca\(^{2+}\) channel blockers were independent of the distance between the dendritic injection site and the soma.

As shown for AMPA receptor-mediated EPSPs, simEPSPs were markedly enhanced when evoked at depolarized membrane potentials (Fig. 6, C and D). The increase in simEPSP area was also larger than the increase in peak, and amplification also was maximal when the simEPSPs were near action potential threshold (Fig. 6, C and D). In some experiments (n = 5), the simEPSPs were successfully recorded at the site of dendritic current injection (>100 μm from the soma) and at the soma. The dendritic resting potential determined shortly after

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**FIG. 5.** EPSP trains evoked by repetitive stimulation in the absence of NMDA channel antagonists are enhanced by Na\(^{+}\) channels but not by Ca\(^{2+}\) channels. EPSPs were evoked by layer 1 stimulation in the absence of AP5. Interspike intervals of 50 or 25 ms produced identical results. **Left:** averages of 10 traces in a representative experiment. **Right:** bar graphs are summary plots for each experimental group. A: EPSP trains recorded in control bath solution. Bar graph, n = 5 cells. B: amplification of EPSP trains during local application of Cd\(^{2+}\) and Ni\(^{2+}\). Bar graph, n = 5 cells (2 with somatic, 3 with dendritic application). C: EPSP train amplification during application of TTX to the soma. Bar graph: n = 4 cells.
injections at very proximal dendritic sites (<40 µm from the soma) little attenuation was observed (not shown). Indeed, these proximal dendritic regions appear to be essentially iso-potential with the soma. When the cells were depolarized by somatic current injection, the area of both dendritic and somatic simEPSPs increased significantly (Fig. 6E). In contrast, the peak of the dendritic simEPSP did not change with depolarization, although the somatic simEPSP peak increased (Fig. 6E). In addition, the increase in area with depolarization was in general slightly larger for the somatic than the dendritic simEPSP (Fig. 6E). These results are consistent with the idea that the voltage-dependent EPSP amplification takes place by activation of TTX-sensitive Na\(^+\) channels located at or near the soma. Activation of these Na\(^+\) channels, appears to generate a subthreshold potential that prolongs the decay of the somatic EPSP and propagates, with some attenuation, back into the apical dendrite to prolong the decay of the dendritic EPSP.

Similarly to the AMPA-EPSPs evoked by stimulation of layer 1, the increase in simEPSP peak and area by depolarization was strongly reduced when the layer 5 PFC neurons were loaded with QX-314 (2–5 mM). Once fast Na\(^+\) spikes were abolished by QX-314, the amplitude and area of the simEPSPs did not change significantly with depolarization of the cell elicited by simultaneous injection of current into the soma (simEPSP peak at −75 mV: 2.77 ± 0.09 mV; simEPSP peak at −50 mV: 2.70 ± 0.03 mV; simEPSP area at −75 mV: 106.81 ± 9.31 mV\(^2\)ms; simEPSP area at −50 mV: 110.66 ± 17.08 mV\(^2\)ms; \(n = 3\) cells; differences not significant by paired t-test, \(P > 0.05\)).

We next bath-applied TTX to examine the effect of selectively blocking voltage-activated Na\(^+\) channels on the shaping of simEPSP waveforms by depolarization. For each neuron, simEPSPs with small amplitudes at the soma when recorded at RMP were evoked at a range of hyperpolarized and depolarized membrane potentials, first in control bath solution and then with TTX (1 µM) in the bath. The action of TTX was verified by the blockade of evoked excitatory synaptic transmission or action potentials (APs) elicited by somatic current steps. Figure 7A shows that the peak and area of small simEPSPs recorded at hyperpolarized membrane potentials, were unaffected by TTX. In contrast, TTX strongly attenuated or removed completely the enhancement of the peak and area of small simEPSPs evoked at depolarized membrane potentials in the same neurons (Fig. 7A).

The removal of voltage-dependent EPSP amplification by bath-applied TTX suggests that activation of Na\(^+\) channels accounts completely for the voltage-dependent enhancement of EPSPs in PFC neurons. However, it was still possible that activation of Ca\(^{2+}\) channels, secondary to Na\(^+\) channel activation, also was required. To test this possibility, we bath-applied a mixture of the Ca\(^{2+}\) channel blockers Cd\(^{2+}\) and Ni\(^{2+}\), at concentrations sufficient to block most of the available voltage-dependent Ca\(^{2+}\) channels, regardless of subtype (200 µM each or 200 µM Cd\(^{2+}\) plus 2 mM Ni\(^{2+}\)). As in the case of TTX, small simEPSPs recorded at RMP or more hyperpolarized potentials were not affected by application of Cd\(^{2+}\) and Ni\(^{2+}\) (Fig. 7B). In contrast to TTX, however, enhancement of simEPSPs by depolarization was not affected during extensive block of Ca\(^{2+}\) channels by bath application of Cd\(^{2+}\) and Ni\(^{2+}\) (Fig. 7B). The action of Ca\(^{2+}\) channel blockers was verified by its block of excitatory synaptic responses or of the afterhyper-
polarizations (see following text). In three of these neurons, 1 μM TTX was applied after washout of Cd²⁺ and Ni²⁺ and strongly reduced the voltage-dependent amplification of simEPSPs at depolarized potentials (data not shown).

Previous studies have shown that T-type nickel-sensitive Ca²⁺ channels contribute to EPSP waveforms in both hippocampal and neocortical pyramidal neurons (de la Peña and Geijo-Barrientos 2000; Deisz 1991; Urban et al. 1998). Because T-like currents inactivate rapidly with depolarization, it is possible that a contribution of these currents to EPSPs was underestimated when EPSPs were evoked at depolarized holding potentials. Small EPSPs (<5 mV peak) evoked at hyperpolarized potentials that would remove T-channel inactivation were unaffected by Ni²⁺, but their small amplitudes may have not been sufficient to gate the Ca²⁺ channels. To test if Ca²⁺ channels could contribute to EPSPs with large amplitudes, we compared the effects of Cd²⁺ and Ni²⁺ on small and large EPSPs. In our experimental conditions, large amplitude EPSPs (peak >4 mV) evoked by superficial layer stimulation were in most cases associated with polysynaptic EPSPs and IPSPs. Therefore the effect of channel block on EPSPs with large amplitude was examined in small and large simEPSPs, generated in the same neuron by adjusting the size of the synaptic-like current injected into the apical dendrite. The kinetics of large and small simEPSPs waveforms was generally similar, except for a tendency of the decay phase of the large EPSPs to hyperpolarize a few millivolts below the cells’ resting potential (Fig. 8A). By increasing the size of the current injected by the dendritic electrode, the amplitude of the somatic simEPSPs could be progressively incremented up to the point of reaching firing threshold from a hyperpolarized membrane potential of −70 mV (Fig. 8B). When the same current injection protocol was repeated after applying TTX (1 μM) to the bath solution, the amplitude of small EPSPs was unaffected, whereas that of large simEPSPs was reduced significantly (Fig. 8C). Interestingly, in three of three cases in which more than three magnitudes of dendritic current injection could be employed, simEPSP peak was observed to increase linearly with injected current in control bath solution and sublinearly in the presence of TTX (Fig. 8C). As shown in Fig. 8, C and D, neither small nor large simEPSPs were significantly affected by adding Ca²⁺ channel blockers (2 mM Ni²⁺ plus 200 μM Cd²⁺) to the external solution. Similar results were found when the cells were held at a potential of −80 mV, thus further removing channel inactivation (data not shown). These results suggest that, within the middle-proximal compartment of the apical dendrite of layer 5 PFC neurons, Ca²⁺ channels do not contribute to propagation of EPSPs that had amplitudes in the whole subthreshold range of membrane potentials. In contrast, TTX-sensitive Na⁺ channels do contribute to depolarization elicited by large simEPSPS evoked at −70 mV. This contribution, however, was less prominent than that found for small EPSPS evoked at depolarized potentials, suggesting that different mechanism may be involved in shaping large and small EPSPS, at least regarding the effect of Na⁺ channels.

Overall, our results seem different from those reported in a
previous study of layer 5 PFC pyramidal neurons, in which it was suggested that a Ca\textsuperscript{2+}-dependent potential generated in the proximal apical dendrite or soma amplifies single distal EPSPs (Seamans et al. 1997). However, in the previous study the recordings were performed after Na\textsuperscript{+} and K\textsuperscript{+} channels were inhibited by application of QX-314 and Cs\textsuperscript{+} or TEA. Therefore altogether the results open the interesting question of whether Ca\textsuperscript{2+} channel contribution to EPSPs is secondary to a reduction of K\textsuperscript{+} currents. In pyramidal and nonpyramidal neurons in the CA1 region of hippocampus, inhibition of K\textsuperscript{+} currents uncovers or enhances EPSP amplification (Andreasen and Lambert 1999; Fricker and Miles 2000; Hoffman et al. 1997). In neocortical layer 5 pyramidal neurons, it has not been examined whether a similar balance between inward and outward currents takes place.

To examine this possibility, we tested the effects of inhibiting K\textsuperscript{+} currents on the voltage-dependent amplification of EPSPs. When applied in the presence of K\textsuperscript{+} channel antagonists, layer 1 stimulation elicited epileptiform activity, therefore the effects of K\textsuperscript{+} channel inhibition were tested only on simEPSPs that were elicited by injection of current into the proximal apical dendrite (up to 100 μm from the soma), with excitatory and inhibitory synaptic transmission blocked by CNQX (10 μM), AP5 (50 μM), and bicuculline (10 μM). In layer 5 neurons from somatosensory cortex, transient and sustained K\textsuperscript{+} conductances found in dendrites and soma are blocked by millimolar concentrations of 4-AP and TEA, respectively (Bekkers 2000a,b; Korngreen and Sakmann 2000). In the presence of 4-AP and TEA (2 mM each), the peak and amplitude of simEPSPs were not significantly different from those recorded in control bath solution (Fig. 9A). As a consequence of this enhanced amplification by K\textsuperscript{+} channel blockers, EPSPs reached firing threshold at potentials equal to or more depolarized than −65 mV (Fig. 9A). As a result of this enhanced amplification by K\textsuperscript{+} channel blockers, EPSPs reached firing threshold at potentials more hyperpolarized than in control conditions (Fig. 9A).

If the EPSP enhancing effect of K\textsuperscript{+} current inhibition results from a contribution of voltage-dependent Ca\textsuperscript{2+} channels, then the presence of K\textsuperscript{+} channel blockers should reveal an effect of the Ca\textsuperscript{2+} channel blockers Cd\textsuperscript{2+} and Ni\textsuperscript{2+}. To test this possibility, we examined the effects of Cd\textsuperscript{2+} and Ni\textsuperscript{2+} (200 μM Cd\textsuperscript{2+} plus 2 mM Ni\textsuperscript{2+}) when applied in the presence of 4-AP and TEA (2 mM each). As shown in Fig. 9B, the enhanced amplification of simEPSPs by 4-AP and TEA was not significantly affected by the presence of Cd\textsuperscript{2+} and Ni\textsuperscript{2+}. In contrast,
the effect of the K⁺ channel antagonists was strongly inhibited by bath application of 1 mM TTX, which actually removed completely the voltage-dependent amplification (Fig. 9B). These results suggest that K⁺ currents counteract the enhancing effect of Na⁺ channels but do not enable a contribution of Ca²⁺ channels to the EPSPs. However, at 2 mM, 4-AP and TEA do not fully inhibit K⁺ currents, therefore it is possible that the residual K⁺ current was sufficient to shunt a depolarization otherwise generated by Ca²⁺ currents. To test this possibility, in a separate series of experiments, we employed 4-AP and TEA at concentrations (4-AP: 5 mM; TEA: 20 mM) above those that inhibit 50% of the K⁺ currents mediated by a variety of K⁺ channels (Coetzee et al. 1999), including transient and sustained K⁺ currents found in the soma and dendrites of hippocampal and neocortical pyramidal neurons (Bekkers 2000a; Hoffman et al. 1997; Korngreen and Sakmann 2000). SimEPSPs were elicited by dendritic current injection at distances from the soma of ±120 μm, first in control conditions and then in the presence of 5 mM 4-AP and 20 mM TEA. Small simEPSPs were not significantly affected by the K⁺ channel blockers when recorded at hyperpolarized potentials. In contrast, at potentials equal to or more depolarized than −55 mV, the simEPSPs were enhanced relative to control conditions. Repeated-measures ANOVA (P < 0.05) indicated that simEPSP areas recorded at −55 or −50 mV were significantly different between control and each experimental group. No significant differences were observed between 4-AP and TEA in the absence or presence of Cd²⁺ and Ni²⁺. Shown are means ± SE; Control: n = 7; 4-AP: n = 7; 4-AP + TEA: n = 4; 4-AP + TEA + TTX: n = 3. Cd²⁺ and Ni²⁺ were applied at 200 mM and 500 mM or 1 mM TTX was applied at 1 μM. C: Experimental paradigm was as in B, except that 4-AP and TEA concentrations were 5 and 20 mM, respectively. Repeated-measures ANOVA (P < 0.05) indicated that simEPSP areas recorded at −55 or −50 mV were significantly different between control and each experimental group. Control: n = 7; 4-AP + TEA: n = 7; 4-AP + TEA + Cd + Ni: n = 5; 4-AP + TEA + TTX: n = 4.

The secondary role of Ca²⁺ channels in shaping the EPSPs seemed not to be due to significant run-down of postsynaptic Ca²⁺ currents. Interestingly, bath application of TTX (1 mM) together with 4-AP and TEA (5 and 20 mM) removed almost completely the EPSP amplification (Fig. 9C), suggesting that the contribution of Ca²⁺ channels to EPSPs is secondary to Na⁺ channel activation.

The presence of the AHPs suggests that Ca²⁺ currents involved in its generation, probably N, P, and Q (Pineda et al. 1998). The presence of Ca²⁺ currents coupled to Ca²⁺ influx through voltage-gated Ca²⁺ channels (Marrion and Tavalin 1998) therefore the presence of the AHPs suggests that Ca²⁺ currents found in the soma and dendrites of hippocampal and neocortical pyramidal neurons (Bekkers 2000a; Hoffman et al. 1997; Korngreen and Sakmann 2000). SimEPSPs were elicited by dendritic current injection at distances from the soma of ±120 μm, first in control conditions and then in the presence of 5 mM 4-AP and 20 mM TEA. Small simEPSPs were not significantly affected by the K⁺ channel blockers when recorded at hyperpolarized potentials. In contrast, at potentials equal to or more depolarized than −55 mV, the simEPSPs were enhanced relative to control conditions. Repeated-measures ANOVA (P < 0.05) indicated that simEPSP areas recorded at −55 or −50 mV were significantly different between control and each experimental group. No significant differences were observed between 4-AP and TEA in the absence or presence of Cd²⁺ and Ni²⁺. Shown are means ± SE; Control: n = 7; 4-AP: n = 7; 4-AP + TEA: n = 4; 4-AP + TEA + TTX: n = 3. Cd²⁺ and Ni²⁺ were applied at 200 mM and 500 mM or 1 mM TTX was applied at 1 μM. C: Experimental paradigm was as in B, except that 4-AP and TEA concentrations were 5 and 20 mM, respectively. Repeated-measures ANOVA (P < 0.05) indicated that simEPSP areas recorded at −55 or −50 mV were significantly different between control and each experimental group. Control: n = 7; 4-AP + TEA: n = 7; 4-AP + TEA + Cd + Ni: n = 5; 4-AP + TEA + TTX: n = 4. 

**FIG. 9.** Effects of inhibition of K⁺ currents on simEPSPs. **A**. *top* averages of 10 consecutive simEPSPs recorded in control solution. SimEPSPs were generated by injecting current at −30 μm from the soma. Shown is voltage recorded by the dendritic electrode. *Bottom* simEPSPs recorded in the same neuron in the presence of 2 mM 4-AP and 2 mM TEA. **B**. simEPSP area was plotted against membrane potential for the experiment shown in A. At −50 mV in the presence of K⁺ channel antagonists, each EPSP elicited a spike, therefore area is not shown at this potential. **B**. simEPSP area normalized to the area measured at −70 mV was averaged across neurons, for each experimental condition an plotted against membrane potential. Repeated-measures ANOVA (P < 0.05) indicated that simEPSP areas recorded at −55 or −50 mV were significantly different between control and each experimental group. No significant differences were observed between 4-AP and TEA in the absence or presence of Cd²⁺ and Ni²⁺. Shown are means ± SE; Control: n = 7; 4-AP: n = 7; 4-AP + TEA: n = 4; 4-AP + TEA + TTX: n = 3. Cd²⁺ and Ni²⁺ were applied at 200 mM and 500 mM or 1 mM TTX was applied at 1 μM. C: Experimental paradigm was as in B, except that 4-AP and TEA concentrations were 5 and 20 mM, respectively. Repeated-measures ANOVA (P < 0.05) indicated that simEPSP areas recorded at −55 or −50 mV were significantly different between control and each experimental group. Control: n = 7; 4-AP: n = 7; 4-AP + TEA: n = 7; 4-AP + TEA + Cd + Ni: n = 5; 4-AP + TEA + TTX: n = 4.
neurons. We compared the effects of Na$^+$ and Ca$^{2+}$ channel blockers on simEPSPs evoked in neurons obtained from rats in the two extremes of the range of postnatal days employed in our study. The results showed no significant differences between the age groups: the area of simEPSPs recorded at depolarized potentials ($-55$ to $-50$ mV) was, at $22-23$ days postnatal: $29.8 \pm 12.2\%$ of control ($n = 5$) in the presence of $1 \mu$M TTX and $121.5 \pm 19.0\%$ of control ($n = 5$) in the presence of Cd$^{2+}$ and Ni$^{2+}$ (200 $\mu$M and 5 mM, respectively); at $31-33$ days postnatal, $25.4 \pm 8.2\%$ of control ($n = 4$) in the presence of TTX and $97.5 \pm 4.1\%$ of control ($n = 3$) in the presence of Cd$^{2+}$ and Ni$^{2+}$. These results suggest that between postnatal days 22 and 33, developmental changes in Na$^+$ and Ca$^{2+}$ channel distribution within the proximal and middle compartments of the apical dendrite examined here are not significant regarding propagation of EPSPs. Indeed, the recent study of Zhu (2000) showed that the main changes in morphology and electrophysiology of layer 5 neocortical pyramidal neurons happen between postnatal days 0 and 14 and are near or at plateau around 30 days. In addition, the developmental changes described by Zhu (2000) take place in the most distal portions of the apical dendrite.

**DISCUSSION**

By selectively inhibiting currents mediated by Na$^+$, Ca$^{2+}$, and K$^+$ channels, independently of their effects on glutamate release, we have investigated the role of these channels in shaping subthreshold EPSPs in neocortical layer 5 neurons from rat medial PFC. We found that in cells resting at membrane potentials typical for pyramidal neurons in vitro, EPSPs with small amplitude at the soma and evoked in the apical dendrite were unaffected by inhibition of Na$^+$, Ca$^{2+}$, or K$^+$ channels. At depolarized subthreshold potentials, both peak and area of EPSPs were enhanced by Na$^+$ currents. Potassium currents also participated in shaping the EPSPs during depolarization. Calcium current contribution, in contrast, occurred only when K$^+$ currents were strongly inhibited by high concentrations of 4-AP and TEA and seemed to be secondary to Na$^+$ channel activation.

** Ionic mechanisms of EPSP amplification**

Our results suggest a predominant role of Na$^+$ channels in shaping subthreshold EPSPs in layer 5 pyramidal neurons from rat PFC. The contribution of Na$^+$ currents was revealed under conditions (depolarized holding potentials) similar to those required for pyramidal cells in other brain regions, namely CA1 (Andreasen and Lambert 1999; Hoffman et al. 1997; Lipowsky et al. 1996) and layer 5 somatosensory neurons (Stuart and Sakmann 1995). Moreover, in layer 5 PFC neurons, Na$^+$ channels shaping the EPSPs appear to be located near the soma, as in other cell types (Andreasen and Lambert 1999; Stuart and Sakmann 1995; Urban et al. 1998). Pyramidal neurons in PFC exhibit Na$^+$-dependent plateau potentials and persistent Na$^+$ currents (Geijo-Barrientos and Pastore 1995; Gorelova and Yang 2000; Maurice et al. 2001; Yang et al. 1996). Thus it is likely that voltage-dependent enhancement of EPSPs in PFC neurons, similarly to other neurons, is mediated by Na$^+$ channels in a persistent or slowly inactivating gating mode (Alzheimer et al. 1993; Andreasen and Lambert 1999;
Lipowsky et al. 1996; Schwindt and Crill 1995; Stuart and Sakmann 1995). However, subthreshold EPSPs can also gate fast Na channels (Magee and Johnston 1995), which are activated during depolarization-dependent amplification of EPSPs, since full-amplitude action potentials are triggered frequently during the decay of the amplified EPSPs. Because both fast and persistent Na+ channels are TTX-sensitive, at present it is difficult to discern the relative contribution of these two channel gating modes.

Compared with the ubiquitous role of Na+ currents in shaping subthreshold EPSPs, the role of Ca2+ currents seems controversial. Calcium channels were reported to amplify EPSPs in CA1 and CA3 hippocampal (Gillesen and Alzheimer 1997; Urban et al. 1998) and neocortical (de la Peña and Geijo-Barrientos 2000; Deisz et al. 1991; Markram and Sakmann 1994) pyramidal neurons. In contrast, no role of Ca2+ channels in shaping small subthreshold EPSPs was suggested for neocortical layer 5 (Stuart and Sakmann 1995) or CA1 neurons (Andreasen and Lambert 1999; Magee and Johnston 1995; Magee et al. 1995) in other studies. Blockade of Ca2+ channels attenuates EPSPs in layer 5 somatosensory neurons from 14- to 18-day-old (Markram and Sakmann 1994; Stuart and Sakmann 1995) but not from 28 day-old rats (Schiller et al. 1997; Stuart and Sakmann 1995), suggesting that maturation of cell properties can explain the different results in some cases. Inhibition of inward currents affected the small EPSPs mainly when they were evoked at subthreshold depolarizing holding potentials. If the voltage dependence and kinetics of inactivation of K+ channels in layer 5 PFC neurons is similar to that in layer 5 cells from somatosensory cortex (Bekkers 2000a; Korngreen and Sakmann 2000), then part of the effect of depolarizing holding potentials might be due to inactivation of K+ channels. Consistently with this idea, single EPSPs with large peak amplitudes (20–25 mV at the soma) evoked at hyperpolarized holding potentials were affected much less by blockade of Na+ channels than small EPSPs evoked at depolarized potentials. Pharmacological blockade of K+ currents enhanced the amplification of EPSPs by increasing the contribution of Na+ channels. In our experiments, Ca2+ currents did not contribute to either single or trains of EPSPs unless K+ currents were extensively reduced by pharmacological inhibition, and this contribution seemed secondary to the activation of Na+ channels. In the previous study of Seamans and colleagues (1997), nickel-insensitive Ca2+ channels were found to amplify EPSPs in these same neurons after Na+ channels were blocked. The differences between the results could be explained if the experimental conditions of Seamans and colleagues (1 M Cs+, and 80–100 mM QX-314 in sharp-electrode internal solution, plus 20 mM TEA in the bath solution) produced a reduction of K+ currents greater than that attained by 5 mM 4-AP and 20 mM TEA in our experiments. Perhaps an even larger inhibition of K+ currents enables a contribution of Ca2+ channels independent of Na+ channels. Another possibility is that the contribution of Ca2+ channels found in the previous study (Seamans et al. 1997) is somehow tightly coupled to the presence of a significant NMDA component in the EPSPs. Indeed, whereas in the previous study pure NMDA EPSPs were recorded, for EPSPs evoked in our experimental conditions, the NMDA component was either absent or extremely weak. This suggests that the synaptic population studied by Seamans and colleagues differed from the synaptic population studied here with respect to the NMDA/AMPA conductance ratio. A small NMDA/AMPA ratio in distal synapses, as found in our experiments, is consistent with recent findings on the distal to proximal distribution of NMDA and AMPA receptors in neocortical layer 5 pyramidal neurons (Dodt et al. 1998). Therefore a plausible explanation for the differences in NMDA component is that the synapses stimulated in the previous study were located in dendritic compartments significantly more proximal than those studied here. Surprisingly, in both studies electrical stimulation was applied to superficial layers 1 and 2 to activate synapses distally. The mechanisms by which Ca2+ channel contribution to EPSPs would be related to activation of NMDA receptors are at present unclear. Duration of the depolarizing synaptic input seems not to be the cause, since Ca2+ channels still did not contribute to amplify trains of EPSPs lasting ~500 ms. Another difference with the recordings from the previous study, is the presence of Cs+, which is a blocker of the hyperpolarization-activated H conductance. Until now, the role of H channels in shaping EPSPs has not been examined in PFC neurons. In other neurons, H channels shorten the EPSP duration, thus deactivation of this conductance at depolarized potentials may explain in part the effect of depolarization on EPSPs. It is possible that a contribution of Ca2+ channels is favored by the combined inhibition by Cs+ and deactivation by depolarization of H channels, but this remains to be addressed.

Functional implications

Our present results confirm previous findings in other neuronal types, showing that, at rest, neither Na+ or Ca2+ channels amplify small EPSPs in cortical pyramidal neurons. The depolarization-dependent enhancement of EPSPs by Na+ channels found here and in previous studies is dependent on somatic or axonal Na+ channels. Thus it can boost EPSPs only after they invade the soma/axon region, therefore not counteracting dendritic filtering. Because our drug applications and dendritic current injections were done at proximal/middle portions of the primary apical dendrite (≈280 μm from the soma), we cannot rule out that in more distal dendritic compartments Na+ or Ca2+ channels act to counter dendritic filtering of EPSPs. In other cell types, activation of Na+ and Ca2+ channels in the distal dendrites provides an all-or-none electrogensis mechanism that seems to detect coincident activation of multiple synaptic connections, rather than amplifying subthreshold EPSPs (Larkum et al. 1999a,b; Zhu 2000). Interestingly, recent experiments have shown that, in CA1 pyramidal neurons, distal EPSPs are not usually amplified by voltage-gated channels but that the AMPA synaptic conductance actually increases with distance from the soma, compensating for the dendritic attenuation of the EPSPs (Magee and Cook 2000). Whether a similar scaling of distal synaptic conductance occurs in pyramidal neurons from neocortex remains to be demonstrated.

The ionic mechanisms found here to shape subthreshold EPSPs may be important to PFC-specific patterns of activity in a number of ways. The EPSP decay time determines the degree of temporal summation (Koch et al. 1996), which must be important to synaptic excitation and sustained firing in the PFC during working memory. Computer simulations in biophysically realistic network models (for a review, see Durstewitz et al. 2000) suggest that this may be indeed the case. Specifically,
Wang and colleagues have shown that EPSPs with kinetics, such as those determined by just an AMPA synaptic conductance, decay too fast for the network to generate stable sustained firing at rates consistent with delay period activity (Compte et al. 2000; Wang 1999). Increasing the EPSP decay time constant, by adding NMDA synaptic conductance, made sustained firing more stable (Compte et al. 2000; Wang 1999). Here we demonstrate that, in addition to the synaptic conductance, in PFC neurons the EPSP decay is shaped by voltage-gated channels at subthreshold potentials whereby a large fraction of synaptic NMDA channels would be blocked by Mg2+ (Hestrin et al. 1990). Thus, we predict that incorporation into network models of the voltage-dependent slowing of EPSP decay by active conductances would make less critical the contribution of NMDA channels, perhaps eliminating the requirement (for the models to reproduce delay-related firing) of synapses with very high NMDA/AMPA receptor ratio (Durstewitz et al. 2000; Lisman et al. 1998), which are uncommon in the adult neocortex.

Shaping of EPSPs by voltage-gated channels also suggests an important mechanism by which neuromodulatory systems can regulate activity in PFC. Input from midbrain dopamine neurons is particularly strong in the PFC and other regions of frontal cortex but is very weak or absent in posterior cortical regions, like somatosensory and visual areas (Fuster 1997). Dopamine signaling in PFC is necessary for correct performance in working memory tasks (Brozoski et al. 1979; Sawaguchi and Goldman-Rakic 1991). Dopamine, via G-protein-linked receptors, may modulate the voltage-dependent channels that shape subthreshold EPSPs, having a critical effect on temporal summation, and thus on the stability of sustained firing in PFC. If neuromodulators were to affect EPSP decay and temporal summation, then our results clearly point to Na+ channels as a crucial target. In most neurons, dopamine downregulates Na+ currents; however, results obtained up to date with PFC cells are contradictory since different groups report up- or downregulation (Geijo-Barrientos and Pastor 1995; Gorelova and Yang 2000; Maurice et al. 2001; Yang and Seamas 1996). Modulation of some K+ currents also could affect EPSP kinetics and summation and could potentially enable control of Ca2+ channels. It remains to be demonstrated if neuromodulation can reduce K+ currents to the levels found to be necessary to reveal an effect of Ca2+ channels in shaping EPSPs in vitro.

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