Dopamine D1/D5 Receptor Activation Modulates a Persistent Sodium Current in Rat Prefrontal Cortical Neurons In Vitro

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Gorelova, Natalia A. and Charles R. Yang. Dopamine D1/D5 receptor activation modulates a persistent sodium current in rat prefrontal cortical neurons in vitro. J Neurophysiol 84: 75–87, 2000. The effects of dopamine (DA) on a persistent Na+ current (I_{NaP}) in layer V-VI prefrontal cortical (PFC) pyramidal cells were studied using whole cell voltage-clamp recordings in rat PFC slices. After blocking K+ and Ca2+ currents, a tetrodotoxin-sensitive I_{NaP} was activated by slow depolarizing voltage ramps or voltage steps. DA modulated the I_{NaP} in a voltage-dependent manner: increased amplitude of I_{NaP} at potentials more negative than −40 mV, but decreased at more positive potentials. DA also slowed the inactivation process of I_{NaP}. The D1/D5 dopamine receptor agonist SKF 38393, SKF 81297, and dihydrexidine (3–10 μM), but not the dopamine D2/D3 receptor agonist quinpirole (1–20 μM), mimicked the effects of DA on I_{NaP}. Modulation of I_{NaP} by D1/D5 agonists was blocked by the D1/D5 antagonist SCH23390. Bath application of specific protein kinase C inhibitor, chelerhythrine, or inclusion of the specific protein kinase C inhibiting peptide (19–36) in the recording pipette, but not protein kinase A inhibiting peptide (5–24), blocked the effect of D1/D5 agonists on I_{NaP}. In current-clamp recordings, D1/D5 receptors activation enhanced the excitability of cortical pyramidal cells. Application of the D1/D5 agonist SKF 81297 induced a long-lasting decrease in the first spike latency in response to depolarizing current ramp. This was associated with a shift in the start of nonlinearity in the slope resistance to more negative membrane potentials. We proposed that this effect is due to a D1/D5 agonist-induced leftward shift in the activation of I_{NaP}. This enables DA to facilitate the firing of PFC neurons in response to depolarizing inputs.

INTRODUCTION

Mammalian medial prefrontal cortex (mPFC) is functionally involved in higher cognitive processes that underlie planning and organization of forthcoming behavior (Fuster 1995; Goldman-Rakic 1995). The mPFC in both primates and rodents receives a mesocortical dopaminergic projection that arises from the ventral tegmental area (VTA) of the midbrain (Björklund and Lindvall 1984). Dopamine (DA), via D1/D5 receptor activation in the mPFC, has been shown to modulate behavior that requires working memory (Brozowski et al. 1979; Murphy et al. 1996; Sawaguchi and Goldman-Rakic 1991). During short-term working memory processing, a DA-dependent increase in active firing of PFC neurons has been shown. This sustained period of firing typically occurred during a brief delayed period when previously acquired memory has to be “held” temporarily and be used later to guide forthcoming behaviors (Fuster 1995; Goldman-Rakic 1995). An optimal level of DA in the mPFC is critical for this type of cognitive functions (Kimmberg et al. 1997; Murphy et al. 1996; Zahrt et al. 1997). Too high or too low level of DA present in the mPFC can significantly disrupt cognitive processes that utilize working memory.

An understanding of the ionic bases of DA actions that mediate an increase in firing of mPFC pyramidal neurons is still incomplete. In vivo electrophysiological studies in mPFC pyramidal neurons show that microiontophoretic application of DA, or stimulation of the VTA, primarily suppress an on-going spontaneous firing activity (Bunney and Aghajanian 1976; Pirot et al. 1992; Sawaguchi and Matsumura 1985; Sesack and Bunney 1989; Yang and Mogenson 1990). In contrast, a very low level of DA potentiates the firing activity induced by iontophoretic application of glutamate and acetylcholine in cortical neurons (Cépeda et al. 1992; Yang and Mogenson 1990). Recent in vitro intracellular recordings in rat mPFC slices have revealed that DA modulates voltage-dependent Na+, K+, and Ca2+ currents to alter neuronal excitability and, ultimately, synaptic integration (Geijo-Barrientas and Pastores 1995; Gulløede and Jaffe 1998; Penit-Soria et al. 1987; Shi et al. 1997; Yang and Seamans 1996; reviewed in Yang et al. 1999). Several of these currents are active at the subthreshold voltage range and play an active role in determining firing threshold and amplification of subthreshold synaptic inputs.

In mammalian neocortical neurons, one of the major ionic conductances that are active at the subthreshold voltage range is the slowly inactivating, or “persistent,” Na+ current (I_{NaP}) (Connors et al. 1982; Crill 1996; Stafstrom et al. 1985). Results from single Na+ channel recordings in acutely isolated cortical pyramidal neurons suggest that the slow I_{NaP}, as well as the transient Na+ current (responsible for spike firing), is conducted via a single population of Na+ channels. The Na+ channels can conduct the two currents by undergoing two kinetically different gating modes (Alzheimer et al. 1993; Brown et al. 1994; Moorman et al. 1990). Furthermore activation of both protein kinase A (PKA) and C (PKC) have been shown to modulate the transient Na+ current as well as the I_{NaP}
The slices were placed in ice-cold oxygenated (95% O2 -5% CO2) artificial cerebrospinal fluid (Medical System Corps) when recording commenced.

**METHODS**

**Brain slice preparations**

The experiments were performed in brain slices prepared from young adult (5–7 wk old, 80–150 g) male Sprague-Dawley rats. Similar in all our previous studies, acute decapitation by a guillotine with no prior anesthesia was performed using Decapicane plastic resin (BrainTree, MA). The brains were quickly removed and placed in ice-cold oxygenated (95% O2-5% CO2) artificial cerebrospinal fluid (ACSF) for 1–2 min. The ACSF contained (in mM): 125 NaCl, 3 KCl, 2.4 CaCl2, 1.3 MgCl2, 26 NaHCO3, and 10 glucose. The lateral portions of the cortex from both hemispheres were trimmed away, leaving the medial portions of the frontal cortex of both hemispheres still joined together by the corpus callosum. Four-hundred-μm-thick bilateral PFC slices were then cut on a vibratome (Campden, World Precision Instruments). The slices were placed in continuously oxygenated ACSF at room temperature. After ≥1 h of incubation, a single slice was transferred to a submersion recording chamber (Medical System Corps) when recording commenced.

**Recordings**

The patch-clamp technique in whole cell configuration was used to study the effects of DA and its agonists on the INaP. Patch pipettes were fabricated from borosilicate tubing (1.5 mm OD, 1.1 mm ID) on a horizontal microelectrode puller (P-87, Sutter Instruments). They had a resistance of 3–5 MΩ when filled with the patch pipette solution.

In voltage-clamp experiments, to pharmacologically isolate the INaP, the patch pipette solution was slightly modified from that used by Fleidervish et al. (1996). The internal pipette solution contained (in mM): 135 CsCl, 2 MgCl2, 1 EGTA, 10 HEPES, 2 Na2ATP, and 0.3 Na2GTP, adjusted to pH 7.3 by CsOH and had an osmolality of 285–295 mOsm (Advanced Instruments). In addition, 200 μM CdCl2 was added to the ACSF to block Ca2+ currents. Under these recording conditions, two protocols were used to activate the INaP.

**Depolarizing voltage ramp.** The membrane potential was clamped at −70 or −80 mV, and slow (20–50 mV/s) voltage ramps from −70 to 10 mV, or −80 to 0 mV were applied.

**Depolarizing voltage steps.** Depolarizing voltage steps were applied (from various holding potentials, 1-s duration) to examine the steady-state INaP mainly in the voltage range from −70 to −50 mV. At membrane potentials more positive than −50 mV, unclampable fast Na+ currents were elicited and they obscured the measurement of the INaP.

To achieve adequate space clamp of the INaP, Cs+ was used as a main cation to block most of the K+ currents and to make the neuron electronically more compact to minimize “space-clamp” error. In addition, we monitored the access resistance very closely and rejected all recordings with access resistance >20 MΩ.

In whole-cell recordings, the patch pipette internal solution contained (in mM): 130 K+ gluconate, 10 KCl, 2 MgCl2, 1 EGTA, 10 HEPES, 2 Na2ATP, and 0.3 Na2GTP, adjusted to pH 7.3 by NaOH and had an osmolality of 290–300 mOsm (Advanced Instruments). Depolarizing current ramp (800 ms) or steps (600 ms) were applied. Membrane potential was continuously current-clamped to control level during drug applications.

Both voltage and current signals were amplified by an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). During voltage-clamp recording, current was low-pass filtered at 1 kHz with the signal gain increased ≥25 nA/mV. All signals were digitized with a 12 bit A/D converter (Digidata 1200B) and stored in the computer hard disk for off-line analysis.

Access resistance in current-clamp “bridge” mode was monitored by recording the instantaneous voltage responses to given repetitive −100-pA (100 ms) pulses. “Bridge” balance was maintained throughout the experiments to eliminate the time-independent component of the voltage responses occurring at the onset and offset of the rectangular current pulses applied through the electrode. Series resistance (10–20 MΩ after “break-in”) compensation in voltage-clamp experiments was first made in current-clamp mode and then monitored periodically in voltage-clamp mode by applying repetitive constant voltage steps while observing the changes in capacitative current amplitude and the associated time constant. Liquid junction potentials between bath and pipette solutions were calculated using the build-in software in pClamp7 and approximately equal to −6 mV for Cs+-based pipette solution and approximately equal to −15 mV for K+-gluconate-based pipette solution. Current-clamp recordings were corrected for the difference between these junction potentials. All experiments were conducted at room temperature (22–24°C).

**Drug applications**

All drugs were bath-applied. Stock solutions of tetrodotoxin (TTX, Alomone Lab., Israel), SKF 38393, SKF 81297 (RBI), PKC inhibitor chelerythrine (RBI), PKC inhibitor peptide[19–36] (Calbiochem) and PKA inhibitor peptide[5–24] (Calbiochem) were prepared in de-ionized water and stored as frozen aliquots at −20°C. The full agonist for dopamine D1/D5 receptors, dihydrexidine (Tocris), was freshly dissolved in ethanol prior to application. Stock solution of DA was also prepared fresh just before application for each experiment. To reduce oxidation of DA, sodium metabisulfite (0.002% final concentration in ACSF) was used. Vehicle controls showed no apparent changes in INaP or neuronal excitability. All drugs were diluted to desired concentrations in the perfusate immediately before application.
Data analysis

In voltage-clamp recordings, a “leak” current was estimated off-line by fitting the linear portion of the current during a depolarizing ramp to a straight line using pClamp6. It was then subtracted off the trace digitally using SigmaPlot software. Group data are presented as means ± SE.

In current-clamp recordings from regular spiking neurons or intrinsic bursting neurons (neuronal response types were classified according to Yang et al. 1996), the threshold of action potential generation was examined by injecting current ramps (800 ms) with an amplitude just sufficient to evoke single or doublet action potentials. In between these current ramps (applied every 30 s), we monitored the input resistance of the cell by its voltage response to a small hyperpolarizing current pulses (100 ms; 25 pA). The slope of the voltage trace during the ramp protocol in the voltage range from −80 to −65 mV (where the $I_{\text{NaP}}$ was largely inactive) was also used to calculate the slope resistance.

Results

Voltage-dependent changes of the $I_{\text{NaP}}$ in layer V–VI pyramidal neurons

Whole cell Na$^+$ currents were recorded using Cs$^+$-filled electrodes (to block K$^+$ currents) in the presence of extracellular Cd$^{2+}$ (to block Ca$^{2+}$ currents) in layer V–VI mPFC neurons. Only neurons that had a resting membrane potential more negative than −65 mV and spike height exceeding 80 mV immediately on achieving whole cell mode were taken into consideration. After switching from current- to voltage-clamp mode, employing a slow depolarizing voltage ramp (34 mV/s) resulted in an initiation of a negative slope conductance at membrane potentials more positive than −60 mV. After leak subtraction, a net inward current with a mean peak current of 290 ± 75 pA (range: 180–500 pA, $n = 20$) between −35 and −30 mV was revealed (Fig. 1). The inward current had a tendency to increase gradually (i.e., “run-up”) during the first 3–5 min after achieving whole cell recording. Then it stabilized and in most cases, the inward current did not show any sign of a “run down” during the next 40–60 min into the experiment.

Addition of 1 μM TTX to the bath ($n = 4$) completely abolished this inward current, thus strongly suggesting that it represents a TTX-sensitive $I_{\text{NaP}}$ (Fig. 1A). At membrane potentials more positive than −25 mV, an outward current was revealed in all cells recorded. This outward current has previously been characterized as a nonselective cationic current (Alzheimer 1994).

The amplitude of inward current evoked by the ramp depolarization was dependent on the rate of voltage ramp: the slower the rate of the voltage ramp, the smaller the peak amplitude of the ramp current (Fig. 1B). This is consistent with the data that have been obtained in mouse layer V pyramidal neurons in neocortical slices (Fleidervish and Gutnick 1996) and suggests that $I_{\text{NaP}}$ in rat layer V–VI mPFC pyramidal neurons is also subjected to inactivation during slow voltage ramp. Holding cells at more negative potentials than −70 mV (up to −90 mV) and employing depolarizing voltage ramps from these potentials did not change the peak amplitude or voltage dependence of inward ramp current (not shown).

Effects of DA on $I_{\text{NaP}}$ and a “leak” current

Using the voltage-ramp protocol, DA (1–30 μM) did not induce a consistent change in the peak amplitude of the $I_{\text{NaP}}$. From the 10 cells tested, DA increased the peak amplitude in 2 cells, reduced it in 1 cell, and did not induce any changes in 7 cells. However, in 9 of 10 cells tested using the depolarizing voltage-ramp protocol, application of 1–30 μM DA induced voltage-dependent changes in the $I_{\text{NaP}}$: there was an increase $I_{\text{NaP}}$ amplitude at potentials more negative than −40 mV and a decrease $I_{\text{NaP}}$ amplitude at potentials more positive than −20 mV (Fig. 2). For example, at a membrane potential of −50 mV, DA increased the $I_{\text{NaP}}$ by 30 to 50% (mean = 34 ± 9%, $n = 10$; Fig. 2, A–C).

![Fig. 1. TTX-sensitive $I_{\text{NaP}}$ recorded in rat medial prefrontal cortex (mPFC) layer V–VI pyramidal neurons. A: I–V relationship obtained during a slow (34 mV/s) depolarizing voltage ramp (from −70 to +10 mV). Each trace represents an average from 5 consecutive recordings. TTX (1 μM, after 10 min application) blocked the inward component of current, indicating that it represents the TTX-sensitive $I_{\text{NaP}}$. Leak current subtracted. B: inactivation of $I_{\text{NaP}}$ during depolarizing ramps delivered at varying rates. The amplitude of the inward $I_{\text{NaP}}$ decreased with a slowing of the depolarizing voltage-ramp rate. Each trace represents an average from 3 consecutive recordings. To avoid a cumulative effect of inactivation, the interval between trials with different ramp rate was 1 min. Note that at fast ramp rate (102.4 mV/s) transient fast Na$^+$ current was also activated (with peak truncated).](http://jn.physiology.org/doi/10.1152/jn.01220.2016)
In 6 of 10 cells tested, DA also induced an increase of a voltage-independent leak current (Fig. 2D). Following subtraction of this leak current, a leftward shift in the voltage dependence of ramp $I_{\text{Na}}$ was also observed (Fig. 2E). This DA-sensitive leak current is expressed as an increase in the slope of the linear portion of the current trace in the voltage range between -80 and -65 mV (where the $I_{\text{Na}}$ was inactive; Fig. 2D). To assess the reversal potential of the “leak” current affected by DA in the six cells, DA (30 µM) was applied when all Na$^+$ currents were blocked by adding 1 µM TTX in the perfusate. Slow depolarizing voltage ramp from -100 to -10 mV was then injected. In five of these cells, DA induced an increase in the leak current that has a reverse potential of -30 mV (Fig. 3). In most cases, changes in the leak current were completely reversible on 15–20 min of wash, while the DA-induced shift in activation of the $I_{\text{Na}}$ persisted for the duration of the recording in the experiment (≤1 h).

**Effects of DA on the inactivation kinetics of the $I_{\text{Na}}$**

Since $I_{\text{Na}}$ is subjected to a time-dependent inactivation during the slow depolarizing ramp (Fleidervish and Gutnick 1997), the ramp $I_{\text{Na}}$ current is a result of an interplay of activation and inactivation processes. Taking this into consideration, a leftward “shift” in the voltage dependence of ramp Na$^+$ current may be due to a combination of a leftward shift in activation, a slowing (time dependence) of $I_{\text{Na}}$ inactivation, and a rightward shift in steady-state inactivation.

To determine if DA affects the time dependence of inactivation of the $I_{\text{Na}}$, depolarizing voltage steps from -80 to -30 mV with increasing duration were injected prior to application of a slow depolarizing voltage ramp from -80 to 0 mV (Fleidervish et al. 1996). We have used prepulse voltage step only to -30 mV to avoid activation of the cationic outward current. Prepulses with duration 1 s induced a duration-dependent reduction of the peak amplitude of the $I_{\text{Na}}$ (Fig. 4A).
Effect of DA receptor agonists on $I_{NaP}$

Bath application of the D1/D5 agonists SKF 39393, SKF 81297, or dihydrexidine (1–10 μM) did not induce consistent changes in the peak amplitude of the $I_{NaP}$ activated by a depolarizing voltage ramp. Of 34 cells tested, the peak amplitude of the $I_{NaP}$ (evoked by a depolarizing voltage ramp) was reduced by the D1/D5 agonists in 12 cells, increased in 5 cells (e.g., Fig. 5C), and not changed in 17 cells.

In 30 of 34 cells tested, the D1/D5 receptor agonist applications shifted the voltage dependence of ramp $I_{NaP}$ to more negative potentials despite having different effects on the peak amplitude of $I_{NaP}$ in some of these cells (Fig. 5, A and C). Since SKF38393, SKF81297 and dihydrexidine are equally effective in inducing a leftward shift in the activation of the ramp $I_{NaP}$, we have pooled the D1/D5 agonists data together for analysis.

At −50 mV, $I_{NaP}$ activated by slow depolarizing ramp was increased by 27 ± 12% (n = 34 cells). As in the case with DA, the D1/D5 agonist-induced leftward shift in the activation of ramp $I_{NaP}$ in most cases lasted for 30 min (Fig. 5, B and D). However, when the brain slices were preincubated for 10 min with D1/D5 receptor antagonist SCH23390 (9 μM), application of the D1/D5 agonist dihydrexidine (3 μM) no longer induced any significant shift in the voltage dependence of ramp $I_{NaP}$.

The DA-induced increase in inward $Na^+$ current can be seen not only at the end but also at the beginning of the voltage step (Fig. 4D) where the inactivation process (in response to depolarization) has not yet played a significant role. It suggests that although DA-induced changes in time dependence of inactivation can contribute to the shift in voltage dependence of the ramp $I_{NaP}$, the DA-induced increase of ramp $I_{NaP}$ at subthreshold membrane potentials are mainly not due to slowing of the $I_{NaP}$ inactivation by DA. Taking together, these data suggest that DA-induced leftward shift in the voltage dependence of the $I_{NaP}$ is primarily due to a leftward shift in the activation of the $I_{NaP}$ by DA.
$I_{\text{NaP}}$ (not shown). Ramp $I_{\text{NaP}}$ measured at $-50$ mV was not changed ($-1.3 \pm 4.2\%$, $n = 4$). The D2/D3 agonist quinpirole at a concentration of $\approx 20 \mu M$ failed to affect the peak amplitude of $I_{\text{NaP}}$ or to induce any shift in the voltage dependence of $I_{\text{NaP}}$ evoked by a voltage ramp ($n = 11$). Taken together, these results strongly suggest that DA affects the activation of the $I_{\text{NaP}}$ through D1/D5 receptor stimulation.

Similar to the effects induced by DA, D1/D5 agonists also slowed the inactivation of the $I_{\text{NaP}}$. Figure 6 illustrates an experiment when the $I_{\text{NaP}}$ activated by a long (7.5 s) depolarizing pulse was used to record the full extent of the inactivation of $I_{\text{NaP}}$. The $I_{\text{NaP}}$ evoked following D1/D5 agonist application was normalized against baseline current in the control (Fig. 6A). Following exposure to SKF81297 (5 $\mu M$), the time constant (tau) of full inactivation of the $I_{\text{NaP}}$ was enhanced by $43 \pm 16\%$ ($n = 6$).

**Effects of D1/D5 receptor stimulation on the activation of the $I_{\text{NaP}}$ required PKC but not PKA**

Activation of PKC has been shown to induce a leftward shift in the voltage dependence of ramp-activated $I_{\text{NaP}}$ and slows the inactivation of the transient Na$^+$ current (Astman et al. 1998; Numann et al. 1991; Sancini et al. 1999; Taverna et al. 1999). In the present study, we have examined the possibility that D1/D5 receptor stimulation activated an intracellular PKC pathway to induce a shift in the voltage dependence of ramp $I_{\text{NaP}}$ in two series of experiments.

In the first series of experiments ($n = 8$), the PKC inhibitor peptide$\text{tr}_{19-30}$ (20 $\mu M$) was added to the patch pipette solution immediately before recording. In six of eight cells tested after 13–30 min of diffusion of the inhibitory peptide into the
recorded cell, bath application of D1/D5 agonists no longer induced an increase in the ramp $I_{\text{NaP}}$ at membrane potentials more negative than $-40$ mV (Fig. 7). When PKC activity was blocked, amplitude of ramp $I_{\text{NaP}}$ measured at $-50$ mV at 10 min after D1/D5 agonist application was even slightly reduced in most cases (mean changes $-4 \pm 5\%$, $n = 6$). In the remaining two cells, we observed a delayed shift in the activation of the $I_{\text{NaP}}$, starting at 20 min after the D1/D5 agonist application. The peak amplitude of the ramp $I_{\text{NaP}}$ was reduced slightly by the D1/D5 agonists following PKC inhibitor peptide [19–36] treatment (Fig. 7, A and B). Control experiments revealed that the PKC inhibitor peptide [19–36] alone could reduce the peak amplitude of the ramp $I_{\text{NaP}}$ in two of four tested neurons, but the peptide alone failed to induce a shift in the $I_{\text{NaP}}$ activation.

In the second series of experiments, another PKC inhibitor, chelerythrine (15 μM), was bath applied for 30–50 min. Subsequent application of D1/D5 agonists failed to induce a shift in the activation of ramp $I_{\text{NaP}}$ to more negative potentials ($n = 3$, not shown). These data suggest that PKC may be involved in mediating the effects of D1/D5 receptor activation on the $I_{\text{NaP}}$ evoked by a voltage ramp in rat mPFC pyramidal cells.

Since it was shown that DA also modulates the transient Na$^+$ current in pyramidal neurons of hippocampus through the cAMP-dependent activation of PKA (Cantrell et al. 1997), we have tested the possible role of this intracellular pathway in modulating ramp $I_{\text{NaP}}$ by D1/D5 receptor stimulation. Inclusion of the PKA inhibitor peptide [5–24] (20 μM) in the internal patch pipette solution did not block the leftward shift in the voltage dependence of ramp $I_{\text{NaP}}$ in response to application of D1/D5 agonists (Fig. 7, C and D, $n = 4$). It resulted in an increase in the amplitude of ramp $I_{\text{NaP}}$ measured at $-50$ mV. Average value of current increase was 21 ± 4%, which is not significantly different from the value of current increase by D1/D5 agonist without the PKA peptide inhibitor in the pipette solution (27 ± 12%). However, the D1/D5 agonist did reduce the peak amplitude of the $I_{\text{NaP}}$ (Fig. 7, C and D). Collectively, these data suggest that the PKC, but not PKA, pathway is likely to mediate the effects of D1/D5 receptor activation on the shift of voltage dependence of $I_{\text{NaP}}$ evoked by voltage ramp in rat layer V–VI pyramidal cells from the mPFC.

**Effect of D1/D5 receptor stimulation on excitability of cortical pyramidal neurons**

$I_{\text{NaP}}$ is known to play an important role in setting the threshold for firing action potentials (Crill 1996). Therefore we have investigated how modulation of $I_{\text{NaP}}$ by D1/D5 agonists might affect the excitability of mPFC neurons in current-clamp experiments. To avoid indirect effect of the D1/D5 agonists on the cell excitability through modulating glutamate and/or GABA release (Gulledge and Jaffe 1998; Zhou and Hablitz 1999), APV (50 μM), 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM), and bicuculline (10 μM) were added to the perfusate to block the N-methyl-D-aspartate (NMDA), AMPA and GABA$\_A$ receptor channels, respectively.

Bath application of D1/D5 agonist SKF81297 (3–10 μM) induced a small membrane depolarization (2–3 mV) accompanied by a transient reduction in the input resistance ($n = 4$ of 8 cell tested) (see also Shi et al. 1997; Yang and Seamans 1996). Under this condition, the number of spikes evoked by injection of depolarizing current steps increased (Fig. 8A1). However, if the membrane potential was current-clamped continuously (via DC injection) to predrug value, injection of the same depolarizing current steps evoked less number of spikes (Fig. 8A1). With this bias current continuous holding the cell, injection of a depolarizing current ramp resulted in a biphasic change in the latency of the first spike initiation (Fig. 8A, 2 and 3). An initial brief increase was followed by a long-lasting pronounced decrease in first spike latency. The late, long-lasting effect on spike firing was associated with the starting of nonlinearity in the voltage slope at more negative membrane potential and a decrease in spike firing threshold, thus giving rise to an overall increase in neuronal excitability (Fig. 8A, 2 and 3). The graph in Fig. 8A3 shows the time course of the D1/D5 agonist-induced responses to current ramp injection.

In mPFC neurons that were not depolarized by SKF81297, they only showed a decrease in first spike latency evoked by a depolarizing current step and ramp (Fig. 8B, 1 and 2) and an increase in the number of spikes evoked by a depolarizing step ($n = 4$ of 8 cells tested; Fig. 8B1). These long-lasting effects were accompanied by no significant changes in input resistance (Fig. 8B3).
DISCUSSION

In the present study in mPFC layer V–VI pyramidal neurons, slow depolarizing voltage ramp activated a TTX-sensitive $I_{\text{NaP}}$. DA induced a leftward shift in the voltage dependence of this ramp $I_{\text{NaP}}$. This has resulted in an increase of $I_{\text{NaP}}$ in the subthreshold voltage range for spike generation but a decrease of this current at more depolarized membrane potentials. DA also slowed the development of a full inactivation of $I_{\text{NaP}}$ during sustained depolarization. The DA effects on the $I_{\text{NaP}}$ was mimicked by D1/D5 agonists SKF 81297, SKF38393, and dihydrexidine but not by the D2/D3 receptor agonist quinpirole. The effects of DA on the voltage-dependent activation of $I_{\text{NaP}}$ were blocked by selective PKC, but not PKA, inhibitors. These data suggest that following D1/D5 receptor activation, DA exerted its effects on the voltage dependence of the $I_{\text{NaP}}$ via intracellular mechanisms requiring the activation of PKC.

Several mechanisms have been proposed to account for the generation of $I_{\text{NaP}}$ in neurons. $I_{\text{NaP}}$ may be the result of 1) a "window" current arising from an overlap between Na$^+$ channel steady-state activation and inactivation (Attwell et al. 1979); 2) switching of an inactivating Na$^+$ channel into a noninactivating state—mode switching (e.g., transition of the transient Na$^+$ channel to nonactivating gating) (Alzheimer et al. 1993; Brown et al. 1994; Keynes 1994; Patlak and Ortiz 1986); 3) slow closed-state inactivation if Na$^+$ channels, especially at intermediate membrane potentials (Cummins et al. 1998); and 4) the presence of a distinct channel isoform with a different unitary conductance (Crill 1996; Magistretti et al.)

**FIG. 7.** Protein kinase C (PKC), but not protein kinase A (PKA), inhibitor abolished the leftward shift in voltage dependence of ramp-activated $I_{\text{NaP}}$ current in response to D1/D5 receptor stimulation. A: ramp-activated $I_{\text{NaP}}$ before (black trace) and 12 min after (gray trace) bath application of 10 μM SKF 81297 when 20 μM PKC inhibitor peptide [19–36] was included in the internal patch pipette solution. Note there is no increase in $I_{\text{NaP}}$ at membrane potentials more negative than −45 mV in response to SKF. Leak current has been subtracted. B: time course of changes in the ramp-activated $I_{\text{NaP}}$ at −50 mV (open circle) and −20 mV (%) for the cell from A. C: $I_{\text{NaP}}$ induced by a slow depolarizing ramp before and 9 min after bath application of 6 μM SKF 81297 when 20 μM PKA inhibitor peptide [5–24] was included in the internal pipette solution. Leak subtracted. Note that SKF induced a shift in voltage dependence of ramp $I_{\text{NaP}}$. D: time course of changes in ramp $I_{\text{NaP}}$ at −50 mV (open circle) and −20 mV (%) for the cell from C.
According to the first three hypotheses, $I_{\text{NaP}}$ is conducted through the same channels as the fast inactivating Na$^+$ current. Data from direct single channel recordings support the hypothesis that the same Na$^+$ channels from neocortical neurons that give rise to fast transient Na$^+$ current for spike firing can also switch to a slowly inactivating/persistent mode and give rise to $I_{\text{NaP}}$ (Alzheimer et al. 1993; Brown et al. 1994). However, it is still unclear under what physiological conditions the soma-dendritic Na$^+$ channels of mPFC neurons will preferentially switch to this slowly inactivating mode.

Our present results of the effects of DA on $I_{\text{NaP}}$ at first appeared to contradict with reported findings of the effects of DA on the $I_{\text{NaP}}$ in striatal and mPFC pyramidal neurons (Cépeda et al. 1995; Geijo-Barrientos and Pastore 1995). In striatal neurons in rat brain slices, whole cell patch-clamp recordings showed that DA reduced the peak amplitude of a putative $I_{\text{NaP}}$ activated during a slow depolarizing ramp protocol (Cépeda et al. 1995). Since Ca$^{2+}$ and K$^+$ channels were not blocked and the effects of DA on the activation and inactivation relationships of the putative $I_{\text{NaP}}$ were not investigated in that study, it is not possible to make a detail comparison with our present results. This becomes more complicated especially when taking into account that in some cells in our study, DA also reduced the peak amplitude of the $I_{\text{NaP}}$ in mPFC pyramidal neurons.

In another study from mPFC pyramidal cells in rat brain slice preparation, putative $I_{\text{NaP}}$ was recorded (also in the absence of any Ca$^{2+}$ and K$^+$ channel blockers) using single sharp electrode voltage clamp (Geijo-Barrientos and Pastore 1995). DA (10 µM) was shown to suppress a persistent inward current during depolarizing steps in the voltage range from −50 to −45 mV. It should be noted that several ionic conductances contribute to the generation of the inward current in this voltage range subthreshold to spike generation. Activation of the low-threshold Ca$^{2+}$ conductance (Sutor and Ziegler-Berger 1987) as well as inactivation of the mixed K$^+$ and Na$^+$ conductance (so called $I_{\text{NaP}}$, which was shown to be active at resting membrane potential in cortical pyramidal neurons) (Spain et al. 1987) resulted in inward currents during depolarizing steps to −50 mV. Furthermore leak currents, and out-
wardly rectifying $K^+$ currents (Yang et al. 1996) can affect the net amplitude of the inward current. Since neither $Ca^{2+}$ nor $K^+$ current blockers were used in the cited studies in the preceding text, it is very difficult to assess which ionic current(s) was suppressed or enhanced by DA.

The actions of DA are mediated via activation of multiple second-messenger signal transduction pathways. D1-like receptor is known to be coupled with the adenylate cyclase system (Kebabian et al. 1972). Subsequent increases in cAMP formation (via stimulation of this cyclase) lead to activation of PKA and the associated biochemical cascades downstream. An additional signal transduction pathway coupled to D1-like receptors was shown by the finding that rat striatal mRNA encodes the expression of D1-like receptors coupled to phospholipase C (PLC) stimulation (Mahan et al. 1990). In rat brain and renal tissues, D1-like receptor activation of PLC leads to increase in inositol phosphate (IP) formation and diacylglycerol (DAG) formation (Felder et al. 1989; Undie and Friedman 1990; Undie et al. 1994). This resulted in a cAMP-independent increase in intracellular $Ca^{2+}$ (by IP) and activation of PKC (by DAG). In mice with D1a receptor subtype “knock-out,” there is a continual expression of D1 receptor-stimulated PI metabolism but not adenylate cyclase activation, further suggesting that the D1-like receptor that couples to stimulation of IP is distinct from the classic D1 receptor which is coupled to stimulation of adenylate cyclase (Friedman et al. 1997). In addition, while D1 receptors stimulation in the rat amygdala induced a large increase in IP formation but no cAMP formation, D1-like receptor activation in the frontal cortex and striatum stimulated both transduction systems (Friedman et al. 1997). These data suggest that there are also distinct differences in the regional distribution of these two signal transduction systems that are coupled to the D1 receptor in the brain (Undie and Friedman 1990).

Modulation of Na$^+$ channel functions in mammalian brain can be achieved by phosphorylation of the channels via activation of the cyclic AMP-dependent protein kinase A (PKA), as well as by calcium-dependent PKC (Numann et al. 1991; Smith and Goldin 1997). Activation of PKA reduced the peak amplitude of the fast Na$^+$ current without affecting either the kinetics or the voltage-dependent properties of the Na$^+$ channels (Gershon et al. 1992; Li et al. 1992; Smith and Goldin 1997). Single-channel recordings in striatal and hippocampal neurons show that DA, through activation of PKA but not PKC, reduced the open probability of Na$^+$ channels that conduct fast transient Na$^+$ current (Cantrell et al. 1997; Schiffmann et al. 1995). Recently it was shown that modulation of the fast Na$^+$ current by D1/PKA pathway is voltage dependent (Cantrell et al. 1999) with no detectable effect at −110 mV but a progressively increasing suppressive effect at more depolarized membrane potentials. Since PKA stimulation induced a small shift in the voltage-dependence of the steady-state inactivation of this Na$^+$ current, the authors suggested that membrane voltage can directly alters the extent or pattern of PKA phosphorylation.

In contrast, activation of PKC not only modulates the peak amplitude of the transient fast Na$^+$ current but also substantially slows its inactivation. In MM14 muscle cells, slowing of inactivation of Na$^+$ current requires a lower concentration of 1-oleoul-2-acetyl-sn-glycerol (OAG, a diacylglycerol analog) than that needed for a reduction of the peak Na$^+$ current, suggesting that these events may be caused by independent phosphorylation events (Numman et al. 1994). Activation of PKC was further shown to increase the life-time of the single Na$^+$ channel spent in the open-state and the probability of its reopening during prolong depolarization (Numann et al. 1991). Moreover, recent studies in rodent cortical neurons also show that a PKC-activating phorbol ester, and OAG, induced a shift in the activation of fast transient Na$^+$ current and $I_{\text{Nap}}$ to more hyperpolarized membrane potentials (Astman et al. 1998; Taverna et al. 1999). A shift in activation has led to a significant increase in the transient Na$^+$ current and $I_{\text{Nap}}$ at membrane potentials more negative than −40 mV and a decrease in their amplitudes at membrane potentials more positive than −40 mV. The PKC-mediated increase in Na$^+$ currents in the subthreshold voltage range for spike generation resulted in a subsequent increase in cortical neuronal excitability (Astman et al. 1998; Sancini et al. 1999; Taverna et al. 1999). These PKC effects are remarkably similar to the effects of DA and D1/D5 agonists on the $I_{\text{Nap}}$ in rat mPFC pyramidal cells as shown in the present study. Our present finding shows that PKC inhibitors, but not a PKA inhibitor, blocked the D1/D5 agonist-induced shift in the activation of $I_{\text{Nap}}$. This finding further suggests that the shift in the activation of $I_{\text{Nap}}$ was mediated via D1/D5 receptor activation of the PKC pathway.

$I_{\text{Nap}}$ has been shown to play a significant role in the modulation of neuronal excitability by setting the threshold of firing and shaping of repetitive firing patterns as well as in the nonlinear amplification of excitatory synaptic inputs (Crill 1996; Schwindt and Crill 1995; Stuart 1999; Stuart and Sackmann 1995). While a DA-induced leftward shift in the activation of $I_{\text{Nap}}$ results in a lowering of the threshold of spike initiation, slowing of $I_{\text{Nap}}$ inactivation by DA may also be responsible for increasing the neuronal ability to repetitive firing. Since DA modulates not one but several ionic conductances at subthreshold voltage range in mPFC pyramidal neurons, the net effect of DA on spike initiation and neuronal excitability will depend on a complex interplay of the $I_{\text{Nap}}$ with these conductances (Dilmore et al. 1999; Yang and Seamans 1996; Yang et al. 1999).

An early transient effect of DA in reducing neuronal excitability due to a reduction in input resistance was reported by Gulledge and Jaffe (1998) and by Geijo-Barritos and Pastore (1995). They showed that a significant DA-induced reduction of slope resistance occurs when GABA$_A$ receptors were not blocked adequately (Gulledge and Jaffe 1998, 1999), and the suppression of evoked spikes by DA can be mimicked by D2/D3 agonist quinpirole (Gulledge and Jaffe 1998). These data suggest that DA-induced activation of GABAergic interneurons may also take part in the control of pyramidal neuronal excitability (Yang et al. 1999; Zhou and Hablitz 1999).

In some mPFC neurons in the present study, activation of D1/D5 receptors can also induce a transient decrease in input resistance with no reduction in neuronal excitability. Our voltage-clamp data suggest that this decrease in input resistance is likely to be due to an increase in a leak current that has a reversal potential of approximately equal to −35 mV. This reduction of input resistance by DA in these cells was also accompanied by a small membrane depolarization that can bring the membrane potential closer to the point of activation of the $I_{\text{Nap}}$ in this group of mPFC neurons.

As we have shown in a previous study (Yang and Seamans
1996), activation of D1/D5 receptors induced a long-lasting increase in neuronal excitability which may be mediated by D1/D5 receptor actions on \( I_{\text{NaP}} \) (this study). This delayed increase of excitability in mPFC neurons by DA has recently been replicated independently by several investigators (Ceci et al. 1999; Gulledge and Jaffe 1999; personal communication; Lavin and Grace 1999). These findings suggest that for a given depolarizing input, postsynaptic D1/D5 and D2/D3 dopamine receptor stimulation may modulate time-dependent changes in ionic conductances that alter the threshold for firing in mPFC neurons. In response to depolarizing inputs, activation of D2/D3 receptors may induce an early transient suppression of neuronal excitability by increasing a leak current, while activation of D1/D5 receptors induces a late, prolonged enhancement of neuronal excitability by shifting the activation of \( I_{\text{NaP}} \) to a more negative potential, plus slowing the rate of full inactivation of \( I_{\text{NaP}} \).

**Functional significance of a DA modulation of \( I_{\text{NaP}} \)**

Modulation of the activation and inactivation kinetics of \( I_{\text{NaP}} \) by DA may result in a wide-range of changes in neuronal signal integration. In mPFC pyramidal neurons, subthreshold membrane depolarization by inward currents is known to be opposed strongly by a slowly inactivating outward \( K^+ \) conductance (Yang et al. 1996). Dopamine, via D1/D5 receptor activation, has been shown to attenuate this slow \( K^+ \) conductance, thus allowing a full expression of the effects mediated by \( I_{\text{NaP}} \) (Yang and Seamans 1996; Yang et al. 1996). Subthreshold EPSPs have been shown to be amplified by TTX-sensitive \( I_{\text{NaP}} \), which are generated close to the initial portion of the apical dendrites of deep layer somatosensory cortical and hippocampal CA1 pyramidal neurons (Lipowsky et al. 1996; Schwindt and Crill 1995; Stafstrom et al. 1985; Stuart and Sakmann 1995). More recently, Stuart (1999) has also shown that hyperpolarizing GABAergic inhibitory postsynaptic potentials (IPSPs) can turn off the inward \( I_{\text{NaP}} \), thus resulting in a net enhancement of the outward GABAergic current to ultimately produce an amplification of the GABAergic IPSP.

The effects of DA on \( I_{\text{NaP}} \) shown in the present study suggest that DA may modulate both excitatory postsynaptic potentials (EPSPs) and IPSPs via its interactions with the \( I_{\text{NaP}} \) directly. \( I_{\text{NaP}} \) also contributes to the generation of a narrow bandwidth membrane voltage oscillation when neocortical neurons are depolarized to the subthreshold membrane potential range in a sustained manner (Alonso and Linas 1989; Amitai 1994; Gutfreund et al. 1995; Linas et al. 1991; Stafstrom et al. 1985; Yang et al. 1996). At the subthreshold potentials, this TTX-sensitive oscillation is generated via interactions of the \( I_{\text{NaP}} \) with a TEA- or a dieldrotoxin-sensitive \( K^+ \) current (Gutfreund et al. 1995; Yang et al. 1996) and other incoming synaptic inputs in vivo (Cowan and Wilson 1994; Nuñez et al. 1992; Steriade et al. 1993). This type of oscillation has been suggested to be the cellular substrate that underlie rhythmic synchronized activities recorded from cortical networks during sensory information processing (Singer 1993). Since the subthreshold oscillation itself cannot be transmitted to neighboring neurons, in order for neuronal network synchronization to occur, synaptic inputs could be used to generate spikes that ride on top of the subthreshold oscillation. This may then generate synchronized spike output of a specific frequency to the neighboring interconnected neurons.

Finally, although not directly tested in the present study, it is conceivable from our present findings that the dopamine-induced shift of activation and inactivation kinetics of the \( I_{\text{NaP}} \) will enhance the probability of mPFC neurons to initiate spike firing at a designated time in response to a subthreshold depolarizing input. In this way, specific spike frequency can be transmitted to neighboring interconnected neurons (Kritzer and Goldman-Rakic 1995; Levitt et al. 1993) that may be undergoing subthreshold oscillation at the same frequency and phase. Outputs from these neurons may induce a micro network of interconnected neurons to fire synchronously (Lampl and Yarom 1993; Yang et al. 1999). This may provide a cellular mechanism for “binding” the firing activity between two or more interconnected mPFC neurons to a specific rhythm during working memory processing (Durstewitz et al. 1999; Yang and Seamans 1996; Yang et al. 1999) when certain information has to be held for a short period of time prior to their use to guide forthcoming behaviors. Clearly experimental evidence is needed to support or refute such theoretical framework of the functional roles of DA modulation of \( I_{\text{NaP}} \) in synaptic integration and neuronal network functions.

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