Dopamine Increases Excitability of Pyramidal Neurons in Primate Prefrontal Cortex

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Received 7 June 2000; accepted in final form 10 August 2000

Henze, Darrell A., Guillermo R. González-Burgos, Nathaniel N. Urban, David A. Lewis, and German Barrionuevo. Dopamine increases excitability of pyramidal neurons in primate prefrontal cortex. J Neurophysiol 84: 2799–2809, 2000. Dopaminergic modulation of neuronal networks in the dorsolateral prefrontal cortex (PFC) is believed to play an important role in information processing during working memory tasks in both humans and nonhuman primates. To understand the basic cellular mechanisms that underlie these actions of dopamine (DA), we have investigated the influence of DA on the cellular properties of layer 3 pyramidal cells in area 46 of the macaque monkey PFC. Intracellular voltage recordings were obtained with sharp and whole cell patch-clamp electrodes in a PFC brain-slice preparation. All of the recorded neurons in layer 3 (n = 86) exhibited regular spiking firing properties consistent with those of pyramidal neurons. We found that DA had no significant effects on resting membrane potential or input resistance of these cells. However DA, at concentrations as low as 0.5 μM, increased the excitability of PFC cells in response to depolarizing current steps injected at the soma. Enhanced excitability was associated with a hyperpolarizing shift in action potential threshold and a decreased first interspike interval. These effects required activation of D1-like but not D2-like receptors since they were inhibited by the D1 receptor antagonist SCH23390 (3 μM) but not significantly altered by the D2 antagonist sulpiride (2.5 μM). These results show, for the first time, that DA modulates the activity of layer 3 pyramidal neurons in area 46 of monkey dorsolateral PFC in vitro. Furthermore the results suggest that, by means of these effects alone, DA modulation would generally enhance the response of PFC pyramidal neurons to excitatory currents that reach the action potential initiation site.

INTRODUCTION

Several lines of evidence indicate that the dorsolateral prefrontal cortex (PFC) plays a critical role in working memory. First, surgical removal of tissue from the principal sulcal region of the monkey PFC impairs performance in behavioral tasks that engage working memory (Butters et al. 1971; Funahashi et al. 1993; Goldman et al. 1971; Levy and Goldman-Rakic 1999; Passingham 1975). Second, single units in the monkey PFC exhibit task-related changes in firing rate during delayed-response tasks (Fuster and Alexander 1971; Kubota and Niki 1971). Furthermore PFC units show a delay-related increase in firing rate that correlates with correct task performance (Funahashi et al. 1989; Fuster 1973) and is less sensitive to disruption by intervening stimuli than delay activity in other cortical regions (Miller et al. 1996). Together these data indicate that PFC cell activity is a leading candidate for a cellular basis of working memory (Funahashi and Kubota 1994; Goldman-Rakic 1995).

The neurotransmitter dopamine (DA) appears to play an important role in the regulation of working memory-related processes in the PFC. For example, disruption of the PFC DA system via local depletion or pharmacological antagonism at the principal sulcus region impairs working memory in monkeys (Brozoski et al. 1979; Sawaguchi and Goldman-Rakic 1991, 1994). Also extracellular levels of DA increase significantly in the monkey PFC during performance of delay tasks (Watanabe et al. 1997). Finally, the delay-task-related activity of monkey PFC units is modulated by DA receptor stimulation (Sawaguchi et al. 1990a,b; Williams and Goldman-Rakic 1995). Thus DA in the PFC seems to play an important role in both delay-task-related neuronal activity and in working memory performance.

Present understanding of the cellular mechanisms underlying the electrophysiological actions of DA in monkey PFC is sparse, in part because the currently available evidence comes only from extracellular recordings in vivo (Sawaguchi et al. 1990a,b; Williams and Goldman-Rakic 1995). All previous in vitro studies that examined electrophysiological effects of DA on PFC neurons were performed in the rat medial PFC. Whereas dorsolateral prefrontal cortical areas in macaque monkeys and humans share multiple characteristics (Petrides and Pandya 1999), there are significant anatomical and functional differences between rat medial PFC and monkey dorsolateral PFC (Preuss 1995). Moreover, the prefrontal DA systems of primates and rats differ markedly in a number of respects (Berger et al. 1991; Preuss 1995; Williams and Goldman-Rakic 1998). For example, in rat medial PFC, the density of dopaminergic terminals and receptors is significantly higher in the deep than in superficial layers, whereas in the monkey and human dorsolateral PFC, DA receptors and fibers also are present in high density in the superficial layers (Berger et al. 1991; Lewis and Sesack 1997).

In macaque monkey dorsolateral PFC, the majority (70–
80%) of pyramidal projection neurons that give origin to cortico-cortical output (association and callosal) are located in layer 3 (Andersen et al. 1985; Schwartz and Goldman-Rakic 1984). Layers 2/3 also contain most of the pyramidal cells that provide long-distance intrinsic horizontal connections (Gonzalez-Burgos et al. 2000; Kritzer and Goldman-Rakic 1995; Levitt et al. 1993; Pucak et al. 1996), which mediate intrinsic excitation that may be essential to delay-related activity of monkey PFC neurons (Goldman-Rakic 1995; Lewis and Anderson 1995). These data suggest that dopaminergic regulation of the activity of layer 3 pyramidal neurons could have a significant functional impact on local excitation in PFC and its propagation to other neocortical regions. However, with a few exceptions (Sawaguchi and Matsumura 1985), most previous in vivo studies in monkey dorsolateral PFC did not report the laminar localization of the recorded cells. In addition, because deep layers in rat PFC receive the strongest dopaminergic innervation, the majority of studies of DA actions on pyramidal neurons in vitro have focused on layers 5 and 6. As a result, substantial evidence has accumulated that indicates that in PFC, DA can modulate cell activity in the deep layers, which send output to subcortical targets. In contrast, direct evidence for dopaminergic modulation in superficial layers, which convey output signals to other regions of the cerebral cortex, is scarce.

It is often assumed that the data from the electrophysiological studies of DA actions on rat medial PFC are applicable to the primate PFC. Indeed although significant differences in dopaminergic innervation and receptor distribution separate the PFC of rats and macaque monkeys, DA actions at the single cell level could be similar in both species. Given the limited availability of primate PFC tissue for in vitro studies, it is extremely important to understand which aspects of the cellular actions of DA in the PFC can be generalized from rodents to primates. However, up to this point such comparison has not been possible. In the CNS, DA acts via activation of G-protein-coupled DA receptors, which are highly conserved across mammalian species, but no ligand-gated receptor channels are known for DA (Civelli et al. 1993; Missale et al. 1998). Therefore DA appears not to mediate fast synaptic transmission but to exert neuromodulatory effects. An important mechanism by which neuromodulators act in the neocortex is by altering the intrinsic excitability of neurons (Hasselmo 1995). In rats, recent experiments have shown that DA modulates the excitability of PFC pyramidal neurons in vitro (Ceci et al. 1999; Geijo-Barrientos 2000; Geijo-Barrientos and Pastore 1995; Gorelova and Yang 2000; Gullelde and Jaffe 1998; Yang and Seamans 1996).

To gain new insights into the neurophysiology of monkey PFC neurons, recently we have developed a monkey PFC brain slice preparation (Gonzalez-Burgos et al. 2000). In the present study, we used this preparation to obtain intracellular voltage recordings from pyramidal cells in layer 3 of area 46 of the macaque monkey dorsolateral PFC, using sharp and whole cell patch-clamp electrodes. We examined whether DA had modulatory effects on the intrinsic excitability and found that DA enhances the firing response of these neurons to somatic injection of depolarizing current. The increase in excitability is reflected by a hyperpolarizing shift in action potential threshold and decreased inter-spike interval during depolarizing current steps. These effects were blocked by the D1 receptor antago-
than −65 mV and action potentials that crossed 0 mV. All recordings were digitized at 10 kHz and stored on computer hard-disk for later analysis using custom designed software in LabView (National Instruments) and Origin (Microcal).

Whole cell recordings

To obtain whole cell recordings, pyramidal neurons in superficial/middle layer 3 were identified visually with infrared illumination and differential interference contrast optics (Stuart et al. 1993) as described previously (Gonzalez-Burgos et al. 2000). Patch pipettes (≈4–7 MΩ) were filled with (in mM): 120 K-methylsulphate, 10 KCl, 10 HEPES, 0.5 EGTA, 4.5 ATP, 0.3 GTP, and 14 phosphocreatine. Patch-pipette voltage recordings were obtained with an Axoclamp-2A amplifier (Axon Instruments) operating in bridge mode. Membrane potential was not corrected for changes in junction potential after break-in. Whole cell recordings were accepted only if seal resistance was ≥2 GΩ and if the resting membrane potential was more negative than −65 mV. Signals were low-pass filtered at 3 kHz, digitized at 10 kHz, and stored on disk for off-line analysis. Data acquisition and analysis were performed using LabView (National Instruments, Austin, TX).

Data collection and analysis

Once a satisfactory recording was obtained, input resistance ($R_{\text{input}}$) was measured by passing 300-ms current steps from −0.3 to −0.5 nA in 0.05- or 0.02-nA steps. Three sweeps were collected at each amplitude and averaged. The “steady-state” input resistance reported is the slope of the best-fit line to the linear portion of the relation between the injected current and the membrane potential at the end of the step. In some cells, the larger amplitude steps showed some inward rectification and so were excluded from the fitting procedure. Action potential threshold and peak amplitude were measured for each spike in a train evoked by depolarizing current injections. Action potentials were detected and subsequently measured by first examining the first derivative of the membrane potential for peaks. Once a peak was detected in the first derivative, the actual peak of the AP was determined from the point where the first derivative crossed back through zero. The foot of the AP was determined by looking backward from the peak in the first derivative to the point where the third derivative of the membrane potential changed sign from negative to positive (Fig. 2). If necessary, the RMP was manually clamped at a “constant” value by passing tonic bias current. This “current clamping” of the RMP was needed because $R_{\text{input}}$ varied as a function of RMP (see RESULTS), and thus spontaneous changes in RMP could result in $R_{\text{input}}$ changes.

All group data are presented as means ± SE unless otherwise indicated.

All drugs were prepared as concentrated stocks and added directly to the perfusing medium. DA stock solutions were prepared fresh (1000:1) in boiled distilled water with 75 μM NaMBS added to minimize oxidation. Control bath solution contained 75 μM NaMBS. Dopamine, SCH23390, and sulpiride were obtained from RBI (Natick, MA). All other drugs were from Sigma (St. Louis, MO). In the experiments in which the time course of DA action was examined, the delay introduced by the dead space in the perfusion line was compensated for so that the indicated time of DA application matches the time in which DA starts to enter and leave the recording chamber.

FIG. 1. Prefrontal cortex (PFC) tissue used in these studies. A: schematic drawing of the dorsal surface of monkey PFC. The shaded areas indicate the location and approximate size of the tissue blocks that were removed for these studies. Numerals and thin lines indicate the approximate locations of different regions of the PFC. AS, arcuate sulcus; PS, principal sulcus. B: schematic drawing of a tissue slice used for these experiments. The locations of recordings (all from layer 3 in area 46) are indicated by the dark gray shaded electrodes. C: photomicrograph of a neurobiotin-labeled layer 3 pyramidal cell representative of the neurons recorded in the present experiments. Note the prominent ascending apical dendrite and the high-density of dendritic spines, characteristic features of cortical pyramidal neurons. In addition, a significant number of local axonal arborizations with putative synaptic varicosities or boutons are observed (arrows). Calibration bar = 150 μm.
RESULTS

Data were collected from 86 neurons recorded in layer 3 of area 46 (~350–600 μm from the pial surface) as shown schematically in Fig. 1. None of the recorded cells fired spontaneously at rest. In response to injection of suprathreshold depolarizing currents, all of the cells exhibited a regular spiking firing mode with marked spike frequency accommodation (see Figs. 6A and 7), which is typical of pyramidal cells (Connors and Gutnick 1990). In most cases, the spikes also showed broadening during the depolarization induced firing (e.g., Figs. 3 and 6). In every case in which the cells were labeled intracellularly with neurobiotin, the recovered neuron had the characteristic morphological features of pyramidal cells, including an ascending apical dendrite and a high-density of dendritic spines. A representative example of a neuron labeled intracellularly with neurobiotin is shown in Fig. 1C.

Several interesting membrane properties were observed in response to injected current steps. First, a slight inward rectification was usually observed with large hyperpolarizing steps (Fig. 3A, ↑). In addition, an outward rectification was often observed with depolarizing steps that brought the membrane potential near action potential threshold (Fig. 3B, ↓). Finally, for single or multiple APs following a long inter-spike interval, the afterhyperpolarization (AHP) exhibited a slow transition from the fast to slow varieties of the AHP as can be seen by the lack of a sharp inflection following the AP (Fig. 3, B and C).

The recorded cells (n = 86) had average (±SD) resting potentials of −77.2 ± 6.9 mV, for sharp electrode recordings (n = 64) and −72.7 ± 4.8 mV for whole cell recordings (n = 22). Average action potential peak potential was 15.8 ± 9.2 (SD) mV. The average (±SD) input resistance (R_input) at rest was 66.9 ± 18.1 MΩ for sharp electrode recordings and 56.3 ± 18.8 MΩ for whole cell recordings. The average (±SD) membrane time constant measured with sharp electrodes (0.10-nA steps hyperpolarizing from rest) was 22.6 ± 5.8 and 23.5 ± 7.7 ms when measured with whole cell electrodes.

Histological procedures

After recordings with neurobiotin-filled electrodes were finished, slices were incubated for 1–3 h in low Ca²⁺-ACSF at room temperature to allow for intracellular diffusion and extracellular washout of the label. The tissue slices were then immersed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline for 12–16 h. The fixed slices were then transferred to 0.1 M phosphate-buffered saline for 12–16 h. The fixed slices were incubated for 1–3 h in low Ca²⁺-ACSF at room temperature to allow for intracellular diffusion and extracellular washout of the label. The tissue slices were then immersed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline for 12–16 h. The fixed slices were then transferred to 0.1 M Na-phosphate buffer, serially resected by the black arrow was used to assign the peak of the AP (adjacent dotted line). The point at the gray arrow was used to assign the threshold of the action potential (adjacent dotted line).

**FIG. 2.** Determination of action potential threshold. The intracellular action potential ($V_{\text{m}}$) is shown in filled squares and solid line. The 1st derivative of the $V_{\text{m}}$ ($V'_{\text{m}}$) is shown in open triangles and dotted line. The black arrow indicates the point where the 3rd derivative changes from negative to positive values before the peak of the 1st derivative. The point indicated by the black arrow was used to assign the peak of the AP (adjacent dotted line). The gray arrow indicates the point where the 1st derivative crosses 0 after its peak. The point indicated by the black arrow was used to assign the peak of the AP (adjacent dotted line).

**FIG. 3.** Membrane responses from layer 3 pyramidal cells to intracellular current steps and dependence of the input resistance of layer 3 pyramidal cells to intracellular current steps. A: single sweeps showing responses to hyperpolarizing current steps (0.05-nA step increments) from rest (~78 mV). The inward rectification is indicated by ↑. B: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. C: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. D: resting potential (measured at rest (−78 mV)). The action potentials are clipped. E: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. F: resting potential (measured at rest (−78 mV)). The action potentials are clipped. G: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. H: resting potential (measured at rest (−78 mV)). The action potentials are clipped. I: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. J: resting potential (measured at rest (−78 mV)). The action potentials are clipped. K: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. L: resting potential (measured at rest (−78 mV)). The action potentials are clipped. M: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. N: resting potential (measured at rest (−78 mV)). The action potentials are clipped. O: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. P: resting potential (measured at rest (−78 mV)). The action potentials are clipped. Q: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. R: resting potential (measured at rest (−78 mV)). The action potentials are clipped. S: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. T: resting potential (measured at rest (−78 mV)). The action potentials are clipped. U: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. V: resting potential (measured at rest (−78 mV)). The action potentials are clipped. W: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. X: resting potential (measured at rest (−78 mV)). The action potentials are clipped. Y: superimposed single sweeps showing responses to depolarizing steps that bring the membrane potential near threshold for eliciting action potential. Note the outward rectification indicated by ↓. Z: resting potential (measured at rest (−78 mV)). The action potentials are clipped.
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(0.04- to 0.10-nA steps hyperpolarizing from rest). Interestingly, we observed that the $R_{\text{input}}$ varied as a function of the membrane potential of the cell, such that it decreased when the cells were hyperpolarized (Fig. 3, C and D). This was true across all cells for both the resting potential measured without intracellular current injection and for a range of holding potentials for a single cell (Fig. 3, D and E). The changes in $R_{\text{input}}$ with membrane potential were statistically significant: $R_{\text{input}}$ at $-80$ mV = $63 \pm 20 \text{ M}\Omega$ \((n = 13)\); $R_{\text{input}}$ at $-70$ mV = $72 \pm 24 \text{ M}\Omega$ \((n = 12)\); and $R_{\text{input}}$ at $-60$ mV = $79 \pm 23 \text{ M}\Omega$ \((n = 13)\). * (significantly different from $R_{\text{input}}$ at $-80$ mV; # (significantly different from $R_{\text{input}}$ value at $-70$ mV, paired t-test, $P < 0.05$). These results are consistent with previously reported data obtained from hippocampal (Spruston and Johnston 1992) and neocortical pyramidal cells (Deisz et al. 1991; Sutor and Hablitz 1989).

Bath application of DA (0.5–50 µM) produced no significant changes in the RMP of layer 3 pyramidal cells ($-75.1 \pm 2.2$ mV, RMP during DA application: $-76.4 \pm 2.5$ mV, $n = 15$; 2-tailed t-test, $P > 0.05$). Consistent with an absence of DA modulation of conductances opened at or near the resting membrane potential, DA did not produce changes in the $R_{\text{input}}$ of the cells, as shown in Fig. 4.

DA application increased the excitability of the cells in response to depolarizing current steps. Figure 5A illustrates that in the presence of DA at a concentration as low as 500 nM, the number of spikes in response to a current injection of fixed amplitude increased. Significant effects were observed after a 10-min application at the lowest concentration (500 nM). DA decreased the voltage threshold at which the first action potential was initiated and decreased the inter-spike interval (ISI) between the first and second evoked spikes (Fig. 5, B and C). As expected for a receptor-mediated effect, higher concentrations of DA tended to produce larger effects on PFC neuron excitability. However, in most of the experiments, DA concentration was increased sequentially for each cell to increase the yield of collected data, given the limited availability of monkey PFC tissue slices. Therefore the trend toward greater effects with larger DA concentrations could be explained by the total duration of DA exposure. Alternatively, because each concentration was applied for $\geq 10$ min (although not 30 min), the prolonged exposure to DA could have lead to desensitization of DA receptors and underestimation of the effects observed at higher concentrations. Indeed as observed in Fig. 5B, in some neurons the decrease in action potential threshold for a given DA concentration seemed to be attenuated when the cell experienced a previous exposure to DA.

In a separate series of experiments, we examined the time course of changes in layer 3 pyramidal cell excitability induced by a 5-min bath application of DA at a single concentration, followed by washout. As shown in Fig. 6, the changes in excitability developed late compared with the timing of application, being generally observed only after ~10 min following the onset of application. Therefore with 5-min applications, the effect was observed during the early washout period (Fig. 6). In addition, in five of six layer 3 neurons the DA effect was not reversed after as much as 30–40 min of washout (Fig. 6).

Figure 7 shows the frequency-current (F-I) curves for the waveforms shown in Fig. 5A. The data are presented as instantaneous frequency for each interspike interval since the firing rate varied during the 350-ms steps. Note that the frequency of all spikes increased in the presence of DA. DA had no consistent effect on the amplitude or time course of either the inward or outward rectification that was sometimes observed in control conditions (see Fig. 3).

We then asked whether activation of D1- or D2-like receptors was necessary for the observed increase in cellular excitability by DA. To address this question, DA was applied in the presence of either the D1 antagonist SCH23390 (3 µM) or of the D2 antagonist sulpiride (2.5 µM). Figure 8 illustrates that when 50 µM DA is added in the continued presence of SCH23390, there is no statistically significant effect on cell excitability as measured by changes in threshold (Fig. 8A) or first ISI (Fig. 8B). In addition, when DA (50 µM) was applied during blockade of D2 receptors by 2.5 µM sulpiride, neuron excitability as measured by spike threshold was still enhanced significantly. In the presence of sulpiride, DA tended to shorten the first ISI, although this effect was not statistically significant. Therefore our data suggest that SCH23390-sensitive D1-like receptors are necessary for the effect of DA, whereas sulpiride-sensitive D2-like receptors are not (Fig. 8).

**DISCUSSION**

In summary, we have demonstrated that the excitability of layer 3 pyramidal cells in monkey dorsolateral PFC is modulated by DA. Interestingly, although DA does not directly alter the resting state of these neurons, it can increase their excitability at low concentrations (as low as 500 nM). The increase in excitability is reflected by a hyperpolarizing shift in first spike threshold and a decrease in first ISI for action potentials evoked by somatic depolarizing current injections. These effects of DA were prevented by SCH23390, suggesting that they require activation of D1-like receptors but not of receptors of the D2 family.
Potential mechanisms of DA-mediated enhanced excitability

In cortical pyramidal cells, action potentials are usually initiated in the axon near the soma, suggesting that this membrane region is a critical target for regulation of excitability (Colbert and Johnston 1996; Stuart et al. 1997). Interestingly, DA receptor proteins are present in the axon hillock and adjacent segments of the axon of layer 3 pyramidal cells in monkey PFC (Bergson et al. 1995). However, dopaminergic fibers do not frequently contact the soma or proximal axon of monkey PFC pyramidal neurons (Sesack et al. 1995; Smiley and Goldman-Rakic 1993). Therefore most axonal or somatic DA receptors must be located at a distance from the DA terminals. Indeed most of the DA receptors located in the dendrites of monkey PFC neurons are located at a distance from DA containing terminals (Smiley et al. 1994). Therefore activation of all DA receptors in vivo must be nonsynaptic with endogenous DA activating receptors far away from the release sites via a volume transmission mechanism (Zoli et al. 1999). In general, volume transmission requires binding to high affinity receptors, like the G-protein-coupled DA receptors (Civelli et al. 1993; Missale et al. 1998). The D1 excitatory actions of DA in the rat striatum in vivo have been reported to be mediated via such a volume transmission mechanism (Gonon 1997).

The conductances present in the initial portions of the axon are poorly characterized, but Na⁺ channels are known to be present (Colbert and Johnston 1996). In pyramidal neurons of rat PFC, the D1-receptor-mediated enhancement of excitability was proposed to be mediated in part through a shift in the activation voltage of a persistent Na⁺ current to more hyperpolarized voltages (Gorelova and Yang 2000; Yang and Seamas 1996; but see Geijo-Barrientos and Pastore 1995). Theoretically such a shift in Na⁺ current gating could by itself explain the change in threshold for action potential initiation observed in the present study (Dilmore et al. 1999). In neurons from rat neostriatum, DA exerts a complex effect on excitability via a combined modulation of Ca²⁺ and K⁺ conductances (Hernandez-Lopez et al. 1997). In rat PFC neurons, DA also

FIG. 5. Dopamine increases the excitability of layer 3 pyramidal cells. A: responses from a representative pyramidal cell evoked by a range of depolarizing current steps (0.3–1.0 nA steps in 0.1-nA increments) before and in the presence of DA. B: decrease in action potential threshold in the presence of increasing concentrations of DA. C: group data for action potential threshold (±SE). D: decrease in the 1st inter-spike interval in the presence of increasing concentrations of DA. E: group data for 1st inter-spike interval (±SE).
appears to regulate Ca\(^{2+}\) - and K\(^{+}\)-dependent potentials that may regulate neuronal excitability (Yang and Seamans 1996). Further work is required to determine the ionic mechanism of the excitatory effects of DA in primate PFC.

Both D1- and D2-like receptors are present in monkey dorsolateral PFC, but D1-like binding sites are much more abundant than D2-like (Goldman-Rakic et al. 1990; Lidow et al. 1991). Consistent with this receptor distribution, D1- but not D2-like receptor antagonists impaired working memory function and antagonized the electrophysiological effects of DA in vivo (Sawaguchi and Goldman-Rakic 1991, 1994; Sawaguchi et al. 1990a), suggesting a predominant role of receptors with D1-like pharmacology in mediating DA effects in PFC.

However, other work indicates that the pharmacology of D1 and D2 receptors in PFC may be more complex in that the effects of so called “specific” agonists and antagonists do not always agree with one another (Ceci et al. 1999; Godbout et al. 1991; Sesack and Bunney 1989; Shi et al. 1997). Also certain effects of DA in rat PFC neurons are mediated by D1-D2 receptor co-activation (Otani et al. 1998, 1999; Rorig et al. 1995; Sugahara and Shiraishi 1999; Vincent et al. 1995). In monkey PFC, individual pyramidal neurons express both D1- and D2-like receptors (Bergson et al. 1995; Mrzljak et al. 1996), and iontophoresis of either D1 or D2 antagonists can inhibit cell firing of PFC neurons recorded in vivo (Williams and Goldman-Rakic 1995). The limited availability of monkey PFC tissue precluded the ability to perform an extensive pharmacological study, therefore the main goal of the present study was to determine whether DA had any effect on the excitability of monkey PFC neurons. Our present results suggest that, in layer 3 neurons of the monkey dorsolateral PFC in vitro, DA-induced changes in intrinsic excitability are mediated by D1-like receptors.

In agreement with previous studies in rat PFC (Gorelova and Yang 2000; Zheng et al. 1999), we found that in most of the neurons in which we examined the time course of the actions of DA, the effect was persistent after DA washout. However, in previous studies of striatal neurons, D1-like receptor-mediated effects were found to be readily reversed after drug wash out (Surmeier et al. 1995; Zhang et al. 1998). The mechanisms for...
FIG. 8. Effects of DA receptor antagonists SCH23390 and sulpiride on the effects of DA in layer 3 pyramidal cell excitability. Action potential threshold and 1st inter-spike interval were measured sequentially, first in control artificial cerebrospinal fluid, then after 10 min application of the antagonists alone and then after a 10 min co-application of the antagonist and DA. A1: blockade of D1 receptors by SCH23390 5 μM prevents the change in spike threshold by DA 50 μM. A2: SCH23390 blocks the effect of DA on first inter-spike interval. B1: sulpiride 2.5 μM did not prevent the decrease in spike threshold induced by DA (50 μM). B2: in the presence of sulpiride (2.5 μM), DA tended to produce a decrease in first inter-spike interval, although this change was not significant. (*, significantly different from control, P < 0.05. Repeated-measures ANOVA, followed by comparison by Dunnett’s method; n = 7 cells for SCH23390 experiments, n = 9 for sulpiride experiments.)

The long-lasting effects are at present unclear. D1-like receptors are traditionally associated with activation of the adenylate cyclase-cAMP-protein kinase A signaling cascade (Missale et al. 1998). In striatal neurons, protein kinase A phosphorylates DARPP32, and phospho-DARPP-32 inhibits phosphatase 1 activity, contributing to the electrophysiological effects of DA (Schiffmann et al. 1998). Simultaneous protein kinase A activation and phosphatase 1 inhibition may result in persistent phosphorylation of substrates and thus persistent effects. Interestingly, however, DARPP32 could not be detected in pyramidal neurons from adult monkey PFC in a previous study (Berger et al. 1990). Layer 3 monkey PFC neurons also seem to lack D2 receptors that can inhibit adenylate cyclase activity (Missale et al. 1998) that may reverse the D1 effects in other neurons. An interesting idea that remains to be tested is that D1 effects are reversed by signal transduction cascades activated by receptors for other neuromodulators that have been proposed to be critical for correct PFC-dependent short-term memory function, among them norepinephrine and serotonin (Arnsten 1998; Goldman-Rakic 1999). Activation of G-protein-linked neurotransmitter receptors typically requires release by bursts of presynaptic action potentials (Gonon 1997), suggesting that there is little activation of G-protein-linked receptors by spontaneous release of endogenous modulators in brain slices. Therefore since neuromodulators other than dopamine were not applied, it is probable that nondopaminergic receptors were not activated in our slice preparation.

Comparison with previous results in monkey PFC

All the previous studies on the effects of DA on monkey PFC cell activity were performed using in vivo extracellular recordings and iontophoretic application of drugs (Sawaguchi et al. 1988, 1990b; Williams and Goldman-Rakic 1995). In some of these studies, DA receptor agonists and antagonists were shown to enhance or depress, respectively, the firing of PFC units in awake monkeys (Sawaguchi et al. 1988, 1990a,b). These findings are consistent with the increased excitability found in the present in vitro experiments. Williams and Goldman-Rakic (1995) reported that unit firing is decreased by high doses and increased by low doses of D1 antagonists. One possible explanation for those results is that depending on the level of receptor occupancy, DA triggers different mechanisms at the single-cell level, leading to opposite changes in firing rate. Alternatively, the changes in firing observed during different levels of D1 antagonism in vivo may result from other effects of DA in the PFC network, like modulation of excitatory and inhibitory synaptic inputs, as suggested recently based on immunohistochemical findings (Muly et al. 1998). The lack of a differential effect of DA concentration in our studies suggests that the complex effects reported by Williams and Goldman-Rakic (1995) are mediated by network effects.

In rat PFC, dopaminergic input is most dense in the deep layers (Berger et al. 1991), therefore the majority of previous studies in vitro have been focused on layers 5 and 6. Furthermore early studies have suggested that pyramidal cells in superficial layers of rat PFC are not responsive to DA receptor activation (Bunney and Aghajanian 1976; Sesack and Bunney 1989). In contrast, more recent studies indicate that a proportion of neurons in the superficial layers of rat medial frontal cortex do express DA receptors and are responsive to DA receptor stimulation (Ariano et al. 1997; Gaspar et al. 1995; Le Moine and Gaspar 1998; Zhou and Hablitz 1999). These and our present results support the idea that dopaminergic modulation of superficial layer cell activity is generally found in PFC across species but that perhaps it is more robust in primates than in rodents.

Our present results, together with recent data obtained from rat brain slices (Ceci et al. 1999; Gorelova and Yang 2000; Yang and Seamans 1996), show that an important effect of DA on pyramidal neurons in PFC is an enhancement of intrinsic pyramidal cell excitability, mediated by activation of D1-like receptors. However, different effects of DA on cellular excitability in rat PFC in vitro were reported across laboratories, making cross-species comparisons difficult. We (González-Burgos, unpublished results) and others observed that activation of DA receptors can decrease the excitability of pyramidal neurons in rat medial frontal cortex (Geijo-Barrientos 2000; Geijo-Barrientos and Pastore 1995; Gullidge and Jaffe 1998; Zhou and Hablitz 1999). Our present data are in agreement with the enhanced excitability reported previously by several groups (Ceci et al. 1999; Gorelova and Yang 2000; Penit-Soria et al. 1987; Yang and Seamans 1996). An interesting possibility is that the decrease in cell excitability is mediated by D2-like receptors, as suggested by Gullidge and Jaffe (1998). In monkey PFC layer 3 pyramidal neurons, this D2-like effect was not observed with bath application of DA alone or during blockade of D1-like receptors by SCH23390, suggesting that D2-like receptors do not depress excitability in these cells. Indeed in the monkey dorsolateral PFC, D2-like receptors are
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found in layer 5 but are barely detectable in the superficial layers (Goldman-Rakic et al. 1990; Lidow et al. 1991, 1998). Thus it remains to be tested if in pyramidal neurons from the deep layers of monkey PFC, DA has D2-like inhibitory effects such as those reported by Gulledge and Jaffe (1998) for deep layer PFC neurons in rats.

Functional implications of DA modulation of layer 3 pyramidal cells in primate PFC

The present results are the first in vitro demonstration that DA has modulatory effects on the activity of monkey PFC layer 3 pyramidal neurons. Under our experimental conditions, the observed effects of DA are most likely due to direct effects on single cells. However, under physiological conditions, the DA effects on intrinsic cell excitability would be combined with any effects it might have on synaptic transmission. Other data from our laboratory suggest that DA depresses transmission at glutamatergic synapses onto layer 3 pyramidal cells in monkey PFC (unpublished results). Whether DA depresses transmission at excitatory synaptic connections in general or only at a specific subset of synapses in monkey PFC is still not clear. In either case, the impact of certain excitatory inputs, those that are more strongly active or that are not modulated by DA, will be enhanced relative to that of less active or selectively depressed inputs. This is consistent with a DA-induced enhancement of the signal-to-noise ratio during working-memory tasks, which was shown experimentally with in vivo extracellular recordings from monkey PFC units (Sawaguchi et al. 1990a,b). Computational models of network activity in PFC during working memory tasks have shown that noise interference can make working-memory storage unreliable (Camperi and Wang 1998). Therefore by increasing the signal-to-noise ratio, DA could make delay-related cell firing less sensitive to noise and intervening stimuli and thus specifically improve the storage aspect of working memory (Camperi and Wang 1998; see also Durstewitz et al. 2000).

In addition to its role in working memory, PFC neuron activity is important to other cognitive functions (Miller 1999). In both working memory and other cognitive processes, PFC is likely to act in dynamic association with other cortices (Fuster 1997; Miller 1999; Quintana and Fuster 1999; Tomita et al. 1999). Our present results show that DA modulates the activity of pyramidal cells in the superficial layers, which contain most of the cortico-cortical projection neurons of the monkey PFC (Schwartz and Goldman-Rakic 1984). Therefore these findings suggest that DA release in layer 3 is likely to modulate significantly the interaction between dorsolateral PFC and other regions of the neocortex.

We thank D. Melchitzky and M. Brady for histology and reconstruction of cell morphology.

This work was supported by National Institute of Mental Health (NIMH) Predoctoral Fellowship MH-10474 to D. A. Henze, a Howard Hughes Medical Institute Predoctoral Fellowship to N. N. Urban, NIMH Grant MH-51234, and NIMH Center for the Neuroscience of Mental Disorders Grant MH-45156.

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REFERENCES


