Local Application of Dopamine Inhibits Pyramidal Tract Neuron Activity in the Rodent Motor Cortex

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INTRODUCTION

The mesocortical dopamine system was previously thought to target only a few select cortical areas, particularly in rodents. Sensitive staining techniques, however, reveal a widespread and dense dopaminergic innervation to the primate (Gaspar et al. 1989; Williams and Goldman-Rakic 1993) and rodent neocortex (Berger et al. 1985, 1991; Descaries et al. 1987). Regional variations in density and distribution patterns exist and suggest a heterogeneous role for dopamine in different neocortical areas. In primates, the motor regions receive the densest dopamine innervation of all cortical regions (Gaspar et al. 1989; Williams and Goldman-Rakic 1993), indicating a potent influence over motor cortex activity. The rodent motor cortex is also innervated by dopaminergic projections, albeit to a lesser degree than in primates (Berger et al. 1991), and the pattern of dopamine terminal distribution is similar in both species. It is bilaminar with the densest input to superficial (layer I) and deep (V–VI) layers (Berger et al. 1985; Descaries et al. 1987; Gaspar et al. 1989; Williams and Goldman-Rakic 1993).

Dopamine’s effects on cortical neurons are mediated in part through five identified subtypes of receptors (Civelli et al. 1993; Gingrich and Caron 1993), categorized as D1 (including D1a and D5 receptors) or D2 like (including D2, D3, and D4 receptors) based on pharmacological and biochemical properties (Kebabian and Calne 1979; Seeman and Van Tol 1994). Binding to the D1 receptor activates adenyl cyclase which subsequently increases intracellular cyclic 3’,5’-AMP (cAMP), whereas binding to the D2 receptors inhibits adenyl cyclase, suggesting that the two types elicit different postsynaptic responses (Albert et al. 1990; Kebabian and Calne 1979; Neve et al. 1989; Seeman and Van Tol 1994). Dopamine receptors are widespread throughout the rodent and primate neocortex and are distributed similarly to dopaminergic axons (Ariano and Sibley 1994; Ariano et al. 1993). Members of both subfamilies are found in primate and rodent motor cortex and display distinct laminar distribution patterns (Goldman-Rakic et al. 1990; Huntley et al. 1992; Joyce et al. 1993; Sawaguchi and Goldman-Rakic 1994). Moreover, different receptor subtypes...
appear to be expressed in distinct populations of cortical neurons (Bergson et al. 1995b; Gaspar et al. 1995).

The functional role of the dense dopaminergic projection to the motor cortex has yet to be determined. Dopamine-depleted rodents exhibit motor impairments (Bures and Bracha 1990), which could be attributed to motor cortex dysfunction, such as deficient temporal sequencing of complex, skilled motor tasks (Salamone et al. 1990; Whishaw et al. 1986), and diminished accuracy and rate of skilled movements (Sabol et al. 1985; Whishaw et al. 1986). Dopamine’s potential to modulate cortical activity is reflected in the observation that neurons in all cortical layers respond to it (Sawaguchi et al. 1986a), but an understanding of its effects is complicated by the varied responses to locally applied dopamine. Both inhibitory and excitatory responses are induced by dopamine in neurons of the prefrontal and motor cortices, although inhibitory responses predominate (Bernardi et al. 1982; Bradshaw et al. 1985; Sawaguchi et al. 1986a). Response types are somewhat laminar specific in that inhibitory responses are elicited in neurons in all cortical layers, whereas excitatory responses to dopamine are elicited, for the most part, only in layer V neurons. These laminar dependent effects may reflect the distinct distribution patterns of dopamine receptor subtypes.

The widespread distribution of DA receptors in neurons of the motor cortex indicate that dopamine may act directly through receptor-mediated mechanisms, especially on pyramidal neurons where dopamine receptors predominate (Bergson et al. 1995a; Gaspar et al. 1995; unpublished observations) and most dopaminergic terminals synapse (Krimer et al. 1997; Segula et al. 1988; Smiley and Goldman-Rakic 1993; van Eden et al. 1987; Verney et al. 1990).

This study was designed to elucidate dopaminergic influences on pyramidal tract neurons (PTNs), which represent the most direct coupling of motor cortex output with spinal cord motor neurons. Electrophysiological techniques were used to determine the effects of dopamine on the spontaneous and glutamate-induced activity of PTNs and the receptors that mediate these effects.

METHO DS

Surgical procedure

Fourteen adult Sprague-Dawley rats were anesthetized with Xylazine (2–4 mg/kg, i.m.) and Ketamine HCl (60 mg/kg, i.m.). Dexamethasone (2 mg/kg, i.m.) was administered 1 h prior to surgery to prevent cortical swelling. The animals received supplemental anesthetics (30 mg/kg of Ketamine HCl, i.m. and 1.0 mg/kg Xylazine i.m./h) during the experiment to maintain a steady state of anesthesia. They were checked regularly for the absence of withdrawal reflexes. The animals were placed in a stereotaxic apparatus. Body temperature was maintained at 37°C with a thermoregulating heating pad. Xylocaine (1 ml, 20 mg/ml, s.c.) was injected around the incision sites. A midline incision, unilateral craniotomy, and durotomy were performed to expose the right motor cortex. The exposed cortex was covered with sterile mineral oil. A laminectomy was made at the cervical (C) 1–2 spinal cord level to expose the corticospinal tract (CST). All procedures were done in accordance with the National Institutes of Health guide for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee.

Electrophysiological paradigm

Ten animals were used for the electrophysiological experiments. A tungsten-in-glass microelectrode (exposed tip of 10–15 μm) was mounted onto a motorized hydraulic microdrive and lowered into the forepaw representation of the motor cortex according to stereotaxic coordinates (Hall and Lindholm 1974). The electrode was lowered to a depth of 1200 μm where most PTNs are located. Intracortical microstimulation (constant current, 200 μs pulses at 200 Hz, 90 ms train at 1 Hz) was delivered through the electrode until contralateral forepaw movement was elicited. The current intensity (voltage drop across a 1 KΩ resistor) was kept below 60 μA to avoid cortical damage. These steps were repeated until a low threshold site for forepaw movement was identified and marked on a drawing of the cortical surface vasculature. The electrode was withdrawn and replaced with a five barrel pipette (total tip diameter 12–20 μm). The central barrel contained 3.0 M KCl and a carbon fiber ( extending <5 μm from tip) for recording extracellular activity. In the first set of experiments the four surrounding barrels were filled with either NaCl (1.0 M; balancing current), DA (0.1 M), the D1 selective receptor antagonist SCH23390 (10 mM), or the D2 selective receptor antagonist eticlopride (10 mM). In the second set of experiments, the barrels were filled with NaCl (1.0 M), DA (0.1 M), or glutamic acid (0.1 M). Electrodes were connected through a silver wire to an iontophoresis Module (World Precision Instruments) to monitor electrode resistance and iontophoresis solutions. The electrode was placed at the appropriate surface location under microscope guidance. A slight change in electrical noise when the electrode encountered the oil/cortical surface interface confirmed the surface contact. To accurately determine cortical depth, extracellular activity was recorded as the electrode was lowered into layer V (1,000–1,300 μm below the pial surface). Neuronal activity was amplified (>1,000) and displayed on an oscilloscope and audiomonitor.

A tungsten-in-glass microelectrode was inserted with a stereotaxic unit into the left CST (200–400 μm depth) at the C1–C2 spinal level (Paxinos and Watson 1986). Antidromic responses from PTNs in the motor cortex were recorded in response to CST stimulation (200-μs duration, 1 Hz). Criteria for determining that action potentials were antidromic included; short refractory periods (<1.5 ms) in response to dual/high-frequency stimuli (750 Hz), no significant decrease in response latency with increased current intensity (>2 times threshold); and brief response latencies (1.5–4.0 ms = axonal conduction velocity/distance between electrodes), which were stable at threshold stimulation. Figure 1 shows a diagram of the experimental paradigm.
Data acquisition

Extracellular recordings were made of the neuronal activity of 30 identified PTNs. Signals were amplified (×1,000), filtered (low-pass = 10,000 and high-pass = 300 Hz), displayed on an oscilloscope, and transmitted to an audiostreamer. The action potentials of single neurons were isolated through an amplitude discriminator online and later through a waveform discriminator offline. Each discriminated action potential triggered a pulse to generate the rate meters, which are graphic displays of stimuli/unit time in histogram form, with the DataWave acquisition and analysis system. A baseline recording of spontaneous activity for each PTN was recorded over a 150 s recording interval at the start and finish of each recording session to assess stability of the neurons. Data were stored as amplitude-discriminated spikes/time in digital format with the DataWave acquisition software. Only those neurons whose baseline spontaneous activity levels were consistent throughout the recording trials were included in the data analysis. Randomly ordered and repeated recordings (3 times for each drug/co-application and for baseline) of activity were recorded over 150 s intervals. An interval of at least 3 min was allowed between each 150 s recording period to ensure recovery of baseline activity. PTNs were subjected to one of two experimental treatments at different ejection currents. For example, dopamine concentrations at high current intensity (190 and 210 nA), which caused electrode blockage, were applied alone to ensure that they had no effect on spontaneous baseline activity. PTNs were subjected to one of two experimental treatments with receptor antagonists; either dopamine and dopamine D1 or D2 receptor antagonists, or dopamine and glutamate.

Drug application

Effective dosages were determined by applying varying concentrations at different ejection currents. For example, dopamine concentrations of 0.001, 0.01, and 0.1 M were iontophoresed at 20, 60, 90, 120, 170 190, and 210 nA. Lower concentrations were effective only at high current intensity (190 and 210 nA), which caused electrode clogging. Therefore 0.1 M dopamine was ejected with 170 nA current throughout the experiments. Microelectrode resistance was monitored during the recording sessions to ensure that blockage did not occur. A retention current (10–20 nA) of opposite polarity to the ejection current was applied continuously (except during ejection) to each solution to prevent leakage. In the first experiment dopamine or dopamine with receptor antagonists were iontophoresed while recording spontaneous activity of PTNs. Initially, SCH23390 and eticlopride were applied alone to ensure that they had no effect on spontaneous activity at the selected doses. Each PTN was subjected to repeated trials (3) of all the following randomly ordered treatment schedules, with 3-min intervals between treatments:

1) 0–30 s = no drug, 30–60 s = dopamine (+170 nA), 60–150 s = no drug
2) 0–30 s = no drug, 30–90 s = SCH23390 (+90 nA) (preceding and succeeding co-application with dopamine by 10 and 20 s respectively), 40–70 s = dopamine + SCH23390, 90–150 s = no drug
3) 0–30 s = no drug, 30–90 s = eticlopride (+90 nA) (preceding and succeeding co-application with dopamine by 10 and 20 s respectively), 40–70 s = dopamine + eticlopride, 90–150 s = no drug

Spontaneous activity was monitored after every trial to ensure that it returned to pretreatment levels. If activity failed to recover to baseline levels within 3 min after cessation of dopamine application, the recordings were terminated for that neuron.

In the second set of experiments, the activity of PTNs in response to iontophoresis of dopamine and glutamate was assessed. Each PTN was subjected to repeated trials (3) of all the following randomly ordered treatment schedules, with 3-min intervals between treatments:

1) 0–30 s = no drug, 30–60 s = dopamine, 60–50 s = no drug
2) 0–30 s = no drug, 30–60 s = glutamate, 60–150 s = no drug
3) 0–30 s = no drug, 30–80 s = glutamate (preceding and succeeding co-application with dopamine by 10 s), 40–70 s = dopamine, 80–150 s = no drug

Neurons that did not respond to either glutamate or dopamine alone were excluded. At the end of the recording sessions, the animals were sacrificed by intracardial perfusion with saline, followed by 4% paraformaldehyde in phosphate buffer.

Data analysis

Data analysis was performed off-line. Single neuron activity was isolated by waveform pattern discrimination using the DataWaves Experimentor’s Workbench software. Rate meters of PTN activity over 150 s for each recording trial were generated from the time stamps of discriminated action potentials. The firing rate (spikes/s) of each PTN during the entire baseline, drug application, and recovery periods of each recording interval was calculated to determine the effects of drugs on neuronal activity. Furthermore, firing rates during sequential 10 s intervals following the onset and offset of dopamine iontophoresis were determined because neuronal responses to each of these events were delayed.

Firing rates were averaged over equivalent time periods from repeated recording trials of like-treatment schedules for each PTN. Changes in neuronal activity were compared between the pretreatment (control) and drug application periods and between the pre- and posttreatment periods for individual PTNs and across the population of PTNs. A Student’s t-test was applied to the population data to determine whether drug application induced significant changes in firing rate.

Immunohistochemical paradigm

Four animals were anesthetized and prepared for surgery. The corticospinal tract was exposed at the C1–C2 level. A glass pipette (tip diameter 20 μm) filled with the retrograde fluorescent tracer, Fast Blue (FB, 2.0% in saline), was mounted on an electrode carrier and inserted into the stereotaxic coordinates for the corticospinal tract (Paxinos and Watson 1986). FB was injected bilaterally into the corticospinal tract by air pressure with a Picospritzer (40 psi; 50 ms). The pipette was withdrawn and the wound area was closed. The animals were monitored during recovery and given postoperative analgesics as needed.

Eight days after the FB injection, rats were deeply anesthetized with Ketamine HCl (60 mg/kg, i.m.) and xylazine (4 mg/kg, i.m.) and perfused intracardially with saline and 4% paraformaldehyde (0.9% NaCl sodium phosphate, pH 7.3). The brains were removed and cryoprotected overnight at 4°C in PB containing 20% sucrose. The motor region of the frontal cortex was cut in the coronal plane with a freezing microtome (−20°C) into 40-μm-thick sections. Three alternating series of sections through both hemispheres were collected and processed for immunohistochemical labeling of dopamine D1a, D2, or D5 receptors.

Free-floating sections were washed (3 × 10 min) in phosphate-buffered saline (PBS: 0.1 M sodium phosphate buffer, pH 7.3 with 2.0% NaCl) and incubated in blocking serum [PBS + 3.0% normal horse serum (NHS)] for 1 h. Sections were then incubated with the appropriate primary antibodies (1:500 for rabbit anti-D1a polyclonal antibody, a gift from Dr. Marjorie Ariano; 1:1000 for goat anti-D5 polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA) in PBS containing 3% NHS and 0.1% Triton X 48 h at 4°C. Subsequently, the sections were washed (3 × 10 min) in PBS with 3.0% NHS and incubated in PBS containing 3.0% NHS, with the appropriate secondary antibody (anti-rabbit IgG conjugated to Cy3 for D1a or D2, 1:400, or anti-goat IgG conjugated to Cy3 for D5, 1:400, Jackson Immunoresearch, West Grove, PA) for 30–90 min. The sections were washed (3 × 10 min), mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA), sealed, and stored at 4°C in the dark.
**Morphological analysis**

The area delineated as primary motor cortex was carefully examined with a Nikon Microphot fluorescent microscope for the presence of neurons with retrograde labeling and immunolabeling for one of the dopamine receptors. The Cy3 fluorochrome was visualized with the excitation filter range of 510–560 nm and barrier filter at 590 nm. Fast blue was visualized through a filter with the excitation range 355–375 nm and barrier filter at 400 nm.

**RESULTS**

**Dopamine inhibits spontaneous activity of PTNs**

Responses to iontophoretically applied dopamine were recorded from 30 spontaneously active PTNs. Their response latencies to antidromic activation by stimulation of the CST ranged from 1.6 to 3.7 ms [2.7 ± 0.13 (SE) ms], which fell within the predetermined range of 1.5 to 4.0 ms. Thirty neurons met our criteria for PTNs as defined by their antidromic response characteristics to stimulation of the CST. The mean baseline spontaneous activity for all PTNs was 5.66 ± 0.52 spikes/s.

Recording trials in which only dopamine was applied were included in both sets of experiments (antagonist and glutamate trials). No differences in response to dopamine were noted between the groups, and therefore the data were combined. Dopamine reduced the spontaneous firing rate during the iontophoretic application for each of the 30 PTNs. The reduction was observable over repeated trials for each neuron as seen by the responses of one PTN to dopamine in Fig. 2, A–C. The spontaneous action potential occurrence during a single recording trial of one PTN is shown in Fig. 3A. The firing rate declined to 74% of baseline during the initial 10 s of dopamine ejection, and to 35% of baseline during the next 10 s of ejection, and remained at this latter level for the final 10 s. Recovery to pretreatment levels (94% of baseline) was nearly complete within the first 30 s after cessation of dopamine ejection current. These responses were consistent over repeated recordings, as illustrated by the firing rates averaged over repeated trials during the pretreatment, dopamine application, and recovery. The activity gradually decreased during the treatment phase and returned to baseline firing levels during the 30-s recovery phase.

**FIG. 3.** A: rate meter, showing the spontaneous occurrence of action potentials from 1 PTN, over a 140-s recording interval (binwidth = 400 ms). The onset (on) and offset (off) of the ejection current are marked by arrows. Dopamine (DA) was iontophoresed for 30 s. B: chart showing the firing rate (spikes/s) averaged from repeated recording trials of the same PTN as in A, during pretreatment, dopamine application, and recovery. The activity gradually decreased during the treatment phase and returned to baseline firing levels during the 30-s recovery phase.
application also was significantly different from pretreatment levels. The percent change from baseline firing rate during the drug application and recovery intervals is shown in Fig. 5B. Spontaneous activity returned to within 81% of pretreatment levels during the initial 30-s recovery period. Often full recovery did not occur until 60 s or more after cessation of dopamine ejection. The 3-min interval between recordings, however, ensured full recovery to baseline. The differences in spontaneous activity between the pretreatment period of dopamine trials one and two, and between trials two and three were not statistically different (paired Student’s t-test, \( P = 0.71 \) and 0.70, respectively).

Effects of dopamine are mediated by D1 and D2 receptors

Selective antagonists to the D1 and D2 receptors were effective in blocking the action of dopamine. Prior to the dopamine and antagonist trials, eticlopride and SCH23390 were applied in the absence of dopamine for 30 s to ensure that they did not directly affect PTN activity. No immediate or prolonged changes were observed, as shown by the example in Fig. 6. Neither the D1 antagonist, nor the D2 antagonist, affected baseline spontaneous activity when they were applied alone (Student’s t-test; \( n = 16 \), baseline vs. antagonist application over repeated trials, \( P = 0.268 \) for D1 and 0.432 for D2).

Sixteen PTNs were subjected to repeated trials of co-application of dopamine and the D1 or D2 receptor antagonist. In each case it was established that dopamine was effective in reducing spontaneous activity (mean = 25.3% below baseline) prior to co-application with an antagonist. Typical responses during single recording trials to dopamine, dopamine and the D1 antagonist, or dopamine and the D2 antagonist are shown in

- **Fig. 4.** Summary diagram of PTN spontaneous activity during dopamine application. Line graph shows averaged spontaneous activity over repeated trials, during pretreatment, dopamine application, and recovery periods for 16 PTNs. Only a subset of neurons, those in the antagonist trials, are included for clarity. Dopamine caused a reduction in spontaneous firing rate for each neuron, which returned partially to pretreatment levels during the initial 30-s recovery period shown. The responses to dopamine varied somewhat over time, but to a greater extent in magnitude, among PTNs.

- **Fig. 5.** A: table showing the mean spontaneous activity (SA; spikes/s) from repeated recording trials of all PTNs (\( n = 30 \)) during the baseline, dopamine (DA) application (divided into 3 10-s intervals), the entire 30 s of DA application, and the recovery interval. Significance levels (\( P \)) are shown for comparison of each interval with baseline activity (paired Student’s t-test). B: graph of the percent change from mean baseline spontaneous activity (0–30 s; 100%) of all PTNS compared with spontaneous activity during the 1st (DA 1 = 30–40 s), 2nd (DA 2 = 40–50 s), and 3rd (DA 3 = 50–60 s) 10-s intervals of dopamine application, and during the recovery period (60–90 s). The precise change is marked on each bar. Bars in graph denote SEs.

- **Fig. 6.** Rate meters showing the activity of 1 PTN during recording intervals in which eticlopride (Etic; Fig. 5A) or SCH 23390 (SCH; Fig. 5B) were applied for 30 s. The baseline remained stable during and after application of each antagonist.
Fig. 7. Dopamine reduced the activity by 74% of baseline (Fig. 7A; 8.1/6.0 spikes/s; baseline/treatment, respectively). The baseline levels remained stable, however, when dopamine was co-applied with the D1 antagonist (Fig. 7B; 8.2/8.0 spikes/s) and with the D2 antagonist (7.8/7.8 spikes/s).

The D1 antagonist blocked dopamine induced inhibition in nearly all cells at the selected dosage. Most pyramidal tract neurons showed either no decrease (n = 11) or only slight reductions (3–10%; n = 4) from baseline spontaneous activity during co-application. The activity of one PTN during a single recording interval is shown in Fig. 8. Dopamine caused a decrease in baseline firing rate (Fig. 8A; 4.2 spikes/s during dopamine application), which was blocked by SCH23390 co-application (Fig. 8B; 7.3 spikes/s during pretreatment; 7.4 spikes/s during the initial SCH23390 application; 7.1 spikes/s during the 30 s of dopamine and SCH23390 co-application; 6.3 spikes/s during the final 20 s of SCH23390 application; 6.4 spikes/s during the recovery period). The variation in mean firing rate, compared with baseline, over repeated trials during pretreatment, treatment, and recovery periods for this neuron is shown in Fig. 8C. A slight decrease in firing rate occurs at the end of the dopamine ejection for this neurons, but the mean firing rate of all PTNs did not differ significantly (P > 0.05) from pretreatment levels during the first SCH23390, the co-application, the second SCH23390, or the recovery period (Fig. 9A). The D1 antagonist was ineffective in blocking dopamine-induced inhibition in two PTNs. These two neurons exhibited 17% and 22% reductions in pretreatment activity levels during repeated trials of the co-application period. The percent change in mean firing rates relative to baseline, over repeated trials of drug applications for all neurons is shown in Fig. 9B.

The D2 antagonist blocked dopamine-induced inhibition in nearly all cells at the selected dosage in that no decrease (n = 9/16) or only a slight reduction (1–10%; n = 4/16) in spontaneous activity occurred. The spontaneous activity of a single PTN during one recording interval is shown in Fig. 10A. No decrease in firing rate during dopamine and eticlopride co-application is evident (12.8 spikes/s during pretreatment; 13.1 spikes/s during the initial 10 s eticlopride
The change in mean pride co-application; 11.4 spikes/s during the recovery period. 

Fi pride co-application; 11.1 spikes/s during the application; 12.6 spikes/s during the 30 s dopamine and eticlopride co-application (SCH23390, SCH; or eticlopride, Etic), and the antagonist and DA co-applications (SCH + DA or Etic + DA), and the recovery intervals are shown. Time of drug application are in the heading (s = seconds). Significance levels (P) are shown for comparison of each interval with baseline activity (paired Student’s t-test). B: graph of the percent change from mean baseline spontaneous activity (0–30 s; 100%) of all PTNs compared with spontaneous activity during the first antagonist (SCH or Etic = 30–40 s), the antagonist and dopamine co-application (DA + SCH or DA + Etic = 40–70 s), 2nd antagonist application (SCH or Etic = 70–90 s), and during the recovery period (90–120 s). The precise change is marked on each bar. Bars in graph = SE.

Dopamine blocks glutamate-induced excitation

Twelve PTNs were subjected to trials of dopamine and glutamate co-application. Iontophoresis of glutamate alone markedly increased the baseline spontaneous activity of each PTN, whereas application of dopamine alone decreased the activity of each neuron included in the trials. The response of a single PTN to glutamate during one recording interval is shown in Fig. 11A. The firing rate increased from 6.7 spikes/s during the pretreatment period to 15.1 spikes/s during the glutamate application and then decreased to 8.3 spikes/s during the early recovery period. The change in mean activity during pretreatment, treatment, and recovery periods over repeated intervals is shown in Fig. 11B. Dopamine reversed glutamate-induced increases (mean = 41% over baseline, n = 12) in activity in all 12 neurons. The activity of a single PTN during one recording interval with glutamate, followed by dopamine and glutamate co-application, is shown in Fig. 12A. Baseline firing of 3.85 spikes/s increased to 4.85 spikes/s during glutamate application, decreased to 4.25, 3.15, and 3.15 spikes/s during three consecutive 10-s periods of glutamate and dopamine co-application, increased to 4.1 spikes/s during the sec-
ond glutamate application, and then recovered to baseline levels (4.65 spikes/s). The change in mean activity from baseline over repeated trials for this neuron during treatment and recovery periods are shown in Fig. 13

B

In all 12 neurons, glutamate induced a significant increase in activity relative to the pretreatment period, which diminished to baseline levels when dopamine was applied concurrently with glutamate (Fig. 13 A). Overall, the glutamate-induced increase in activity levels was reduced significantly (Student’s t-test, P = 0.00066 for glutamate vs. glutamate + dopamine) to 70% (98% of baseline) of its peak level by dopamine. No significant increase relative to baseline firing rate occurred during the 10 s of glutamate application that immediately followed the cessation of dopamine iontophoresis. The percent change in mean activity over repeated trials for all neurons during the dopamine and glutamate applications is shown in Fig. 13 B.

Pyramidal tract neurons possess dopamine receptors

A dense band of retrogradely labeled PTNs was evident in layer V of the motor cortex after FB injections into the CST. The FB label was clearly visible in the somata and proximal dendrites of many PTNs.

PTNs express D1a receptors

Labeling for dopamine D1a receptors was observed in numerous PTNs in layer V of the motor region of the frontal cortex. Immunostaining was observed primarily in the somata but also in the most proximal segments of dendrites. Not all of the FB-labeled PTNs were immunoreactive for D1a receptors, suggesting that only a subpopulation of these PTNs contains this receptor subtype. Examples of FB-labeled PTNs immunoreactive for the D1a receptor are shown in Fig. 14, A and B. Numerous layer V neurons that did not contain FB were immunoreactive for the dopamine D1a receptor. The lack of retrograde tracer suggested that they were not likely to be PTNs, but their identity was not determined.

PTNs express D2 receptors

Immunolabeling for dopamine D2 receptors also was observed in numerous PTNs. Not all PTNs were immunoreactive for the receptor, indicating that only a subpopulation of PTNs contains D2 receptors. Labeling was observed in the somata and proximal portions of dendrites. Examples of FB-labeled PTNs immunoreactive for the D2 receptor are shown in Fig. 14, C and D. Immunoreactivity for the D2 receptors was observed in numerous neurons that did not contain FB. These neurons were not likely to be PTNs.
PTNs express D5 receptors

Immunolabeling for dopamine D5 receptors also was observed in numerous PTNs. Here again, not all PTNs were immunoreactive for the receptor, indicating that only a subpopulation of PTNs contains D5 receptors. Labeling was observed in the somata and also extended well into the apical dendrites. Examples of FB-labeled PTNs immunoreactive for the D5 receptor are shown in Figs. 14, E and F. Many neurons in layer V did not contain FB but were immunoreactive for the D5 receptor, with label sometimes extending into apical dendrites that indicated that these neurons are pyramidal cells. Their projection sites were not identified.

DISCUSSION

The effects of locally applied dopamine on spontaneous activity and glutamate-induced activity in PTNs of the intact rodent motor cortex were studied. Dopamine was effective in significantly reducing the spontaneous activity of PTNs after 20 s or more of iontophoretic application. Both the D1 and the D2 antagonists were effective in blocking the actions of dopamine on most, but not all, neurons. Dopamine blocked glutamate-induced increases in firing rates of PTNs.

Technical considerations

The volume of a drug expressed at recording sites by iontophoresis is difficult to determine accurately because some ejection factors (e.g., tip diameter) and cortical diffusion rates are variable. However, dopamine concentrations at an electrode tip (1.0 mm cortical depth) produced by iontophoresis at different current intensities have been assayed. Extrapolation of calibration curves from these tests indicates that iontophoresis of 0.1 M dopamine over 60 s at 100 nA current intensity yields a tip concentration of 0.001 M dopamine (Millar et al. 1981). Therefore the dopamine concentration in our study was likely to be exponentially less than the 0.1 M solution in the pipette. At any rate, the molarity and current intensity were consistent and the tip diameters and electrode resistance were carefully monitored and maintained within a limited range throughout the experiments to minimize dosage fluctuations. Despite these precautions to minimize drug spread, neurons in close proximity to the electrode tip were likely to be exposed to drugs. Similar curves are not available for the receptor antagonists, but the tip concentration is likely to be several factors lower than that in the pipette. The slight reductions in spontaneous activity observed during co-application of dopamine and the antagonists in some PTNs were considered to be within the range of normal variations in spontaneous activity, but our injection parameters for the antagonists may have yielded tip concentrations that were too low to completely block dopamine-induced inhibition. Blockade by only the D1 or only the D2 antagonist in some cells indicated that their tip concentrations were within ranges that were selective for their appropriate receptor.

Our initial dose-response tests determined the effective parameters for dopamine ejection and showed that the transmitter was effective in altering neuronal activity only after a prolonged ejection period. During the first 10 s of iontophoresis, a notable decrease in spontaneous activity occurred, but further changes that were statistically significant were observed after 10 s. Other studies also show gradual or even delayed reductions of 15 s or more in firing rate from the onset of dopamine application (Bassant et al. 1990; Reader et al. 1979). Retaining currents which are applied to prevent leakage cause ion depletion at the electrode tip and a subsequent delay of several seconds before efflux occurs (Purves 1979). The recording intervals used in our study were prolonged to accommodate for this phenomenon. Conversely, at the end of the ejection period the ion concentration is relatively high at the electrode tip, causing a delay before the retaining current effectively minimizes efflux (Stone 1985). This phenomenon is likely to contribute to the continuation of dopamine-induced inhibition beyond cessation of the iontophoretic current. Another factor may contribute to the delay. A marked glutamate-induced increase in spontaneous activity was seldom achieved when glutamate followed dopamine application, suggesting that dopamine’s effects outlast those of glutamate. Both drugs should be affected similarly by the ejection current, but dopamine clearance may be delayed because its transporters are sparse and sometimes distant from synaptic release sites in the cortex (Sesack et al. 1998). These morphological features may account for the relatively slower clearance rate and prolonged receptor activation in the prefrontal cortex compared with the striatum (Garris and Wrightman 1994; Garris et al. 1993).
the current study, a 3-min interval between recordings allowed for adequate dopamine clearance, as evidenced by recovery of baseline spontaneous activity prior to each recording trial.

Variations in anesthesia levels and resulting changes in cortical activity are problematic in nearly all in vivo paradigms. Our attempts to maintain stable levels may not have been fully successful. However, because recording of baseline activity levels prior to each drug application allowed us to exclude neurons whose activity did not return to baseline levels of the previous trial, the potential influence of anesthetic variations was minimized. The prolonged baseline recording prior to drug application in each instance also served as a within-trial control for effects of drugs.

**Dopamine inhibits the SA of PTNs**

Spontaneous activity was selected as a measure of cortical activity in this study for several reasons. With careful control of variability, it can be monitored continuously over prolonged periods of time, including during the drug application, and assessed at all or selected time points. Given the relatively slow effects and clearance of iontophoresed drugs, this paradigm clearly reveals the time line for neuronal responses to treatment. Spontaneous activity can be considered as a measure of cortical excitability, particularly effective when monitoring substances that are likely to have broad modulatory effects. Dopamine targets a large population of pyramidal neurons, including PTNs, but acts through numerous mechanisms, including multiple receptors and probably endocrine-like secretion. Monitoring spontaneous activity allowed us to show that dopamine has a global inhibitory effect on a subpopulation of cortical neurons. This modulation may serve to dampen cortical excitability and refine responses to specific inputs. In fact, the magnitude of PTN evoked responses to specific input pathways, to date callosal and thalamocortical inputs have been...
investigated, are attenuated by dopamine (Huda et al. 1999, 2001). The coupling of such decreases in evoked responses and in spontaneous activity may serve to improve the signal-to-noise ratio of PTN responses to excitatory inputs and refine the output signal of the motor cortex. Although, the mechanisms for inhibition of either evoked (Huda et al. 1999, 2001) or spontaneous activity were not determined, our findings suggest that dopamine suppresses PTN responses to incoming input in a nonspecific manner.

Inhibition of neuronal activity in response to dopamine has been observed in neurons across all cortical laminae. Inhibitory responses include increased firing thresholds, coupled with decreased firing frequency of pyramidal neurons in rat prefrontal cortex (Geijo-Barrientos and Pastore 1995) and decreased task-related firing rates during voluntary movements in primate frontal cortex (Bernardi et al. 1982; Matsumura et al. 1990; Sawaguchi et al. 1986a, b). In slices of ferret prefrontal cortex, dopamine increases the failure rate and decreases the amplitude of EPSPs elicited in pyramidal neurons by activation of a synaptically paired neuron (Gao et al. 2001). On the other hand, dopamine-induced excitation occurs in a subpopulation of neurons in layer V (Sawaguchi et al. 1986b; Yang and Seamans 1996). Increased spontaneous activity was noted in vivo (Sawaguchi et al. 1986a) and increased firing rates in response to current induced depolarization were noted in vitro (Penit-Soria et al. 1987). In addition, dopamine D1 activation decreases the action potential threshold and the interspike interval of some layer III pyramidal neurons. (Henze et al. 2000). In these studies, target neurons were identified only by cortical layer or in a few cases, by pyramidal or nonpyramidal morphology.

Pyramidal neurons are the primary, but not exclusive, targets of dopaminergic axon terminals (Smiley and Goldman-Rakic 1993). Nonpyramidal neurons that express the calcium-binding protein parvalbumin also receive dopaminergic input (Porter 1995; Sesack et al. 1998). Dopaminergic modulation of GABAergic neurons occurs in the cortex and could mediate indirectly the dopamine-induced inhibition of PTNs. Although, our experimental paradigm cannot rule out this possibility, several lines of evidence suggest that dopamine-induced changes are mediated directly through receptors on PTNs. Activation of dopamine D1 receptors enhances GABAergic neuron excitability, whereas D2 receptor activation decreases the release probability of GABA (Seamans et al. 2001). Bidirectional effects of the antagonists were not observed in PTNs. Furthermore, the GABA antagonist, bicuculline has no affect on dopamine-induced depression of EPSPs in pyramidal neurons, indicating that this response results from direct receptor activation (Gao et al. 2001).

The heterogeneity of mesocortical dopamine innervation and laminar-specific distributions of dopamine receptor subtype may account for the different responses of neuronal subpopulations. Indeed, even individual populations of cortical neurons, may have the potential for varied responses. For example, corticostriatal and corticocortical neurons contain mRNA for both D1 and D2 receptors (Gaspar et al. 1995). Furthermore, our findings show that the PTN population contains three distinct dopamine receptor subtypes, D1a, D2, and D5. Methodological constraints (primary antibodies for 2 receptors from the same species) made it difficult to test for multiple receptor subtypes within individual neurons. Therefore dopamine may activate PTNs through one, or perhaps multiple, dopamine receptors, resulting in activation of different intracellular pathways. Our observations that both D1 and D2 antagonists are effective in blocking dopamine-induced effects in some neurons suggest that they contain more than one receptor subtype. In a few cells, however, only one antagonist was effective, suggesting that these PTNs expressed only one receptor subtype. PTNs’ responses to transcallosal volleys are decreased by dopamine in all but a few cases, in which they increase, suggesting different receptor activation (Huda et al. 1999). In unidentified pyramidal neurons in layer V of prefrontal cortex, D2 receptor activation reduces the number of spikes elicited by depolarizing current steps (Gulledge and Jaffe 1998), whereas D1 receptor activation decreases spike latency and increases firing frequency (Yang and Seamans 1996). In some instances, however, agonists and antagonists to both receptor subtypes are ineffective at mimicking and blocking, respectively, dopamine-induced effects (Shi et al. 1997), suggesting that the transmitter also works through nondopamine receptor mediated interaction with other neurotransmitters.

**Dopamine and glutamate interactions**

Dopamine modulates excitatory input onto cortical pyramidal neurons by both pre- and post-synaptic mechanisms (Reid et al. 1997; Zheng et al. 1999). The notion that dopaminergic and glutamatergic axon terminals might function as interactive units, presynaptic to pyramidal neurons, arose from the discovery of synaptic triads; structures composed of an asymmetric, presumably excitatory terminal and a dopaminergic terminal, both presynaptic to a dendritic spine (Goldman-Rakic et al. 1989). Physiological evidence for presynaptic glutamate modulation by dopamine comes from intracellular recordings of synaptically paired cortical pyramidal neurons: Dopamine increases the synaptic failure rate of one neuron in response to activation of the paired neuron and reduces the probability of glutamate release in response to a second stimulus (Gao et al. 2001). Dopaminergic modulation of glutamate-induced responses appears to depend on the glutamate receptor which is activated, although reports do not fully agree. For instance, Cepeda et al. (1992) found that dopamine increases the amplitude and decreases the latency of excitatory postsynaptic potentials (EPSPs), increasing the firing rate in layer V pyramidal neurons activated by N-methyl-D-aspartate (NMDA). On the other hand, it decreases EPSP amplitude, reducing firing frequency of cortical neurons activated by quisqualate. However, a D1-activated decrease in both the NMDA and the AMPA components of glutamate-induced EPSPs was reported by Law-Tho et al. (1994). Recently, opposing dose-dependent effects of dopamine on NMDA-receptor activated transmission were noted. Low concentrations preferentially activate D1 receptors that postsynaptically enhance NMDA-induced inward current, whereas high concentrations, acting on D2 receptors, suppress NMDA function (Zheng et al. 1999). The current study was designed to assess the combined effects of dopamine and glutamate on a specific population of neurons rather than to determine the mechanism of the interactions. Therefore it is not certain how the dopamine-induced suppression of glutamatergic excitation of PTN firing is mediated.

Dopamine’s influence over PTN activity may provide potent modulation over cortical control of motor behavior. PTNs are

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tightly coupled to spinal cord motor neurons and are an important link between the motor cortex and skilled movements (Phillips and Porter 1977). A balance of inhibition and excitation among groups of PTNs is thought to be a crucial component in coordinating agonist and antagonist muscle contraction for smooth movements (Matsamura et al. 1992). Not only is it important therefore to understand dopamine’s role in normal cortical function, but the potential contribution of cortical dopamine depletion to the symptomatology of disease processes, such as Parkinsonism, should be considered. Motor dysfunction of Parkinson’s patients has been attributed to gradual dopamine loss in the basal ganglia (Bernheimer et al. 1973; Hornykiewicz 1982), but affected individuals also show a marked and selective loss of dopamine in the motor cortex (Bernheimer et al. 1973; Hornykiewicz 1982; Whishaw et al. 1986). It is likely that the significant loss of cortical dopamine contributes to these motor impairments (Playford et al. 1993; Priori et al. 1994; Rascol et al. 1993).

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