Attenuation of Preparatory Activity for Reaching Movements by a D1-Dopamine Antagonist in the Monkey Premotor Cortex

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Sawaguchi, T. Attenuation of preparatory activity for reaching movements by a D1-dopamine antagonist in the monkey premotor cortex. J. Neurophysiol. 78: 1769–1774, 1997. To examine the role of dopamine receptors in the function of the premotor cortex (PM) for preparing for reaching movements, dopamine antagonists (SCH23390 for D1 receptors and sulpiride for D2 receptors) were applied iontophoretically to neurons of the PM of monkeys that performed a delayed-reaching (DR) task with their arms. In the DR task, the monkey made a reaching movement to one of three target levers (left, upper, and right), which had been cued by a visuospatial stimulus before a delay period of 4 s. We focused on neurons (n = 56) that showed a sustained increase in activity during the delay period (delay-period activity; i.e., “set-related” activity), because such activity is considered to play a central role in preparing for forelimb movements. Iontophoretic application of SCH23390 (usually with a current of 50 nA) significantly decreased the activity of most of these neurons (n = 44/56, 79%), and delay-period activity was attenuated during its application. In contrast, application of sulpiride or SCH23388 (an inactive analogue of SCH23390), using the same current intensity, had no effect on most of the neurons tested with these drugs (n = 31/33 and n = 21/23, respectively), despite the fact that their activity was decreased by SCH23390. Furthermore, for neurons that were affected by SCH23390, the percent decrease in delay-period activity was significantly greater than the percent change in background activity. In more than one-half of the neurons (n = 26/44, 59%), background activity was either increased (n = 10) or not affected (n = 16) by SCH23390, even though it significantly attenuated delay-period activity. These findings suggest that the activation of D1-dopamine receptors plays a modulatory role in PM function in preparing for reaching movements.

INTRODUCTION

It is well known that the premotor cortex (PM) of primates is involved in motor-set/preparation for forthcoming forelimb movements based on sensory information (for reviews see Kurata 1994; Wise 1985). For example, a subset of neurons in the monkey PM shows a sustained increase in activity during the delay period between a visual instruction signal and a “go” signal for movement execution in a variety of visually guided delayed-movement tasks with the forelimbs (e.g., Crandall and Kalaska 1994; Kurata 1993; Weinrich and Wise 1982; Weinrich et al. 1984). Such activity has been designated “set-related activity” and is considered to represent a central process in preparing for forthcoming forelimb movements (Kurata 1994; Wise 1985). However, the neurochemical basis of the PM function in preparing for forelimb movements is almost completely unknown. Recent evidence has suggested that dopamine in frontal motor cortices, including the PM, might play a role in controlling movements. Like the basal ganglia, in which dopamine plays an important role in motor control (e.g., Albin et al. 1989), frontal motor cortices of primates receive dopaminergic fibers from midbrain dopamine cells (Gaspar et al. 1992). The density of dopaminergic fibers in the PM is among the highest in several frontal cortical areas, including the motor cortex and the prefrontal cortex (Berger et al. 1988, 1991; Lewis et al. 1987; Williams and Goldman-Rakic 1993). The frontal cortex of primates contains a high level of D1-dopamine receptors (Cortes et al. 1989; Goldman-Rakic et al. 1990; Rischofield et al. 1989), and, in the case of the prefrontal cortex of monkeys, D1-dopamine receptors play a modulatory role in neuronal activities related to manual or oculomotor delayed-response tasks (Sawaguchi et al. 1988, 1990; Sawaguchi and Kubota 1996; Williams and Goldman-Rakic 1995). Therefore it is likely that D1-dopamine receptors might play a modulatory role in PM function in preparing for forelimb movements, but, as yet, there has been no attempt to examine this hypothesis.

To approach this hypothesis, we combined the iontophoretic application of selective dopamine antagonists (SCH23390 for D1 receptors and sulpiride for D2 receptors) with single-neuron recording in the PM while monkeys performed a delayed-reaching (DR) task. The PM is involved in controlling reaching movements with the arms (Crammond and Kalaska 1994; Moll and Kuypers 1977; Weinrich and Wise 1982; Weinrich et al. 1984), and the DR task requires such movement based on a visuospatial cue that has been presented before a brief delay period. Therefore this task was considered to be adequate for assessing the important function of the PM, i.e., preparation of reaching movements based on visuospatial information. We found a considerable number of neurons that showed a sustained increase in activity, i.e., set-related activity, during the delay period between a visual instruction signal and a go signal for the execution of reaching movements. We focused here on this delay-period activity and found that it was preferentially attenuated by iontophoretic application of a D1-dopamine receptor antagonist. Our data strongly suggest that the activation of D1-dopamine receptors plays a modulatory role in PM functions in preparing for reaching movements. A preliminary report of this study has appeared previously (Sawaguchi et al. 1993).

METHODS

Two rhesus monkeys (Macaca mulatta, males, 3.5–4.5 kg) were trained to perform a DR task. Throughout the experiment, the subjects were treated in accordance with the Guide for Care and
Use of Laboratory Animals (National Institutes of Health) and the Guide for Care and Use of Laboratory Primates (Primate Research Institute, Kyoto University, Aichi, Japan). Each monkey was seated in a monkey chair and faced a 21-in. cathode ray tube monitor (CRT) that was positioned ~40 cm from the monkey's eyes. Below the CRT, and ~25 cm from the monkey, three target levers (left, upper, and right) were mounted on a panel. The left and right levers were 20 cm apart and located at shoulder level. The upper lever was located at nose level 10 cm from the left or right lever. Below the panel, and 10 cm from it, a hold lever was located at waist level. The DR task was initiated when the monkey pressed the central hold lever with its left hand. After a 1-s waiting period, a warning cue (green square, 2×2 cm) appeared at the center of the CRT (warning period). One-half second later, one of three visual target cues (left, upper, or right; green square, 2×2 cm) appeared for 0.5 s (cue period) and was followed by a delay period. The delay period lasted for 4 s, and the color of the central cue changed from green to red, signaling “go.” Within 1.2 s after the go signal, the monkey released the hold lever to reach the target lever above which the cue had been presented (go period). A correct response on a target lever was rewarded by a drop of water 0.2 s after the response.

After the training was complete, surgery for the experiment was performed under aseptic conditions by a standard method that has been described previously (Sawaguchi 1987; Sawaguchi et al. 1990a). Briefly, each monkey was anesthetized with pentobarbital sodium (20–30 mg/kg ip or iv), and two stainless steel tubes (8 mm ID) were implanted on the anterior and posterior portions of the skull with dental acrylic for head fixation during recording sessions. To prevent bacterial infections, prophylactic antibiotics were injected intramuscularly on the day of the surgery and daily for 1 wk after surgery. A few weeks after the surgery, the monkeys began retraining for 1–2 wk. As in the experimental sessions, the head of the monkey was rigidly fixed to a stereotaxic frame on the top of the monkey chair by two stainless steel bars (8 mm diam). Under this condition, the monkeys performed the DR task for 500–1,500 trials at a correct-response rate of almost 100% during both the training and experimental sessions. The mean reaction time from the onset of the go signal to movement onset was ~310–380 ms, and the mean movement time, from movement onset to reaching to one of the three target locations (left, upper, or right), was ~340–360 ms.

While the monkeys were performing the DR task, the extracellular activity of single neurons was recorded from the PM contralateral to the hand used for DR performance, and drugs were applied iontophoretically to each neuron to examine their effects on neuronal activity related to DR performance. The iontophoretic technique and the method used to analyze neuronal activity were similar to those described in our previous studies (Sawaguchi 1987; Sawaguchi et al. 1990a). Briefly, multibarreled glass micropipettes were used for extracellular recording of neuronal activity and iontophoretic application of drugs. The central barrel of the micropipette, which contained a carbon-fiber filament (7 μm diam) and was filled with 0.9% saline, was used to record neuronal activity. The surrounding barrels were filled with, and used for iontophoretic application of, solutions of the following drugs: SCH23390 (0.01 M, pH 5–6; Research Biochemicals International, Natick, MA); l-sulpiride (0.01 M, pH 7–8; Research Biochemicals International); SCH23388 (0.01 M, pH 5–6; Research Biochemicals International); and 0.9% saline (to balance the current). The activity of a single neuron related to the DR task was recorded by the micropipette and converted from A/D by a window discriminator (DIS-1, BAK Electronics, Germantown, MD) for analysis with a personal computer (PC9801BX, NEC, Tokyo).Raster displays and time histograms, which were aligned at the onset of the task periods, were made by the computer (usually a 50-ms sampling rate), and the discharge rate during each period of the task was compared with that during the waiting (control) period. When the discharge rate of a neuron during the waiting, cue, delay, and/or go period(s) differed significantly from that during the waiting (control) period (Mann-Whitney U test, P < 0.05), the activity of the neuron was judged to be related to task performance. Among the task-related neurons, those that showed a sustained increase in activity during the delay period (“delay-period activity”) were examined in the present study. When a neuron’s discharge rates during both the early and latter halves (2 s, respectively) of the delay period were significantly higher than the discharge rate during the waiting (control) period (P < 0.05), it was considered to show delay-period activity.

When neuronal activity related to the task was encountered during penetration of the micropipette, it was recorded for >15 successive trials. Each of the drugs was then applied with a current of 30–100 nA for >15 successive trials (i.e., more than ~2 min) to examine the effect of the drug on task-related neuronal activity. When the discharge rate of a neuron changed significantly during the application of a drug (P < 0.05), the neuron was judged to be responsive to the drug. This test was performed using Student’s t-test; when there was a significant difference in variance, the Aspin-Welch method was used for correction. The comparison in activity for predrug trials and trials during drug application was based on overall activities during all of the periods of the task, and when the neuron was found to be significantly affected by the drug, the activities during each of the task periods were compared by the statistical methods mentioned above. The intensity of the current used to apply the drugs was usually 50 nA because previous studies showed that a current of 50 nA induced responses of frontal cortical neurons to drugs such as noradrenaline, dopamine, acetylcholine, and dopamine antagonists (Sawaguchi 1987; Sawaguchi and Matsumura 1985; Sawaguchi et al. 1990a,b), and this current intensity was sufficient to induce responses in most of the neurons that responded to the drugs. Drug applications were separated by >2 min to allow for the recovery of neuronal activity. To prevent leakage of the drugs, a backing current (2–5 nA) was continuously applied, during the predrug control period and between drug applications, through the tip of each barrel containing drug solution.

The areas examined, i.e., PM areas, were estimated physiologically using conventional intracortical microstimulation (ICMS; a train of 11 0.2-ms cathodal pulses at 333 Hz) (cf., Asanuma 1975) during each of the recording sessions. When ICMS (<40 μA) at a cortical site induced a muscle twitch or movement, the site was considered to be located within the motor cortex, and data obtained at these sites were excluded from the present examination. The PM was defined as the rostral portion of the area in which ICMS at an intensity of ≤40 μA did not induce muscle twitch or movement. These areas and their boundaries were later confirmed by histological examination based on cytoarchitectonic characteristics, as described below.

After extensive testing, the monkeys were deeply anesthetized with an overdose of pentobarbital sodium and perfused with physiological saline followed by formalin. The cortical surface was examined to detect the points of penetration. The brains were photographed, and the frontal lobes were cut serially and coronally at a thickness of 50 μm. The sections were stained by the Nissl method, and cortical layers and areas were determined cytoarchitecturally (Barbas and Pandya 1987; He et al. 1993; Watanabe-Sawaguchi et al. 1991). Figure 1 illustrates the points of penetration (n = 36) on the cortical surface of each of the monkeys. As shown in Fig. 1, the points were distributed in the dorsal and ventral aspects of the PM, i.e., PMd and PMv (He et al. 1993), and most of the points (31/36, 86%) were distributed in the dorsal PM (i.e., PMd).

**Results**

We examined a total of 56 neurons in the PM contralateral to the hand used for DR task performance that showed...
a sustained increase in activity during the delay period, i.e., delay-period activity. Iontophoretic application of SCH23390 (usually with a current of 50 nA) significantly decreased the activity of most of these neurons (n = 44/56, 79%). In contrast, application of sulpiride or SCH23388 (an inactive analogue of SCH23399), using the same current intensity, had no effect on the activity of most of the neurons tested with these drugs (n = 31/35 and n = 21/23, respectively), despite the fact that their activity was decreased by SCH23390.

Figure 2 shows raster displays and averaged histograms for the activity of a PM neuron before (control) and during the application of SCH23390 and sulpiride with a current of 50 nA. This neuron showed delay-period activity, particularly for upper trials, and also showed an increase in activity during the warning period. Iontophoretic application of SCH23390 significantly decreased the activity of this neuron (11.0 ± 10.4 vs. 7.8 ± 9.0 spikes/s, mean ± SD; t-test, P < 0.001). SCH23390 had a significant negative effect on delay-period activity (12.4 ± 10.5 vs. 7.0 ± 6.5 spikes/s, P < 0.001), particularly in upper trials (25.7 ± 6.4 vs. 14.1 ± 5.3 spikes/s, P < 0.001). The background activity during the waiting period and activity during the warning period were not significantly decreased by SCH23390 (3.1 ± 2.6 vs. 3.6 ± 3.4 spikes/s, P = 0.34, NS, for background activity; 24.3 ± 9.4 vs. 28.9 ± 10.8 spikes/s, P = 0.09, NS, for the warning period). In contrast to SCH23390, application of sulpiride did not have any clear effect on overall activity (11.0 ± 10.4 vs. 10.6 ± 11.0 spikes/s, P = 0.589, NS) or delay-period activity for left, upper, and right trials (6.3 ± 3.1 vs. 5.9 ± 3.6 spikes/s, P = 0.347, NS, for left trials; 25.7 ± 6.4 vs. 26.8 ± 5.6 spikes/s, P = 0.267, NS, for upper trials; 5.1 ± 3.6 vs. 4.2 ± 2.9 spikes/s, P = 0.009, NS, for right trials). As with sulpiride, iontophoretic application of SCH23388 did not affect the activity of most of the neurons tested, the activities of which were significantly decreased by SCH23390, as shown in the example in Fig. 3. The neuron in Fig. 3 showed delay-period activity, particularly for upper and right trials, and also showed an increase in activity during the cue period. Again, iontophoretic application of SCH23390 significantly decreased the activity of this neuron (7.4 ± 4.9 vs. 3.2 ± 3.1 spikes/s, P < 0.001). The delay-period activity was greatly decreased (7.7 ± 4.5 vs. 3.3 ± 2.9 spikes/s, P < 0.001), and the background activity and activity during the cue period were less strongly, but still significantly, decreased (3.4 ± 2.9 vs. 2.2 ± 2.3 spikes/s, P < 0.05, for background activity; 11.0 ± 5.8 vs. 6.3 ± 5.3 spikes/s, P < 0.01, for the cue period). In contrast to SCH23390, application of SCH23388 did not have any clear effect on the overall activity (7.4 ± 4.9 vs. 7.5 ± 5.0 spikes/s, P = 0.636, NS) or the delay-period activity (7.7 ± 4.5 vs. 7.8 ± 4.6 spikes/s, P = 0.827, NS). Thus SCH23390, but not sulpiride or SCH23388, had a significant attenuating effect on delay-period activity.

To further examine the characteristics of the effect of
SCH23390 on delay-period activity, we compared the percent changes in the discharge rate during the delay period (relative to predrug control activity) during SCH23390 application with the changes in the background activity for each neuron (n = 44) whose activity was significantly decreased by the iontophoretic (50 nA) application of SCH23390. In Fig. 4A, the percent changes in the discharge rates during the delay period are plotted against the percent changes in the background activity for the neurons affected by SCH23390. Most of the data points fall below the 45° line, indicating that the application of SCH23390 had a greater attenuating effect on delay-period activity than on background activity. In more than one-half of the neurons (n = 26/44, 59%), background activity was either increased (n = 10) or not affected (n = 16) by SCH23390, even though it significantly decreased delay-period activity. On average, the percent decrease in delay-period activity was significantly greater than the percent change in background activity (mean ± SD, −35.5 ± 20.7% vs. −7.1 ± 24.3%, paired t-test, P < 0.001), as shown in Fig. 4B. Thus SCH23390 had a greater attenuating effect on delay-period activity than on background activity.

**DISCUSSION**

In the present study, the dopamine antagonists SCH23390 and sulpiride were applied iontophoretically to neurons in the monkey PM, which showed a sustained increase in activity during the delay period (delay-period activity) in a DR task. Iontophoretic application of SCH23390 significantly decreased the activity of most of these neurons. In contrast, the application of sulpiride did not have any clear effect on the activity of most of the neurons tested with this drug, despite the fact that their activity was decreased by SCH23390. In addition, an inactive analogue of SCH23390, SCH23388, had no effect on most of the neurons tested, indicating that the effect of SCH23390 was not due to any nonspecific effects, such as a local anesthetic effect. SCH23390 is a highly selective antagonist of D1-dopamine receptors, whereas sulpiride is a selective antagonist of D2 receptors, which also has a potent affinity for D3 receptors (Christensen et al. 1984; Hyttel 1983; Memo et al. 1986; Sokoloff et al. 1990). The frontal cortex of primates contains a high level of D1 receptors, but a relatively low or negligible level of D2, and probably also D3, receptors (Camps et al. 1989; Cortes et al. 1989; Goldman-Rakic et al. 1990; Lidow et al. 1989; Rischfield et al. 1989). Together, these findings suggest that the activation of D1-dopamine receptors is involved in the delay-period activity of PM neurons during DR task performance.

The present study focused on neurons with delay-period activity, i.e., neurons that showed a sustained increase in activity during the delay period between the visual instruction signal and the go signal for movement execution. Similar sustained activity during such a delay period has been demonstrated for PM neurons in previous single-neuron studies using monkeys that performed behavioral tasks similar to the present DR task, and has been designated “set-related activity” (Crammond and Kalaska 1994; Kurata 1994; Wise 1985). The present study demonstrated that iontophoretic application of SCH23390 attenuated delay-period activity in most of the neurons tested. The percent decrease in delay-period activity was significantly greater than the percent change in background activity, and, in more than one-half of the neurons, SCH23390 significantly decreased the delay-period activity but not the background activity. This suggests that the activation of D1-dopamine receptors plays a modulatory role in neuronal functions of the PM in preparing for reaching movements.

It has been shown in vitro that D1-dopamine receptors modulate the synaptic transmission of the excitatory amino acid glutamate in the frontal cortex of rats (Lawtho et al. 1994). Furthermore, D1-dopamine receptors form asymmetric synapses with dendritic spines of pyramidal neurons in the primate cerebral cortex (Goldman-Rakic et al. 1989; Smiley and Goldman-Rakic 1993; Smiley et al. 1994), which also suggests that D1-dopamine receptors modulate excitatory neurotransmission, probably glutamnergic transmission. Moreover, it has been recently demonstrated in rats that the activation of D1-dopamine receptors modulates dendritic-somatic signal integration of pyramidal neurons of the frontal cortex; activation of D1-dopamine receptors restricts
inputs to apical dendrites and potentiates the influence of local inputs from neighboring neurons (Yang and Seamans 1996). The preparatory activity of at least some PM neurons may be generated or augmented by such dendritic-somatic signal integration. Thus the activation of D1-dopamine receptors in the PM appears to regulate excitatory neurotransmission, thereby modulating or facilitating the preparatory activity of PM neurons for reaching movements.

Finally, the present study might provide insight into symptoms of Parkinson’s disease. This movement disorder is associated with not only a lesion on the substantia nigra pars compacta but also a lesion on the ventral tegmental area (Javoy-Agid and Agid 1980), and dopamine cells in both of these structures send their fibers to the frontal motor corti-

ces in primates (Gaspar et al. 1992). Furthermore, the concentration of dopamine and its metabolites has been shown to be reduced in the frontal cortex of Parkinson’s patients (Scatton et al. 1983). Based on these previous and the present findings, it is plausible that the dysfunction of D1-dopamine receptors in the PM, and probably also in other frontal motor cortical areas, may contribute to some symptoms, such as bradykinesia, in Parkinson’s disease. This possibility is worth considering for the development of improved therapeutic approaches to this disease.

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