Increase of Extracellular Dopamine in Primate Prefrontal Cortex During a Working Memory Task

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Watanabe, Masataka, Tohru Kodama, and Kazuo Hikosaka. Increase of extracellular dopamine in primate prefrontal cortex during a working memory task. J. Neurophysiol. 78: 2795–2798, 1997. The dopamine innervation of the prefrontal cortex is involved importantly in cognitive processes, such as tested in working memory tasks. However, there have been no studies directly investigating prefrontal dopamine levels in relation to cognitive processes. We measured frontal extracellular dopamine concentration using in vivo microdialysis in monkeys performing in a delayed alternation task as a typical working memory paradigm and in a sensory-guided control task. We observed a significant increase in dopamine level in the delayed alternation task as compared both with the sensory-guided control task and the basal resting level. The increase was seen in the dorsolateral prefrontal but not in the arcuate or orbitofrontal areas. The increase appeared to reflect the working memory component of the task and was observed mainly in the lip areas of principal sulcus. Although there was no significant difference in dopamine level between delayed alternation and sensory-guided control tasks in the premotor area, significant increases in dopamine concentration were observed during both tasks as compared with the basal resting level, indicating the importance of premotor dopamine for the motor response itself.

INTRODUCTION

Dopamine in the prefrontal cortex plays important roles in cognitive functions (Berman and Weinberger 1990; Luciana and Collins 1997). Depletion of dopamine impairs the performance of working memory tasks in monkeys (Brozoski et al. 1979; Sawaguchi and Goldman-Rakic 1991), whereas application of its agonist improves reduced working memory (Arnsten et al. 1994). Excessive dopamine activity also causes cognitive dysfunction (Arnsten et al. 1994; Farah 1997; Murphy et al. 1996a,b). Working memory-related neuronal activity in the prefrontal cortex depends on the stimulation of dopamine D-1 receptors (Williams and Goldman-Rakic 1995).

Despite these indications, there have been no studies directly investigating prefrontal dopamine levels in relation to cognitive functions. This may be due to the technical complexities of such studies and the difficulties in quantifying the relatively low cortical basal dopamine levels (Moghaddam et al. 1993; Saunders et al. 1994). Taking advantage of recent technical innovations in “in vivo microdialysis” and employing suitable experimental and control tasks, we examined the changes in prefrontal dopamine levels in behaving monkeys. We employed, as a working memory task, a delayed alternation task rather than the more widely used delayed response task. In delayed alternation, each reward serves as implicit instruction for a subsequent key press, and the animal needs to maintain a representation of the position of the next key press in working memory during almost all periods of task performance except for the time of the response. Thus working memory is engaged during a much longer period than in the delayed response task. This also is reflected in prolonged memory-related neuronal activity during delayed alternation (Kubota and Niki 1971).

METHODS

Three Japanese monkeys (Macaca fuscata) were trained in a delayed alternation (D-ALT) and a sensory-guided control (S-CON) task (Fig. 1). The animal faced a panel, which contained two circular keys and a holding lever below them. On each trial of D-ALT, the animal pressed the holding lever for 5 s (delay period), after which time both keys were illuminated by a white light as a go signal. The animal obtained juice reward by alternating key presses to the right and left, each key press being preceded by the delay period. The S-CON task also employed a 5-s delay period, but the key to be pressed was indicated explicitly by a white light at the right or left key. The same juice reward was delivered for correct key presses. Thus both tasks had the same motor and motivational requirements, but only the D-ALT task contained a working memory component. On completion of training, the same device for head fixation was implanted under pentobarbital sodium anesthesia (Nembutal, 30 mg/kg) as for chronic neuronal recordings (Watanabe 1992).

The dialysis experiment followed previously reported procedures (Kodama et al. 1992). Guide canulas (Type AG, EICOM, Kyoto) were fixed stereotaxically to the skull over the frontal cortical target areas under general anesthesia. At least five days after surgery, coaxial microdialysis probes (Type A-I, EICOM, Kyoto) with a semipermeable membrane of 2 mm at their tips were inserted into the appropriate cortical locations. These were determined by neuronal recording during the implantation of the guide canulas.

About 20 h after insertion of the probes, in vivo dialysis was performed in three behavioral situations, namely D-ALT, S-CON, and a resting period (REST), during which the animal sat quietly. On the first day of experimentation, the order of behavioral situations was S-CON (20 min), REST (20 min), D-ALT (35 min), REST (20 min), S-CON (20 min). On the second day, the order was changed to D-ALT (20 min), REST (20 min), S-CON (35 min), REST (20 min), D-ALT (20 min) to counterbalance the sequence of D-ALT and S-CON situations. Probes were perfused with Ringer solution at a flow rate of 2 μl/min. Dialysate sampling of 15 min duration began 5 min after the beginning of each task or rest period. One sample was obtained during the 20-min periods, whereas two samples were collected during the 35-min periods. Four dialysate samples were obtained in each of the three behavioral situations at each cortical position on two consecutive days. Dialysates collected at 10°C in polypropylene sample tubes under acetic conditions (pH 3.5) were frozen immediately and kept
in the DL area ($P < 0.001$). Compared with the basal REST value, significant increases in dopamine concentration were observed during D-ALT in both DL ($P < 0.05$) and PM ($P < 0.05$) areas, whereas during S-CON, only in the PM ($P < 0.01$) area.

Dopamine levels were further compared among the dorsolateral (DLd, $n = 10$), principalis (PS, $n = 15$), and ventral (DLv, $n = 11$) DL subareas (Fig. 3b). Mean dopamine levels during REST period for DLd, PS, and DLv subareas were $0.084 \pm 0.01$, $0.105 \pm 0.01$, and $0.101 \pm 0.01$ fmol/$\mu$m, respectively. A one-way ANOVA failed to reveal significant differences among the three subareas ($P = 0.43$).

Figure 3b shows the mean dopamine changes during D-ALT and S-CON tasks in the three subareas as compared with the REST situation. A two-way ANOVA (task by area) indicated significantly different dopamine levels between D-ALT and S-CON ($P < 0.001$), but not among the three subareas ($P = 0.18$) or in the interaction ($P = 0.25$). Planned comparisons for each subarea indicated significant dopamine increases during D-ALT as compared with S-CON in DLd ($P = 0.0057$) and DLv ($P = 0.0247$) but not in the PS subarea ($P = 0.079$). The increase amounted to 23% in DLd and DLv combined.
DISCUSSION

The present study revealed a significant difference in extracellular dopamine between a typical working memory task and a sensory-guided control task in the primate dorsolateral prefrontal cortex but not in the other prefrontal areas investigated. In previous studies, the importance of prefrontal dopamine innervation for working memory was demonstrated by the effects of altered dopamine neurotransmission (Arnsten et al. 1994; Brozoski et al. 1979; Diamond 1996; Diamond et al. 1994; Luciana and Collins 1997; Murphy et al. 1996a;b; Sawaguchi and Goldman-Rakic 1991). In line with these studies, the present experiment provides the direct evidence for an increase of dopamine in the dorsolateral prefrontal cortex during a typical working memory task. Our result is consistent with the general notion emerging from ablation and neurophysiological studies, which demonstrate the importance of the dorsolateral area for delayed alternation behavior (Kubota and Niki 1971; Mishkin 1957; Niki 1974).

An unexpected finding of the present study was the absence of significant increases in dopamine levels in the depth of the principal sulcus. The lip areas of this sulcus (DLd and DLv subareas) appear to be involved more critically in delayed alternation performance, which is impaired greatly when only these areas are ablated while sparing substantial parts of the depth of the principal sulcus (Mishkin 1957). This functional difference corresponds to noticeable differences in cytoarchitecture between parts of the lip and depth subareas (Barbas and Pandya 1989).

There were increases in dopamine concentration in the PM area during both D-ALT and S-CON tasks as compared with the basal REST level. The result is in accordance with recently reported impairments in both manual delayed response and sensory-guided control task performance by the injection of a dopamine D-1 antagonist into the PM area (Sawaguchi 1995) and may indicate that premotor dopamine plays important roles for the motor response itself rather than for working memory.

Different from prefrontal neurons, midbrain dopamine neurons that innervate the prefrontal cortex do not show sustained activations during the delay period in delayed alternation (Ljungberg et al. 1991). Although dopamine neurons do not respond to predicted rewards in reaction time tasks (Schultz 1992), they are activated by the reward in delayed alternation, probably because the instructional components of reward delivery arouse the attention of the animal to guide its goal-directed behavior (Schultz 1992). This activation in turn may contribute to the increase in dopamine levels in the prefrontal cortex. In this regard, the difference of attentional demand or that of task difficulty besides that of working memory requirement between D-ALT and S-CON tasks, also may contribute to the observed difference in dopamine levels between these two tasks.

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