Age-related effects of bromocriptine on sensory gating in rhesus monkeys

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Declines in dopamine neurotransmission are a robust characteristic of the process of normal aging. Using neuroimaging, biochemical and cognitive methods, age-related reduction of D2 receptor has been noted in a wide range of species. On the other hand, it is well known that dopamine plays a crucial role in the modulation of sensory gating. Here, we examined age-related alterations of D2 receptor in rhesus monkeys, using a sensory gating paradigm. The direct D2 receptor agonist, bromocriptine, was characterized in young adult and aged monkeys. We found bromocriptine disrupted sensory gating in young adult monkeys but not in aged ones. Our results provided new evidence that there is a functional decline of D2 receptor in aged monkeys. *NeuroReport* 16:603–606 © 2005 Lippincott Williams & Wilkins.

Key words: Aging; Dopamine; Rhesus monkeys; Sensory gating

INTRODUCTION

The dopamine (DA) system is a key neurotransmitter system in the brain, which acts as a powerful regulator and integrator of different aspects of brain functions, such as action, emotion, motivation and cognition [1]. Since 1976, when Miller *et al.* [2] found that hypothalamic content of DA is significantly reduced in aged rats, alterations of the DA system in the process of normal aging have been extensively studied in a variety of species: humans, monkeys, rats, rabbits and dogs. Previous efforts involving neuroimaging, biochemical and cognitive studies have demonstrated that the age-related alterations in the DA system include reduced DA release and loss of DA receptors [3,4].

In the past several years, many studies focused on the action of DA on its various receptors (D1 and D2) during aging. In particular, the D2 receptor has received attention regarding age-related alterations. By in-vitro assay of receptor concentrations and in-vivo imaging techniques, loss of D2 receptors in the aged brain has been noted in many brain regions, such as the striatum, nucleus accumbens (NAC) and hippocampus [5,6]. Also, there is an age-related decline of the function of D2 receptors [4]. Previous studies in our laboratory have assessed the contribution of D2 receptors to age-related loss of the prefrontal cortical (PFC) cognitive function [7]. Here, we planned to examine the age-related alterations of D2 receptors in rhesus monkeys, using a sensory gating paradigm as a measure.

Inhibiting response to repetitive, irrelevant environmental stimuli is an essential protective function of the central nervous system and has been demonstrated in humans and animals. One important aspect of this is the so-called sensory gating. Sensory gating is commonly measured in auditory-evoked potential (AEP) paradigms. Many features of AEPs are consistent across species so that experiments with common laboratory animals and humans may be of help in evaluating sensory gating. Sensory gating can be defined as the reduced midlatency auditory-evoked responses (MLAERs) in response to the second (or test) stimulus compared with the first (or conditioning) stimulus of a click pair separated by a short time (e.g. 500 ms). MLAERs are a series of brain waves that occur between 10 and 250 ms after stimulation and can be recorded at the scalp. This also holds true for another type of gating, prepulse inhibition (PPI), the decrease in startle response that occurs when a startling stimulus is preceded by a weaker stimulus (or prepulse) at intervals between 60 and 120 ms.

The P50 (P1) component, which is a positive wave peaking between 15 and 80 ms following stimulus presentation, is the earliest component of the MLAERs that habituates to stimulus repetition. At this early stage of information processing, attentional influences are minimal, thus making the P50 (P1) component ideal for examination of preattentive sensory habituation mechanisms. Normal participants exhibit robust (e.g., 60% to 80%) suppression in the P50 amplitude to the second auditory stimulus compared with the first one [8]. However, schizophrenic patients do not gate or suppress the P50 response to the second stimulus [9]. A variety of data show that the impairment of sensory gating in schizophrenic patients is caused by overactive DA neurotransmission [10]. Using DA receptor agonists such as bromocriptine and amphetamine, the deficits of sensory gating, similarly to schizophrenic patients, were found in healthy humans and rats [11–13].

In the present study, we examined the effects of the direct D2 receptor agonist, bromocriptine, on sensory gating using the paired clicks paradigm in young adult and aged monkeys to address the age-related alterations of D2 receptor. We hypothesized that after a bromocriptine challenge, sensory gating would be diminished in young adult monkeys, but would be less diminished in aged monkeys.

MATERIALS AND METHODS

Participants and surgery: The experimental participants were two young adult (6–7 years old, M1 and M2) and two old (about 20 years old, M3 and M4) female rhesus monkeys (*Macaca mulatta*), weighing 6.0–7.0 kg. The animals were kept in individual cages in a temperature-controlled (25°C) colony room, with food and water available. This study was performed in accordance with the 'Principles of laboratory animal care' (NIH) and institutional guidelines.

Twelve hours before surgery, each animal was restricted from food and water. The surgery was performed aseptically. The monkeys were anesthetized with sodium pentobarbital (10 mg/kg, intramuscular) following premedication with hydrochloric acidulated ketamine (15 mg/kg, intramuscular) and placed in a stereotaxic apparatus. During the operation, sodium pentobarbital anesthesia was maintained and supplementary doses were administered as needed. The scalp was incised and retracted along with the muscles overlying the skull. The surface of the skull was cleaned of all fasciae and then thoroughly dried. One Teflon-insulated epidural stainless steel recording electrode was inserted through the skull to rest on the dura in the right PFC (dorsal part of area 46) for electroencephalogram (EEG) recording. Previous studies have shown that sensory gating can be recorded in the PFC [14].

Procedures: Animals were allowed at least two weeks to recover from surgery before EEG recording commenced. EEG recording took place in a soundproof room in which the monkey was placed in a primate chair. On three consecutive days before test sessions, the animals were habituated to the experimental chamber each morning for about 2.5 h.

All test sessions began with a baseline session (T0, 20 min), followed by the administration of either bromocriptine or saline (NaCl 0.9%, 2 ml) challenge. Bromocriptine was dissolved in distilled water. Three dosage levels (0.3125, 0.625, 1.25 mg/kg) were tested, and each dose was administered three times on each monkey. Previous experiments in our laboratory have indicated that the AEP and the behavior of the monkeys return to normal two days after each drug injection (unpublished data). Therefore, all washout periods between each drug injection were at least two days. Additionally, when the dose of bromocriptine was increased, there was at least a 1-week interval between tests. Post-drug trials were acquired at 0–120 min after each drug treatment, which were divided into six sessions (20 min per session, T1–T6). The period was selected on the basis of published studies demonstrating physiological activity of bromocriptine at some point during that period [15].

P50 measures: Auditory stimuli were delivered through a speaker located in the front of the primate chair, 50 cm from the animals; a camera was used to monitor the behavior of monkeys. The computer-controlled stimuli consisted of paired clicks, presented 500 ms apart, at 90 dB (sound pressure level). Click pairs were automatically presented at 10-s intervals. Each session (20 min) involved 100 trials. A background white noise (60 dB) was continuously present throughout the experiments.

EEG was amplified, filtered with a band pass of 0.1–120 Hz, with a 50-Hz notch filter. The sampling rate was 1000 Hz and data were stored on disk for offline analysis. Only trials occurring while the animal was motionless and alert were accepted for analysis. Fewer than 10% trials were rejected in each session and the rejection rate was similar for each animal.

The P50 component was identified in a manner consistent with previous studies. The P50 was defined as the most positive deflection 40–80 ms following stimulus presentation. The amplitude was defined as the absolute difference between the P50 peak and the preceding negative trough. Evoked potential waveforms were examined visually; determination of latencies and amplitudes were computer-assisted. A test/condition ratio (TC ratio) was calculated for each averaged recording by dividing the amplitude of the test AEP by the amplitude of the condition AEP.

Statistical analyses: Because the animals served as their own controls, statistical analyses employed repeated measures designs: paired *t*-test (also called dependent *t*-test or *t*-dep) and three-way or four-way analyses of variance with repeated measures (ANOVA-R). To avoid the difference within participants, the TC ratio performance on drug was calculated as percentage difference scores from the baseline. The effect of saline was tested using a three-way ANOVA-R with the within-participants factors of time and repeated times and the between-groups factor of group. And the effect of bromocriptine was tested using a four-way ANOVA-R with the within-participants factors of dose, time and repeated times and the between-groups factor of group. Statistical analysis was conducted using the SPSS 10.0 Statistical Package. Statistical significance was set at the probability level of $p < 0.05$.

RESULTS

No significant differences were observed among P50 amplitudes or latencies across the different conditions (baseline, saline and bromocriptine in different doses) in both young adult and aged animals (data not shown). No significant main effects of group, time and repeated times for the saline condition were found. However, there was a significant main effect of group, $F(1,2)=26.833$, $p=0.035$, for the bromocriptine condition. No significant main effects of dose, time and repeated times were found. An interaction of time by group, $F(6,12)=2.651$, $p=0.071$, was observed. A simple effect analysis revealed that there was an effect of time effects in young monkeys but not in aged monkeys (young monkeys: $p=0.012$; aged monkeys: $p=0.907$). Individual drug comparisons revealed that three doses of bromocriptine all disrupted P50 suppression: 0.3125 mg/kg at T3 and T4 in M1, and at T5 and T6 in M2; 0.625 mg/kg at T4 in M1, and at T1 and T3 in M2; and 1.25 mg/kg at T4 and T5 in M1, and at T3 in M2. Although there was a decrease in TC ratio at T4 after 1.25 mg/kg bromocriptine challenge in M3,

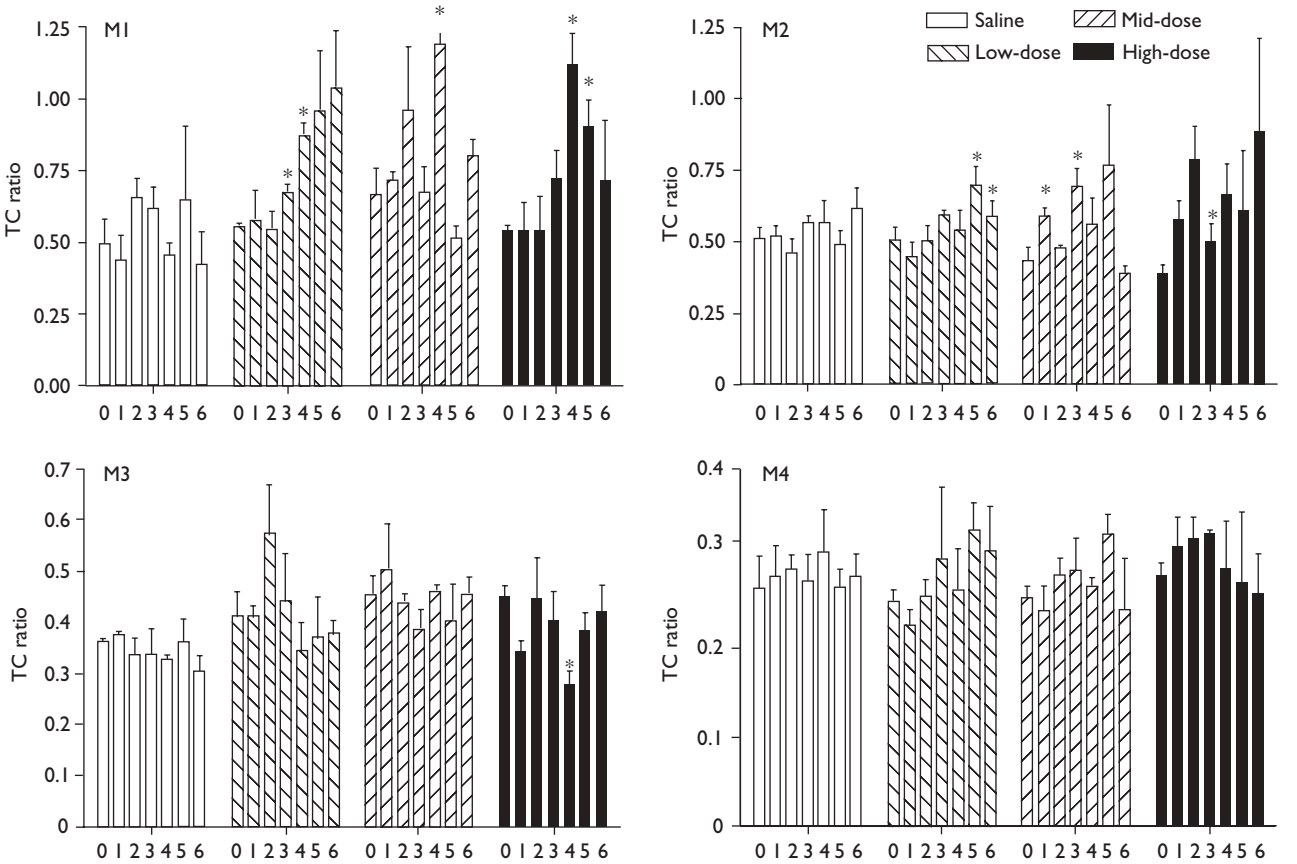

Fig. 1. The effects of saline and bromocriptine on sensory gating in young adult monkeys and aged monkeys. Young adult monkeys: M1 and M2; Aged monkeys: M3 and M4. Low dose: 0.3125 mg/kg; Mid-dose: 0.625 mg/kg; High dose: 1.25 mg/kg. 0–6 labeled in the bottom of each bar represent the sessions from T0 to T6, respectively. Saline did not affect the test/condition (TC) ratio in all experiment animals. Bromocriptine produced the increases of TC ratio in young adult monkeys but not in aged ones. Data are represented as means and SEM. *$p < 0.05$ by paired *t*-test compared with baseline (T0).

none of the three dosage levels of bromocriptine produced significant and consistent changes in TC ratio in aged monkeys (see Fig. 1).

DISCUSSION

As expected, bromocriptine disrupted sensory gating in young adult monkeys, but not in aged ones. In aged monkeys, none of the three dosage levels of bromocriptine produced a deficit in sensory gating. Even at the highest dose (1.25 mg/kg), there was no tendency to reduce sensory gating.

The results in young adult monkeys are consistent with previous studies. In healthy humans, bromocriptine and amphetamine (indirect DA receptor agonist) can reduce P50 suppression [11,12]. Also, bromocriptine has been shown to reduce PPI in healthy humans, and this effect was antagonized by haloperidol (DA D2 antagonist) [16]. In rats, cortical and hippocampal sensory gating can be reduced by systemic injections of amphetamine [13,17]. These studies have identified the role of DA in the regulation of P50 suppression. Our results indicated that systemic injections of bromocriptine influenced the DA system in young adult monkeys but not in old monkeys. This may be due to a decline of dopaminergic function in aged monkeys.

Furthermore, it has been extensively shown that the effects of DA agonists on sensory gating and PPI have been linked to DA hyperactivity in the mesolimbic system, which is formed by neurons that project from the VTA to the ventral striatum, that is, NAC, olfactory tubercle and other limbic regions [18]. A variety of data showed that, specifically, the mesolimbic DA terminal region in the NAC and predominantly D2 receptor regulate sensory gating. Microinjections of quinpirole (DA D2/3 agonist) in the NAC core and shell can disrupt cortical and hippocampal sensory gating [19]. Additionally, local infusion of DA and quinpirole in the NAC has been shown to significantly reduce PPI [20]. Thus, our results suggested that in aged monkeys D2 receptors in the mesolimbic pathway might not be activated by bromocriptine as they were in young adult animals, indicating the functional decline of D2 receptor in the mesolimbic system in the aged primate brain.

A series of data show that the declines of DA neurotransmission are a robust characteristic of the process of normal aging. Using functional imaging methods, such as magnetic resonance imaging, positron emission tomography and single photon emission computed tomography, an age-related change in the brain dopamine system was revealed (for example, see Ref. [21]). In addition, evidence shows that the impairment of cognitive skill learning in normal aging might be accounted for by the age-dependent decline of D2 receptor availability [7]. In particular, the age-related

declines of D2 receptors were manifested in dopaminergic areas with a high density of DA receptors. The mesolimbic pathway is one of the four major DA pathways. Therefore, it is reasonable that the mesolimbic pathway is more vulnerable to age-related declines of D2 receptors. Biochemical studies have shown significant declines of D2 receptors in the mesolimbic pathway in the process of aging. By in-vitro assay of receptor concentrations, age-associated decreases of D2 receptors were found in the striatum, caudate-putamen, olfactory tubercle and NAC [5]. In the present study, using sensory gating as a measure, age-related functional declines of D2 receptors in the mesolimbic system were demonstrated in monkeys.

CONCLUSION

Previous studies in our laboratory have found loss of D2 receptor function in the PFC of the aged brain. In the present study, we provided evidence for the loss of D2 receptor function in the mesolimbic system in aged monkeys, using sensory gating as a measure. Our results demonstrate that age-related declines of DA neurotransmission occur in the process of normal aging in monkeys.

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