Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys

(FG7142/RO15-1788/haloperidol/clozapine/SCH23390)

B. L. Murphy*, A. F. T. Arnesten†, P. S. Goldman-Rakic‡, and R. H. Roth*†§

Departments of *Pharmacology and †Psychiatry and ‡Section of Neurobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510-8001

ABSTRACT The selective activation of the prefrontal cortical dopamine system by mild stress can be mimicked by anxiogenic β-carbolines such as FG7142. To investigate the functional relevance of elevated levels of dopamine turnover in the prefrontal cortex, the current study examined the effects of FG7142 on the performance of spatial working memory tasks in the rat and monkey. FG7142 selectively increased prefrontal cortical dopamine turnover in rats and significantly impaired performance on spatial working memory tasks in both rats and monkeys. Spatial discrimination, a task with similar motor and motivational demands (rats), or delayed response performance following zero-second delays (monkeys) was unaffected by FG7142. Further, biochemical analysis in rats revealed a significant positive correlation between dopamine turnover in the prefrontal cortex and cognitive impairment on the delayed alternation task. The cognitive deficits in both rats and monkeys were prevented by pretreatment with the benzodiazepine receptor antagonist, RO15-1788, which blocked the increase in dopamine turnover and by the dopamine receptor antagonists, haloperidol, clozapine, and SCH23390. These findings indicate that excessive dopamine activity in the prefrontal cortex is detrimental to cognitive functions mediated by the prefrontal cortex.

The unique sensitivity of the prefrontal cortical dopamine system to mild stress has been the focus of intensive investigation (1) due to its potential relevance to psychiatric disorders, including the exacerbation or precipitation of psychotic episodes (2–4). Prefrontal cortical dysfunction has been implicated in schizophrenia (5, 6), and alterations in the function of the prefrontal dopamine innervation are thought to underlie some of the cognitive and affective symptoms associated with this disease (7, 8).

Despite considerable research into the biochemical and pharmacological regulation of dopamine release and turnover in the prefrontal cortex (1, 9), the impact of increased dopamine turnover on prefrontal cortical function has not been directly explored. In the present study, we have examined this question by exploiting the finding that the anxiogenic β-carboline, FG7142, reproduces the biochemical effects of mild stress on prefrontal cortex by eliciting a selective and significant increase in dopamine turnover and release (1, 10–14).

The behavioral effects of FG7142 administration were examined in both rats and monkeys performing working memory tasks which depend on prefrontal cortical functioning (15–22). Previous research has shown that the spatial working memory functions of the prefrontal cortex are dependent upon the integrity of the dopamine neurons: experimental depletion of dopamine restricted to the prefrontal cortex can lead to working memory deficits in monkeys and rats (23–26). The results of the current study suggest that excessive dopamine-receptor stimulation in the prefrontal cortex is also detrimental to prefrontal cortical cognitive functioning in both rodents and monkeys.

MATERIALS AND METHODS

Subjects. Subjects were male Sprague–Dawley (Camm) rats (Charles River Breeding Laboratories) and four female rhesus monkeys. All rats had access to water ad lib. Rats used only in the biochemical study had ad lib access to rat chow, while rats and monkeys in the behavioral studies were limited to small quantities of standard chow immediately following cognitive testing.

Behavioral Testing. Delayed alternation testing was performed in a standard T maze. On the first trial, rats were rewarded for traversing the runway and entering either arm. Thereafter, for a total of 10 trials per session, rats were rewarded only if they entered the maze arm which was not previously chosen. The intertrial delay, during which the rat was confined to the start box of the runway, was adjusted until a rat’s performance stabilized at ~80% correct, to allow for detection of either improvement or impairment during drug administration.

During spatial discrimination testing in the same T maze, rats were rewarded for always choosing either the left or the right arm. Half of the animals were rewarded for entering the right arm, and half were rewarded for entering the left. The intertrial delay was adjusted so that a rat was performing at or below 90% correct, to allow for detection of either improvement or impairment following drug administration.

Monkeys were tested on delayed response according to the methods of Brozowski et al. (23) and Arnesten et al. (15). Delays were adjusted until an individual monkey performed at ~83% correct, to allow for detection of either improvement or impairment with drug administration.

Locomotor Activity. Measurements of motor activity in rodents were conducted in a darkened sound-attenuating chamber equipped with an eight-cage (24.5 mm x 45 mm x 20 mm) infrared photobeam activity monitoring system (Omnitech Digiscan Analyser, Omnitech Electronics, Columbus). Subjects were monitored for 15 min preinjection and 30 min postinjection. Activity counts were combined across 5-min intervals.

Pharmacological Treatment. The behavioral testing was carried out by experimenters who were blind to drug conditions. All injections in rats were given i.p. All drugs were administered to monkeys i.m. except clozapine, which was given p.o. Drug treatment was given only after normal baseline performance was reestablished for at least two consecutive test sessions, with a minimum washout period between consecutive drug treatments (1 week for rats; 10 days for monkeys).

Abbreviations: ANOVA, analysis of variance; DOPAC, 3,4-dihydroxyphenylacetic acid.

*To whom reprint requests should be addressed: Department of Pharmacology, P.O. Box 208066, 333 Cedar Street, New Haven, CT 06520-8066.

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FG7142 was purchased from Research Biochemicals (Natick, MA) and suspended in a saline vehicle containing Tween 80, hydroxybetacyclodextrin, and ethanol. For rats, a dose of 20 mg/kg was administered ~25 min before testing. A dose of 0.2 mg/kg was administered to monkeys 35 min before testing. FG7142 treatments were repeated throughout the study to ensure that animals did not habituate to the effects of repeated FG7142 treatment.

RO15-1788, a gift from Hoffmann–La Roche, was administered to rats in a dose of 20 mg/kg 15 min before FG7142 administration. In the monkey, RO15-1788 was administered in a dose of 2 mg/kg, 15 min before FG7142 administration. Injectable haloperidol (McNeil Pharmaceutical, Spring House, PA) was diluted with sterile saline. Rats were injected with 0.1–0.2 mg of haloperidol per kg 30 min prior to FG7142 injections. In monkeys, 0.005 mg of haloperidol per kg was injected 30 min prior to FG7142 injections.

Clozapine was a gift from the Sandoz Research Institute. For rodent treatments, clozapine (2.5 mg/kg) was dissolved in saline and injected 15 min before FG7142 injections. For monkeys, clozapine (1–6 mg/kg) was mixed with powdered rice and chocolate syrup and fed to the monkey 20 min before injection of FG7142. Although no side effects were noted in rats, individual variation in monkeys was noted in the dose of clozapine which produced impairments when given alone, as well as in the severity of side effects of clozapine when combined with FG7142.

SCH23390 maleate was a gift from Schering, SCH23390·HCl was purchased from Research Biochemicals. For rats, SCH23390·HCl was dissolved in sterile saline and administered (0.035 mg/kg) 15 min before FG7142. As with haloperidol, the motor side effects of SCH23390 were exacerbated when combined with FG7142. However, all animals were able to test without profound motor impairment at the dose of 0.035 mg/kg. For monkeys, 0.0065 mg of SCH23390·HCl per kg or 0.01 mg of SCH23390 per kg maleate (equimolar doses) in sterile saline was injected 30 min before testing. No marked effects on motor coordination of SCH23390, alone or with FG7142, were noted in monkeys.

Biochemistry. Rats used only for biochemical analysis were decapitated 30 min after FG7142 or vehicle administration. In addition, 15 rats were sacrificed immediately after behavioral testing for correlative analysis. Brains were removed and dissected according to Tam and Roth (12). Tissue samples were prepared and analyzed for 3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine levels by HPLC with electrochemical detection according to the methods of Elsworth et al. (27).

Data Analysis. Within-subjects comparisons (paired t test) were employed for delayed alternation and spatial discrimination (rat) and delayed response experiments (monkey). The measures of locomotor activity in rats employed a one-way analysis of variance (ANOVA) to examine drug vs. vehicle over successive 5-min intervals. Biochemical experiments in rats utilized between-subjects comparisons (unpaired t test). Correlative analyses between biochemical and behavioral measures in rats were performed by using the Pearson test. Statistical analyses were performed by using STATWORKS on a Macintosh computer.

RESULTS

Stress Increases Dopamine Turnover in the Prefrontal Cortex and Impairs Behavioral Performance. The pharmacological stressor, FG7142 (20 mg/kg), produced a 150% increase in dopamine turnover in the rodent prefrontal cortex (Fig. 1). In contrast, there was no significant change in the DOPAC/dopamine ratio in the nucleus accumbens or striatum. When FG7142 was administered prior to the delayed alternation (20 mg/kg) or delayed response (0.2 mg/kg) tasks, performance in both the rat and the monkey was significantly impaired when compared with performance in the presence of vehicle (Figs. 2 and 3). Pretreatment with RO-151788, a benzodiazepine antagonist which blocks the stress-induced increase in prefrontal cortical dopamine turnover (12), prevented the delayed alternation deficit induced by FG7142 in the rat (Fig. 2). When given alone, RO15-1788 produced a small but significant impairment of performance in the rat. Pretreatment with RO151788 in the monkey had no effect alone but also attenuated the FG7142-induced cognitive impairment (Fig. 3).

To examine whether animals given FG7142 were impaired as a result of nonspecific changes in behavior, rats were tested...
FIG. 2. Effects of FG7142, RO15-1788, and dopamine antagonists on delayed alternation performance in the rat. FG7142 (FG; 20 mg/kg) produced a significant impairment in the accuracy of response on the delayed alternation task when compared to an animal’s performance on vehicle (VEH; FG vs. VEH, P = 0.001). There was a small but significant impairment of delayed alternation with RO15-1788 (RO; 20 mg/kg) when given alone (RO vs. VEH, P = 0.03). However, pretreatment with RO15-1788 prevented the FG7142-induced impairment of delayed alternation (RO/FG vs. FG, P < 0.001). Haloperidol (HAL; 0.1–0.15 mg/kg) and clozapine (CLZ; 2.5 mg/kg) had no effect on delayed alternation performance when given alone. In contrast, SCH23390 (SCH; 0.035 mg/kg) produced a slight but significant impairment of cognitive performance when given alone (SCH vs. VEH, P = 0.05). Haloperidol, clozapine, and SCH23390 ameliorated the FG7142-associated cognitive impairment when given as a pretreatment (HAL/FG vs. FG, P = 0.004; CLZ/FG vs. FG, P = 0.001; SCH/FG vs. FG, P = 0.002). *, P < 0.001 vs. VEH; **, P < 0.001 vs. FG; ***, P < 0.05 vs. VEH.

on a spatial discrimination task, which has the same motor and motivational requirements as delayed alternation but is not dependent on the prefrontal cortex. In contrast to delayed alternation performance, spatial discrimination was unaffected by FG7142 (Fig. 4). The 0-sec delay condition in delayed response provides a control for examining possible nonspecific effects of FG7142 in monkeys, as this condition does not require spatial working memory. An examination of the 0-sec delay condition found no effect of FG7142 on performance accuracy in monkeys.

In addition, the locomotor effects of FG7142 were examined to determine whether the FG7142-induced impairment might be related to impaired motor abilities. A repeated-measures ANOVA indicated that FG7142 had no effect on the levels of total activity for 30 min following injection.

Dopamine Turnover in the Prefrontal Cortex Correlates with Spatial Working Memory Performance Deficits in the Rat. At the end of the behavioral study, rats were given FG7142 or vehicle, tested on the delayed alternation task, and sacrificed immediately after testing. As with previous biochemical findings, postmortem quantitation of dopamine and DOPAC indicated that FG7142 significantly increased dopamine turnover in the prefrontal cortex. Furthermore, there was a significant correlation between increased dopamine turnover in the prefrontal cortex and impaired accuracy of delayed alternation performance (Fig. 5). Thus, the animals exhibiting the worst performance on the delayed alternation task had the highest levels of dopamine turnover in the prefrontal cortex.

Dopamine Antagonist Pretreatment Prevents FG7142-Induced Cognitive Impairments in the Rat and Monkey. The involvement of excess dopamine in the FG7142-induced prefrontal cortical-dependent cognitive deficits was examined by determining whether dopamine antagonists with three different receptor subtype profiles could block the impairment induced by FG7142 (Figs. 2 and 3).

Delayed alternation performance in rats was protected from FG7142-induced cognitive impairment by pretreatment with low doses of the nonspecific dopamine receptor antagonist and typical antipsychotic drug haloperidol (0.1–0.2 mg/kg). Haloperidol did not impair delayed alternation performance in rats when given alone. However, haloperidol pretreatment blocked the delayed response impairment associated with FG7142. As in rats, haloperidol (0.005 mg/kg) blocked the FG7142-impaired performance in monkeys. As haloperidol impairs delayed alternation performance in monkeys when given alone, additive drug effects cannot account for this improvement.

Delayed alternation performance in the rat was also protected from FG7142-induced cognitive impairment by pretreatment with low doses of the atypical antipsychotic drug clozapine (2.5 mg/kg), a nonspecific dopamine receptor antagonist with 5-HT₂ receptor affinity. The FG7142-associated cognitive deficits in the monkey were also prevented by pretreatment with clozapine (1–6 mg/kg). Clozapine did not impair cognitive performance when given alone in either the rat or the monkey.

The D₄ receptor-selective antagonist SCH23390 (0.035 mg/kg) was also able to attenuate the FG7142-induced cognitive impairment in the rat. In the monkey, SCH23390 (0.01 mg of SCH23390 maleate per kg) ameliorated the cognitive impairment associated with FG7142 administration. In monkeys and rats, SCH23390 treatment produced a significant impairment of performance accuracy when given alone.

DISCUSSION

The pharmacological stressor FG7142 selectively activates the mesofrontal cortical dopamine system and impairs response accuracy on working memory tasks. As expected from previous studies (12, 14, 28), FG7142 treatment in rats pro-
Fig. 3. Effects of FG7142 and dopamine antagonists on delayed response performance in the monkey. FG7142 (FG; 0.2 mg/kg) produced a significant impairment in the accuracy of response on the delayed response task when compared with an animal’s performance on vehicle (VEH; FG vs. VEH, \( P = 0.02 \)). Although there was no change in delayed response performance with RO15-1788 (RO; 0.2 mg/kg) when given alone, pretreatment with RO15-1788 prevented the FG7142-induced impairment of delayed alternation (RO/FG vs. FG, \( P = 0.03 \)). Haloperidol (HAL; 0.005 mg/kg) and SCH23390 (SCH; 0.1 mg/kg) significantly impaired cognitive performance when given alone (HAL vs. VEH, \( P = 0.03 \); SCH vs. VEH, \( P = 0.04 \)). Clozapine (CLZ; 1–6 mg/kg) produced a small nonsignificant impairment in delayed response when given alone. Haloperidol, clozapine, and SCH23390 all ameliorated the FG7142-associated cognitive impairment when given as a pretreatment (HAL/FG vs. FG, \( P = 0.05 \); CLZ/FG vs. FG, \( P = 0.02 \); SCH/FG vs. FG, \( P = 0.04 \)). *, \( P < 0.05 \) vs. VEH; **, \( P < 0.05 \) vs. FG.

Fig. 4. The effect of FG7142 on spatial discrimination in the rat. When given FG7142 (FG; 20 mg/kg), rats were unimpaired in their performance of spatial discrimination, as compared with their performance when given vehicle (VEH).

Fig. 5. The correlation between accuracy of delayed alternation performance in the rat and the ratio of DOPAC to dopamine in the prefrontal cortex. Rats were given vehicle or FG7142 (20 mg/kg) before being tested on delayed alternation, and were sacrificed immediately after testing. Increased dopamine turnover in the prefrontal cortex significantly correlated with impaired performance on the delayed alternation task \( (r = 0.627, P < 0.01) \).
tion in the prefrontal cortex underlies the cognitive deficits produced by FG7142 treatment. This hypothesis is supported by the finding that FG7142-induced cognitive deficits were blocked by pretreatment with dopamine receptor antagonists. Furthermore, the fact that SCH23390 prevents the FG7142-induced cognitive impairment suggests that postsynaptic D1-receptor blockade underlies at least a portion of the amelioration by both haloperidol and clozapine.

The ability of haloperidol, clozapine, and SCH23390 to ameliorate the FG7142-induced cognitive deficit also suggests a dopaminergic mechanism, but it should be noted that these dopamine antagonists have some ability to bind to nondopamine receptors. In addition, FG7142 can alter the activity of other neurotransmitter systems (32, 33). While the involvement of nondopamine systems cannot be ruled out, drugs which target the serotonergic (fluoxetine) and noradrenergic (propranolol) neurotransmitter systems do not appear to alter FG7142-impaired cognitive responses using a delayed alternation paradigm in rats (unpublished results). The interpretation that the cognitive deficits observed in these experiments are due to large increases in dopamine-receptor activation finds support from recent single unit experiments in monkeys. Intracerebroventricular application of moderate doses of the dopamine agonist SKF38393 inhibited the delay-period neuronal activity related to the mnemonic component of a delayed response task (34).

Prefrontal cortical dysfunction is thought to contribute to the symptoms of a number of psychiatric disorders. Patients with schizophrenia, Tourette syndrome, Parkinson disease, and attention defect/hyperactivity disorder exhibit many symptoms of prefrontal cortex dysfunction (5, 35–37). Abnormal dopamine regulation in the prefrontal cortex (7, 38) and hypofrontality (39) have been postulated to be involved in the etiology of schizophrenia. The sensitivity of the prefrontal cortex to stress may underlie part of the sequelae associated with the stress-induced precipitation or exacerbation of psychosis (2–4).

The cognitive changes of Parkinson disease, as well as the hallmark motor impairments, have been linked to dopaminergic-depleted circuits (37, 40, 41). However, in 1-dopa-treated Parkinsonian patients, apomorphine can induce cognitive deficits (42). Consistent with the current study, these findings support the idea that excessive dopamine-receptor stimulation can be detrimental to cognitive function.

Our experimental findings correlating impaired prefrontal cortex cognitive abilities with increased prefrontal cortex dopamine turnover complement previous studies indicating that dopamine depletion impairs prefrontal cortex cognitive function (23). The present study suggests that there may be a critical range of dopaminergic activity for optimal prefrontal cortex-dependent cognitive functioning and that exceeding this range can result in dysregulation and cognitive impairment. These findings highlight the role of dopamine in prefrontal cortex-dependent cognition and may provide new insights into better treatment strategies for psychiatric disorders associated with dysregulation of prefrontal cortical dopamine function.

We give special thanks to Lisa Ciavarella, Tracy White, and J. David Jentsch for their expert assistance. We also thank James Lee for his reliable help. Support for these studies came from National Institutes of Health Grants MH14092 (R.H.R.), MH48866 (P.S.R., A.F.T.A., and R.H.R.), and AG06036 (A.F.T.A.) and from National Science Foundation Fellowship Grant GER9253954 (B.L.M.).