Dose-Dependent Effects of the Dopamine D1 Receptor Agonists A77636 or SKF81297 On Spatial Working Memory in Aged Monkeys

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ABSTRACT

With advancing age, monkeys develop deficits in spatial working memory resembling those induced by lesions of the prefrontal cortex (PFC). Aged monkeys also exhibit marked loss of dopamine from the PFC, a transmitter known to be important for proper PFC cognitive function. Previous results suggest that D1 agonist treatment can improve spatial working memory abilities in aged monkeys. However, this research was limited by the use of drugs with either partial agonist actions or significant D2 receptor actions. In our study, the selective dopamine D1 receptor full agonists A77636 and SKF81297 were examined in aged monkeys for effects on the working memory functions of the PFC. Both compounds produced a significant, dose-related effect on delayed response performance without evidence of side effects: low doses improved performance although higher doses impaired or had no effect on performance. Both the improvement and impairment in performance were reversed by pretreatment with the D1 receptor antagonist, SCH23390. These findings are consistent with previous results demonstrating that there is a narrow range of D1 receptor stimulation for optimal PFC cognitive function, and suggest that very low doses of D1 receptor agonists may have cognitive-enhancing actions in the elderly.

DA has a vital influence on the spatial working memory functions of the PFC. Accumulating evidence indicates that stimulation of PFC DA receptors produces an inverted “U” pattern of response: either insufficient or excessive DA receptor stimulation is detrimental to PFC cognitive function. The importance of DA receptor stimulation to PFC spatial working memory function was first noted by Brozoski et al. (1979), who observed that depletion of DA from the dorsolateral PFC in monkeys markedly impaired spatial working memory performance. Working memory deficits were comparable to those induced by ablation of the PFC, and were ameliorated by replacement therapy with DA agonists (Brozoski et al., 1979). This seminal finding was replicated in rats (Simon, 1981; Bubser and Schmidt, 1990) and marmosets (Roberts et al., 1994) after 6-OHDA lesions of the PFC. Subsequent research demonstrated that working memory deficits also could be induced by acute blockade of DA D1 receptors. Infusions of the selective D1 receptor antagonists SCH23390 or SCH39166 into the PFC of monkeys (Sawaguchi and Goldman-Rakic, 1991) or rats (Seamans et al., 1995) impaired spatial working memory performance, without altering performance of a control task with identical motor and motivational demands but little mnemonic component (Sawaguchi and Goldman-Rakic, 1991). A comparable pattern of impairment was observed after systemic treatment with a D1 receptor antagonist; SCH23390 produced a dose-related impairment in spatial working memory performance in young adult monkeys, but had no effect on performance of “0” sec delay control trials or performance of a fine motor task (Arnsten et al., 1994). Conversely, the full D1 receptor agonist, dihydrexidine, improved working memory performance (Arnsten et al., 1994), underscoring the beneficial effects of DA actions at D1 receptors. Electrophysiological studies in monkeys have indicated that DA may have beneficial actions on PFC pyramidal cells by enhancing cue- and delay-related activity during working memory tasks (Sawaguchi et al., 1988). More recently, in vitro studies of rat PFC neurons have shown that D1 agonist application may facilitate signal transfer from dendrite to the soma by sharpening signals arriving on apical dendrites (Yang and Seamans, 1996).

In contrast to these beneficial DA actions, evidence indicates that excessive DA receptor stimulation in the PFC impairs spatial working memory performance (see Arnsten, 1997 for review). Exposure to environmental or pharmacological...
logical stress preferentially increases DA release and turnover in the PFC (see Deutsh and Roth, 1990 for review), and both environmental stress (Arnsten and Goldman-Rakic, 1990) and pharmacological stress (Murphy et al., 1994; 1996a) induce deficits in spatial working memory. Memory impairment correlated with increased DA turnover in the rodent PFC: rats exhibiting the greatest memory impairment had the largest increase in PFC DA turnover (Murphy et al., 1994; 1996a). Also consistent with a DA mechanism, memory deficits were reversed by pretreatment with D1 and/or D2 DA antagonists (Arnsten and Goldman-Rakic, 1990; Murphy et al., 1994; 1996a), or with agents that prevent the rise in PFC DA turnover (Arnsten and Goldman-Rakic, 1986; Murphy et al., 1996b). Memory impairment in rats also has been observed with ketamine administration, an NMDA noncompetitive antagonist which similarly increases PFC DA turnover (Verma and Moghaddam, 1996). In this study, memory impairment was reversed by D2, but not D1 receptor antagonists, perhaps due to NMDA receptor blockade masking D1 receptor mechanisms. The importance of D1 receptor mechanisms in the PFC has recently been underscored by the finding that bilateral infusion of the D1 receptor agonist, SKF81297, into the rat PFC produced a dose-related impairment in spatial working memory that was reversed by D1 antagonist pretreatment (Zahr h et al., 1996). Electrophysiological studies in awake, behaving monkeys have also shown that iontophoresis of low concentrations of D1 antagonists enhance memory-related neuronal firing (Williams and Goldman-Rakic, 1995). Thus, either insufficient or excessive D1 receptor stimulation impairs PFC cognitive function.

With advancing age, there is a prominent loss of DA and DA metabolites from the PFC (Goldman-Rakic and Brown, 1981; Wenk et al., 1989), and a marked loss of PFC cognitive function (Bartus, 1979; Rapp and Amaral, 1989). Aged monkeys are impaired on tests of spatial working memory such as the delayed response task (Bartus et al., 1978; Rapp and Amaral, 1989; Bachevalier et al., 1991), as well as tests of behavioral inhibition and attention (distractibility) that rely on the PFC (Bartus and Dean, 1979; Rapp, 1990). Interestingly, both PFC cognitive deficits and PFC DA depletion emerge early in the aging process, and progressively worsen with advancing age (Wenk et al., 1989; Bachevalier et al., 1991). Given the importance of D1 receptor mechanisms to PFC functions, it is likely that DA loss contributes to PFC dysfunction in the elderly. This hypothesis is supported by biochemical studies in aged rats, where loss of spatial working memory abilities correlates most strongly with loss of DA metabolites in the PFC (Luine et al., 1990). The importance of catecholamine loss to the aging process is also evident from pharmacological studies of aged monkeys. With advancing age, monkeys begin to show alterations in their response to DA drugs that resemble the changes observed in young monkeys with experimentally induced catecholamine depletion. For example, low doses of the D2 agonist, quinpirole, inhibit DA release and impair spatial working memory in young control monkeys, but do not impair memory in reserpine-treated young monkeys (Arnsten et al., 1995). With advancing age, there is a progressive loss of response to quinpirole, and the oldest monkeys exhibit drug responses similar to reserpine-treated young monkeys (Arnsten et al., 1995). Both old and experimentally depleted monkeys are improved by the partial D1 agonist, SKF38393, although young intact monkeys are not (Arnsten et al., 1994). Importantly, aged monkeys often exhibit a biphasic response to D1 agonist treatment: they are improved by low doses and impaired by higher doses (Arnsten et al., 1994), consistent with an inverted “U” pattern of response. Impairment was particularly prominent with the full D1 agonist, dihydrexidine (Arnsten et al., 1994), which has significant D2 activity as well (Brewster et al., 1990; Mottola et al., 1991).

The finding that D1 agonists can improve working memory function in aged monkeys suggests that this class of drug may have potential clinical utility. The present study characterized the effects of two full D1 agonists, SKF81297 and A77636, on spatial working memory performance in aged monkeys. Unlike dihydrexidine, both SKF81297 (Andersen and Jansen, 1990) and A77636 (Kebaban et al., 1992) are selective for D1 receptors (SKF81297: K_i D1 receptors = 2.2 nM; K_i D2 receptors = >1000 nM; A77636: K_i D1 receptors = 40 nM; K_i D2 receptors = >1000 nM).

Methods

Subjects. The subjects in this study consisted of seven female and two male rhesus monkeys (Macaca mulatta), ranging in age from about 16 to more than 30 years. As actual birth dates were unavailable for most monkeys, ages were estimated on the basis of prior breeding and behavioral testing records, dental records and general appearance. Rhesus monkeys in captivity have been reported to live 20 to 25 yr and occasionally longer (Lapin et al., 1979; Tigges et al., 1988). Animals were housed individually under standard laboratory conditions. Four of the nine monkeys were treated and tested at the Kunming Institute of Zoology, Kunming, China, under the direction of Dr. Cai; the remaining animals were housed and tested at the Yale Medical School, New Haven, CT, under the direction of Dr. Arnsten. Dr. Cai had previously trained at Yale Medical School with Dr. Arnsten, thus ensuring comparable methods between the two institutions.

Delayed response testing. Cognitive testing occurred in a WGTA situated in a sound-attenuating room. Background masking noise (60 dB, wideband) also was used to minimize auditory distractions. Animals were always tested at the same time of day immediately before feeding. Highly palatable food rewards (e.g., peanuts, raisins or chocolate chips) were utilized during testing to minimize the need for dietary regulation. Using these conditions, no problems with motivation were evident.

The monkeys had been previously trained on the two-well delayed response task. During delayed response, the animal watches as the foodwell containing the food reward is raised or lowered. After the interval, reward is presented in either well and the animal is allowed to choose. Reward is randomly distributed between the left and right wells over the 30 trials that make up a daily test session. To observe the effects of drug on memory capacity, the animals were trained on a variable delayed response task. Delays varied between less than 1 sec (“0” sec) and the temporal interval that yielded chance performance for each animal. Five different delay lengths were quasi-randomly distributed over the 30 trials that made up a single test session. For example, the range of delays for aged monkey 30 was “0”, 9, 18, 27 and 36 sec. These delays were termed the A (“0”), B, C, D and E delays, respectively. The mean ± S.E.M. B delay for the aged monkeys was 11.6 ± 2.5 sec. These delays are lower than those needed to produce comparable levels of performance in young adult monkeys testing for approximately the same number of years: mean ± S.E.M. B delay of 27.5 ± 7.5 sec. All monkeys performed near perfectly at “0” sec and had increasing difficulty with progressively longer delays, a pattern that is consis-
tent with memory impairment. Delays were adjusted until the aged animals showed stable baseline performance of approximately 70% correct when collapsed across all five delay intervals. The monkeys were tested twice a week, with 3 to 4 days separating test sessions (e.g., Mondays and Thursdays).

**General.** Changes in arousal and aggression were evaluated during cognitive testing by an experimenter who was familiar with the normative behavior of each animal but was unaware of drug treatment conditions. Sedation and agitation were rated using a nine point scale, where 0 = normal level of arousal, I = quieter than usual, II = sedated (drooping eyelids, slowed movements), III = intermittent sleeping and IV = too sedated to finish testing; -I = more alert than usual, -II = slight agitation, but not sufficient to disrupt testing, -III = agitation disrupting testing and -IV = too agitated to test. Aggression was rated using a 7 point scale, where 0 = normal level of aggression, -I = slightly more aggressive, -II = moderately more aggressive and -III = extremely aggressive; I = slightly more docile, II = moderately more docile and III = very docile.

**Drug administration.** Drug solutions were made fresh each day under aseptic conditions. Animals were injected before every session with either drug or saline vehicle. The experimenter testing or rating the animal was unaware of the drug treatment conditions. SCH23390, SKF81297, and A77636 were diluted in sterile saline; drug or saline was injected intramuscularly 1 hr before delayed response testing. Two animals required 2 hr pretreatment with SKF81297 given the poorer brain penetration of this compound (Abbott Pharmaceuticals, Abbott Park, IL; D. Britton and M. Williams, unpublished data). A77636 was tested in monkeys housed at Kunming (n = 4) and New Haven (n = 1), although SKF81297 was only tested in New Haven (n = 5). Thus, one aged monkey (no. 121) had both A77636 and SKF81297 treatment.

A77636 was generously provided by Abbott Pharmaceutical and SKF81297 and SCH23390 HCl were purchased from Research Biochemicals, Incorporated (Natick, MA).

**Data analysis.** Delayed response performance on drug was compared with matched saline (vehicle) control performance for the same week. As the animals served as their own controls, statistical analyses employed repeated measures designs: one-way analysis of variance with repeated measures (1-ANOVA-R) with user defined contrasts, or paired t test (also called dependent t test or Tdep) for paired comparisons (e.g., SKF81297 vs. SCH23390). The level of significance was P < .05 (two-tailed). Drug treatments that impaired performance were analyzed for effects on "0" sec delay control trials using Tdep test; ceiling effects precluded comparable analysis of treatments which improved performance (2-ANOVA-R for drug and delay interactions could not be used due to the small n). Behavioral rating data were assessed using a nonparametric repeated measures analysis (Wilcoxon test). Statistical analysis was conducted on a Macintosh LC III computer using a statistics package (Systat).

**Results**

A77636. Administration of A77636 to aged monkeys produced a significant effect on delayed response performance [1-ANOVA-R F(3,12) = 7.34, P = .005]. As can be seen in figure 1A, this compound produced an inverted "U" shaped dose-response curve [second degree polynomial contrast: F(1,4) = 19.73, P = .011]. Low doses produced a modest but consistent improvement in performance, although higher doses had no significant effect on performance [user defined contrasts- 0.01 μg/kg vs. saline: F(1,4) = 16.44, P = .015; 0.1 μg/kg vs. saline: F(1,4) = 6.604, P = .062; 1.0 μg/kg vs. saline: F(1,4) = 1.31, P = .32]. The improvement following 0.01 μg/kg was most apparent at the longer delays (fig. 2). Three of the five aged monkeys were impaired by 1.0 μg/kg A77636 (e.g., monkey 3; fig. 1B), performing at or near chance levels of responding. The same pattern of response was observed in monkeys from Kunming (n = 4) and New Haven (n = 1). There were no changes in behavioral ratings of agitation, sedation or aggression following A77636 treatment (all scores 0).

SKF81297. A similar, although weaker pattern was observed after treatment with SKF81297. Pilot studies indicated that the 0.01 μg/kg dose had no effect on behavior (1.5 ± 2.1% change compared to saline control); thus research focused on the 0.1 to 10.0 μg/kg dose range. SKF81297 treatment had a significant effect on delayed response performance [1-ANOVA-R F(3,12) = 3.37, P = .05], producing an inverted "U" shaped dose-response curve (second degree polynomial contrast: F(1,4) = 8.43, P = .04; fig. 3A). Low doses
produced a small but significant improvement in performance, although higher doses tended to impair performance (user defined contrasts- 0.1 μg/kg vs. saline; F(1,4) = 7.53, P = .05; 1.0 μg/kg vs. saline: F(1,4) = 0.9, P = .4; 10.0 μg/kg vs. saline: F(1,4) = 5.23, P = .08). Four of the five aged monkeys were impaired by 10.0 μg/kg SKF81297 (e.g., monkey 446; fig. 3B), each performing only 17 of the 30 trials correct. Further analysis of the deficits induced by SKF81297 demonstrated no effect of drug on performance after “0” sec delay trials (Tdep = 0.4, df = 3, P = .72). These results are consistent with changes in cognitive performance rather than nonspecific performance variable which would be expected to disrupt performance after the “0” sec delay control trials.

There were no significant changes in behavioral ratings of agitation, sedation or aggression after SKF81297 treatment. One aged monkey was given an agitation rating of “-2” (slight agitation) after 0.1 μg/kg, and two aged monkeys were given this rating after 10.0 μg/kg SKF81297 treatment (Wilcoxon: saline vs. 0.1 μg/kg: P = .18; saline vs. 1.0 μg/kg: P = 1.0; saline vs. 10.0 μg/kg: P = .11). One of these animals was also described as “pinker than usual” after SKF81297 treatment. This was the only change in general appearance noted in this experiment.

**Reversal with SCH23390.** The improvement in delayed response performance induced by a very low dose of either A77636 or SKF81297 was significantly reversed by pretreatment with 10.0 μg/kg of the D1 receptor antagonist, SCH23390 (fig. 4). Thus, 0.01 μg/kg A77636 by itself significantly improved performance compared to saline vehicle (11.2 ± 2.7% improvement, Tdep = 4.18, df = 4, P = .014), although in SCH23390-pretreated monkeys A77636 did not significantly improve performance relative to vehicle (2.0 ± 1.4% improvement, Tdep = 1.50, df = 4, P = .11). Similarly the improvement induced by the best dose of SKF81297 (0.1 μg/kg, n = 2; 1.0 μg/kg, n = 1) was significantly reduced by pretreatment with SCH23390 (SKF81297 by itself: 14.3 ± 3.6% improvement relative to saline, Tdep = 4.91, df = 3, P = .039; SKF81297 in SCH23390-pretreated animals: 1.8 ± 3.5% improvement relative to saline, Tdep = .9, df = 3, P = .46). The 10.0 μg/kg (i.e., 0.01 mg/kg) dose of SCH23390 had previously been shown to have no effect on the delayed response performance of aged monkeys when administered on its own (Arnsten et al., 1994).

The impairment in delayed response performance induced by a higher dose (10.0 μg/kg) of SKF81297 was also blocked by 10.0 μg/kg SCH23390 treatment (fig. 5). The 10.0 μg/kg dose of SKF81297 by itself significantly impaired performance relative to saline (-7.7 ± 0.9%, Tdep = 6.33, df = 3, P = .008), but did not impair performance when animals were pretreated with SCH23390. Indeed, pretreatment with SCH23390 lead to a tendency for improved performance relative to saline (6.5 ± 2.7%, Tdep = 2.83, df = 3, P = .066). These findings are consistent with drug actions at DA D1 receptors.
Unfortunately, the same analysis could not be performed for doses which improved performance, due to ceiling effects on the “0” sec delay control trials. Further examination of this issue will require testing low doses of SKF81297 and A77636 on other, non-PFC tasks with similar levels of difficulty.

**Evidence for D1 receptor mechanisms.** The effects of A77636 and SKF81297 were blocked by D1 receptor antagonist pretreatment, consistent with a D1 receptor mechanism. Both the improvement in delayed response performance induced by low doses, and the impairment in performance produced by the higher SKF81297 dose, were blocked by SCH23390 pretreatment. The reversal of the higher dose SKF81297 response was of particular interest, as some monkeys exhibited improved rather than impaired performance after the combined drug treatment. These data suggest that SCH23390 treatment effectively lowered the dose of SKF81297 into the beneficial range. Further evidence for D1 receptor mechanisms arises from the similar response patterns observed for the four D1 receptor agonists examined in this paradigm to date: SKF38393, dihydrexidine, SKF81297 and A77636 all produce an inverted “U” shaped dose-response curve (Arnsten et al., 1994; current study). The most prominent impairment in delayed response performance has been observed with dihydrexidine, perhaps due to its additional D2 receptor actions. The results of our study suggest that more selective D1 full agonists may be better candidates for cognitive enhancers in humans.

**Role of PFC.** Our study was limited to systemic drug administration; however, additional evidence suggests that changes in delayed response performance may involve drug actions in the PFC. In rats, infusion of higher doses of SKF81297 (0.01–0.1 μg/0.5 μl) into the PFC produced a dose-related impairment in spatial working memory that was reversed by SCH23390 pretreatment (Zahrt et al., 1996). These results are similar to those found in our study in monkeys, where higher systemic doses of SKF81297 produced a SCH23390-reversible impairment in spatial working memory performance. Further research is needed to determine whether infusing low doses of SKF81297 into the rodent PFC will improve performance, as has been observed with systemic administration of low doses of D1 agonists in aged monkeys, and with dihydrexidine treatment in young monkeys (Arnsten et al., 1994). Unlike DA D1 receptor antagonists (Sawaguchi and Goldman-Rakic, 1991), D1 receptor agonists have not been infused in the young or aged monkey PFC. These experiments would be needed to provide definitive evidence of D1 agonist actions in the PFC. However, given that DA depletion or D1 receptor blockade in the PFC impairs working memory performance (Brozoski et al., 1979; Sawaguchi and Goldman-Rakic, 1991), it is likely that low dose D1 agonist infusion into the PFC would improve performance in monkeys with PFC DA depletion. Most importantly, the inverted “U” dose/response seen with D1 agonist treatment in aged monkeys underscores the recent findings that either too little or too much DA D1 receptor stimulation is detrimental to PFC cognitive function (Arnsten and Goldman-Rakic, 1990; Arnsten et al., 1994; Murphy et al., 1994; Williams and Goldman-Rakic, 1995; Murphy et al., 1996a; Zahrt et al., 1996).

**Clinical relevance.** Studies of human aging indicate that PFC cognitive deficits contribute prominently to age-related cognitive decline (see Hochanadel and Kaplan, 1984 or West,

**Discussion**

Both A77636 and SKF81297 produced inverted “U” shaped dose-response curves: improving performance after lower doses and impairing or having no effect on performance with increasing dose. SKF81297 was less potent than A77636 by an order of magnitude, perhaps due to the better brain penetration of A77636. Unlike D2 receptor agonists, neither A77636 nor SKF81297 produced significant side effects in the dose range examined.

It is likely that A77636 and SKF81297 altered performance by effecting cognitive functioning rather than nonspecific performance variables. For example, the impairment in performance after high dose SKF81297 administration was not evident on “0” sec delay control trials with little memory impairment. Nonspecific changes in motivation or motor performance should be reflected in performance of these trials.
1996 for reviews). As with monkeys, deficits on PFC tasks such as the Wisconsin Card Sort or Stroop begin early in the aging process, and become pronounced in advanced age (Davis et al., 1990). Biochemical studies of DA levels in the aged human cortex are not reliable, due to the very low levels of DA in cortex, and the problems associated with use of postmortem tissue. However, neuropathological studies have demonstrated loss of DA cell bodies (McGeer et al., 1977). In vivo imaging studies have shown a 40 to 50% loss of striatal DA reuptake sites over the lifespan in humans (van Dyck et al., 1995). Unfortunately, these measures are not sufficiently sensitive to detect the low levels of cortical DA sites. Imaging methods have been able to visualize cortical D1 receptors, and these studies have shown evidence of age-related decline in SCH23390 binding sites in the PFC (de Keyser et al., 1991; Suhara et al., 1991). This loss of receptor may reduce the substrate for D1 receptor agonist actions. However, even the oldest monkey in our study (no. 121, estimated to be ≥ 35 yr of age) showed improvement with D1 agonist treatment, indicating that D1 receptor loss with age may not be a limiting factor.

Although most research on D1 agonists such as dihydrexidine and A77636 has focused on their potential as treatments for Parkinson’s disease (Taylor et al., 1991; Kebeanian et al., 1992; Schneider et al., 1994a), the results of our study, as well as previous research in monkeys (Taylor et al., 1991; Arnsten et al., 1994; Schneider et al., 1994a), suggest that this class of agent may also have use as cognitive enhancers. DA medications can improve cognitive performance in Parkinson’s patients, although, as with our study, higher doses can further improve performance (Gotham et al., 1988). Interestingly, D1 but not D2 agonists have been shown to improve accuracy of cognitive performance in MPTP-treated monkeys (Schneider et al., 1994a, 1994b). In aged monkeys, both D1 and D2 agents can improve working memory performance, but the D2 agonist, quinpirole, additionally induced prominent side effects (e.g., agitation, hypotension, “hallucinatory-like” behaviors) that would preclude clinical use (Arnsten et al., 1995). In contrast, D1 agonists improve cognitive performance with few apparent side effects (Arnsten et al., 1994; current study). This profile recommends this class of drug for further research. However, the development of cognitive impairment at higher doses cautions that clinical studies should target the very low dose range.

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References


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