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A-412997, a selective dopamine D_4 agonist, improves cognitive performance in rats

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Abstract

The recent development of a highly selective dopamine D_4 receptor agonist, A-412997 (2-(3′,4′,5′,6′-tetrahydro-2′*H*-[2,4′] bipyridinyl-1′-yl)-*N*-*m*-tolyl-acetamide), has provided a pharmacological tool with which to conduct systematic investigations into the putative role for dopamine D_4 receptors in the central nervous system. These present studies evaluated the potential cognitive enhancing properties of A-412997 in rat models of ADHD (5-trial repeated acquisition inhibitory avoidance in Spontaneous Hypertensive Rat pups) and short-term memory (Social Recognition), in comparison with the less selective dopamine D_4 receptor agonists PD168077 and CP226269. A-412997 showed significant dose-dependent efficacy in both models. PD168077 repeatedly improved acquisition in the 5-trial inhibitory avoidance model but failed to reach significance at any dose tested, although significantly improved social recognition was observed (albeit less potent than A-412997). CP226269 showed a significant enhancement in the 5-trial inhibitory avoidance model. These results support a role for the dopamine D_4 receptor subtype in cognition.

Keywords: A-412997; CP226269; PD168077; Social recognition; 5-trial inhibitory avoidance

1. Introduction

Dopamine (the major catecholaminergic neurotransmitter in the central nervous system) has been implicated in a number of behaviors including: cognition, motor coordination, reward, and recently, sexual function (Brioni et al., 2004; Grace, 2002). While initial research efforts focused primarily on movement disorders, it is now generally appreciated that the dopaminergic system plays a role in many disorders, including: addiction, schizophrenia, Parkinson's disease, and attention deficit hyperactivity disorder (ADHD). Given the implication of cognitive processes associated with or underlying aspects of these disorders, it is important to understand the relationship between dopaminergic innervation and cognitive capacities (Nieoullon, 2002).

Part of the difficulty in fully elucidating a role for dopamine in cognitive functioning, is that there are a number of different receptor subtypes; to date, 5 distinct subtypes have been identified, members of the dopamine D_1-like family (D_1 and D_5 receptor subtypes) and the dopamine D_2-like subtypes (D_2, D_3, and D_4). In view of the high expression of D_4 receptors in the cortex as well as localization to the hippocampus and hypothalamus (Ariano et al., 1997; Khan et al., 1998), it is of interest to investigate the role of D_4 in cognition.

The dopamine D_4 receptor subtype has been implicated in schizophrenia, ADHD, and may play a role in reactions to novelty (Falzone et al., 2002; Hrib, 2000; Powell et al., 2003; Tarazi and Baldessarini, 1999). Results generated to date, however, with available dopamine D_4 receptor agonists range from suggesting no consistent effects on behavior or associative learning (Clifford and Waddington, 2000; Nayak and Cassaday, 2003), to improving memory consolidation (Bernaerts and

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Tirelli, 2003). The recent development of the highly selective dopamine D_4 receptor agonist A-412997 (2-(3′,4′,5′,6′-tetrahydro-2′*H*-[2,4′] bipyridinyl-1′-yl)-*N*-*m*-tolyl-acetamide), however, has provided a tool with which to systematically investigate the putative role for dopamine D_4 receptors in cognitive processes (Moreland et al., 2005).

To investigate the putative efficacy of A-412997 for the treatment of cognitive disorders, we first selected the 5-trial repeated acquisition inhibitory avoidance model in spontaneously hypertensive rat (SHR) pups (Fox et al., 2002). Since there is no one definitive animal model of ADHD, the SHR was selected, as rats from this strain exhibit increased activity and impulsivity in novel environments, habituate more slowly to inappropriate stimuli, and exhibit impaired sustained and selective attention (Boix et al., 1998) for review, see (Sagvolden, 2000). The SHR is recognized as being a useful model of ADHD by many researchers since these impairments are genetically-based and are not the result of pharmacological or surgical intervention. In addition, we compared these effects of A-412997 on the 5-trial inhibitory avoidance model to the less selective dopamine D_4 receptor agonists CP226269 and PD168077. Finally, to assess broader effects on cognition, we profiled A-412997 and PD168077 in a social memory test, based on the recognition of a juvenile rat by a male adult rat (Carr et al., 1976).

2. Materials and methods

2.1. Animals

Male SHR pups were used in cognition studies according to methods published previously (Fox et al., 2002; Komater et al., 2003). Briefly, rats were obtained from Harlan (Madison, WI, USA) at post-natal day 7 and housed in Abbott facilities until use at post-natal days 20–25. Pups outside of a 35–50 g body weight range were excluded from testing. Pups were housed up to 12 per cage (2 litters combined) and fostered with Long–Evans lactating females, to avoid the poor maternal care of SHR females and possible associated effects on brain and cognitive development (Fox et al., 2002). Male Sprague Dawley rats from Charles Rivers (Portage, MI, USA) were used in the social recognition and locomotor activity experiments. This strain was selected based on previous work in our laboratories profiling this strain in the social recognition model (Fox et al., 2003). Adults weighed 370–500 g, and juveniles weighed 70–120 g at the time of testing. Adult male Wistar rats (weighing 175–200 g upon arrival; Charles River Laboratories, Portage, MI, USA) were also used to test for non-specific effects on locomotor activity; a strain selected for elucidating a role for D_4 receptors in penile erections (see companion paper by Moreland et al., 2005). All animals were housed in a quiet room under conditions of 12 h lights on/12 h lights off (on at 06:00 am) with food and water available ad libitum. Studies were conducted between 08:00 h and 16:00 h and treatment groups were arranged for equal representation of time of day. All experiments were conducted in accordance with Abbott Animal Care and Use Committee and National Institutes of Health Guide for Care and Use of Laboratory Animals guidelines in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

2.2. Chemicals

A-412997 (Moreland et al., 2005), PD168077 and CP226269 (Matulenko et al., 2004) were synthesized at Abbott Laboratories. Clozapine (Tocris Cookson Inc., Ellisville, MO, USA) was dissolved in acetic acid, pH ≈ 6.0 with 1 N NaOH, and brought to a final volume in 0.9% saline. Clozapine was injected intraperitoneally (i.p., in a volume of 5 mL/kg). A-412997, PD168077 and CP226269 were dissolved in a 0.1% ascorbic acid solution and injected i.p. (adult rats) or subcutaneously (s.c.; rat pups) in a volume of 1 (adults) or 5 (pups) mL/kg. Doses presented are μmol/kg for comparison across drugs.

2.3. 5-Trial inhibitory avoidance in SHR pups

Training methods were according to those previously described (Fox et al., 2002). Briefly, pups were placed into a brightly lit (247.3 lx) compartment (14 cm L × 14 cm B × 14 cm H) of a computer-controlled Gemini inhibitory avoidance apparatus (San Diego Instruments, San Diego, CA, USA) 30 min following drug or vehicle injection. After transfer through an open door to a larger (25 cm L × 21 cm B × 17 cm H), dark compartment (1.1 lx), the door closed automatically and a 0.1 mA scrambled, square wave current was applied to a 31 bar stainless steel grid floor (bar diameter ≈ 0.3 cm, distance between bars ≈ 0.5 cm) for 1 s by a precision regulated small animal shocker (Coulbourn Instruments, Allentown PA, USA). The pup was then removed and returned to its home cage and littermates for 1 min. After recording the transfer latency, the floor and walls of the compartments were cleaned and the same pup was then returned to the bright compartment. This process was repeated for a total of 5 trials. A 60 s cut-off time applied for trial 1 and a 180 s criterion was used for all other trials. Animals exceeding 60 s on trial 1 were eliminated from the study. For all other trials, if the animal did not enter within 180 s this time was noted, and the pup was removed and treated as described above without receiving a footshock. An oscilloscope (Hitachi V-212, 20 MHz) and a 100 kΩ resistor were used to ensure correct calibration of the equipment in producing this footshock.

2.4. Social recognition testing

Rats under investigation were placed in a dimly lit experimental room and allowed to acclimatize for at least 1 h before testing. Observations were carried out in a clean cage with similar bedding material to the home cage. Following acclimation, the adult rat was placed into the test cage. After 30 min, a juvenile rat was placed into the test cage with the adult rat for 5 min. The time the adult spent exploring (sniffing, grooming, close following) the juvenile during this test session was recorded, and defined as the first investigation duration. The juvenile was then removed from the test cage, and the adult rat was injected with the vehicle or drug, and then placed into its home cage. Following a further 90 min, the adult was placed back into the same test chamber, for a second 30-min habituation. Following this second habituation the same juvenile (familiar) was again placed into the test cage for a 5-min test session; the time spent exploring the juvenile during this test session was defined as the second investigation duration. Vehicle treated rats do not remember the familiar juvenile following this two hour delay. Immediately following the second investigation the familiar juvenile was removed, and a novel juvenile (housed in a different home cage to eliminate any odor similarity) was introduced for the third investigation testing (unfamiliar juvenile). The amount of time the adult rat explored the juvenile during the third investigation was recorded for 5 min. The purpose of the third investigation was to assess the potential for non-specific effects of the compound.

2.5. Activity testing

Locomotor activity was assessed in one of 16 acrylic open field environments (42 cm L × 42 cm B × 40 cm H; Piper Plastics Inc, Libertyville IL, USA) situated inside Versamax/Digiscan activity monitors. Each monitor was equipped with 32 horizontal and 16 vertical infrared sensors (Accuscan Instruments, Columbus OH, USA), and located in a darkened room. Rats were brought into the testing room in their home cage, allowed to habituate for 30 min, and then were tested in two independent procedures: habituated and non-habituated. For the habituated procedure, rats were placed into activity chambers and left undisturbed for 30 min. Following acclimation to the activity chambers, rats were injected with the appropriate substance and placed back into the chamber at which point their locomotor activity was monitored. For the non-habituated procedure rats were injected and immediately placed into the activity chamber and distance traveled (in cm) was recorded. Locomotor activity for both paradigms was recorded for 60 min in 1 min intervals. To assess the specific contributions of the dopamine D_4 receptor in the habituated procedure, rats were injected with the preferential D_4 receptor antagonist, clozapine, 30 min prior to habituation to the locomotor activity chambers.

2.6. Statistical analysis

Individual Mann–Whitney U tests (due to the trial truncation at 180 s the data are non-parametric, and thus one of the assumptions of an ANOVA are not met) were used to compare performance in the 5-trial inhibitory avoidance task on data summed across trials 2–5. For social recognition, data were calculated using an investigation ratio: [investigation duration during the second or third contact (familiar or unfamiliar juvenile)/investigation duration during the first contact]. A one-way analysis of variance (ANOVA) followed by post hoc Fisher's comparisons was used to determine significant changes in the investigation ratio in drug treated animals compared to vehicle treated controls in the social recognition paradigm. Locomotor activity data were analyzed using either a two-way (for the combination study) or a one-way analysis of variance (ANOVA) with treatment as the factor. Significant effects as indicated by the ANOVA were investigated using Dunnett's post hoc analyses. For all analyses significance was set at $p < 0.05$.

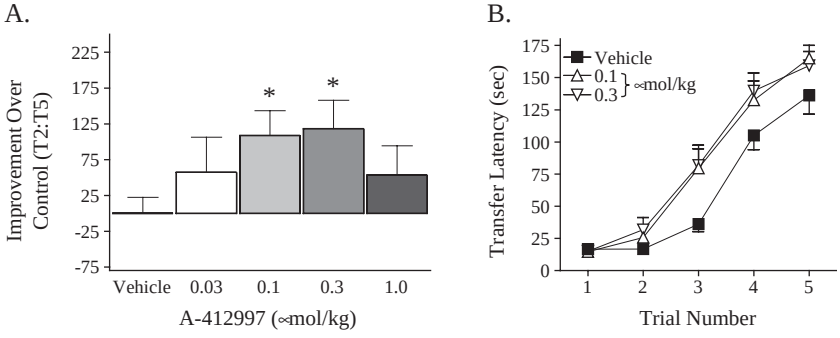

Fig. 1. Effects of the dopamine D_4 receptor agonist A-412997 ($N = 16–17$) in SHR pups on acquisition in the 5-trial repeated avoidance model. Panel A illustrates the data for trials 2–5 (learning trials) summed and expressed as improvement over control. Panel B depicts the response over the five trials (for clarity only the efficacious doses, as per panel A, are depicted). A-412997 significantly improved acquisition in a dose-dependent manner, reaching significance at 0.1 and 0.3 μmol/kg relative to controls. All data are represented by mean ± S.E.M. for clarity: statistical calculations used non-parametric analyses (*$p < 0.05$ compared with vehicle-treated controls).

3. Results

3.1. 5-Trial inhibitory avoidance in SHR pups

Treatment of SHR pups with A-412997 significantly improved acquisition in the 5-trial repeated acquisition model when compared with vehicle-treated littermates. This was exemplified by the prolonged latency to transfer from the brightly lit to the dark compartment (Fig. 1). Panel B illustrates the response over the five learning trials, while panel A depicts the response summed over Trials 2–5 and expressed as improvement over control performance. There was a dose dependent increase in response (Fig. 1B) compared to the vehicle treated rats, reaching significance following the administration of both 0.1 and 0.3 μmol/kg. Similarly, SHR pups treated with 0.01 and 0.1 μmol/kg CP226269 showed a significant increase over control treated pups (Fig. 2A), although the dose response was more variable compared to A-412997. The administration of PD168077 produced consistent increases in transfer latencies that did not reach significance in this model (Fig. 2A; this study was conducted twice with the same result; data from the second experiment are shown).

3.2. Social recognition memory

A-412997 and PD168077 were profiled in the social recognition model of short-term social memory. The administration of A-412997 shortened the second investigation time (Fig. 3A), as indicated by a significant reduction in investigation ratio during time spent with the familiar juvenile, demonstrating improved memory in these rats relative to vehicle treated rats [$F(2,31)=4.084$, $p<0.05$, significant post hoc analyses]. There were no significant effects of A-412997 on the investigation ratio of the unfamiliar juvenile, indicating that the compound produced specific effects on social memory and not behavior in general [$F(2,31)=1.59$, $p=0.22$].

Similarly, PD168077 also significantly reduced the amount of time the adult rat investigated the familiar juvenile (Fig. 3B), although only at the highest dose administered, 3.0 μmol/kg [$F(3,59)=16.85$, $p<0.001$]. When an unknown juvenile rat was exposed to the adult rat, PD168077 did not influence the investigation time, thus demonstrating the specificity of the effects of PD168077 for cognitive components of the test [$F(3,59)=2.05$, $p=0.12$].

3.3. Locomotor activity

A-412997 was profiled in locomotor assays (see Table 1). There were no significant effects on activity in the non-habituated paradigm. In contrast, when rats were allowed to habituate to the activity chambers for 30 min before injection, there was a significant increase following the administration of 3 and 10 μmol/kg [$F(5,59)=9.39$,

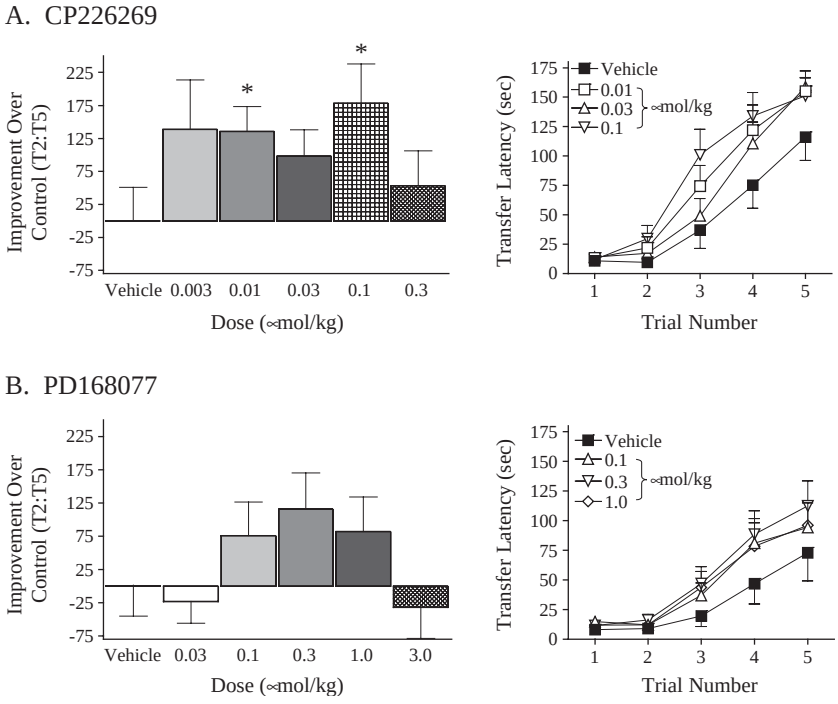

Fig. 2. Illustrates the response of SHR pups to administration of CP226269 (A, $N=9-11$/group) or PD168077 (B: $N=12-14$/group) on acquisition of the 5-trial repeated acquisition inhibitory avoidance task. The right panels show the summed response for trials 2–5 expressed as improvement over control while the left panels depict the response across trials (for clarity only some doses are depicted). CP226269 significantly improved acquisition at doses of 0.01 and 0.1 μmol/kg (2A, left panel), while no significant effects were observed following the administration of PD168077 (B). All data are represented by mean±S.E.M. for clarity: statistical calculations used non-parametric analyses (*$p<0.05$ compared with vehicle-treated controls).

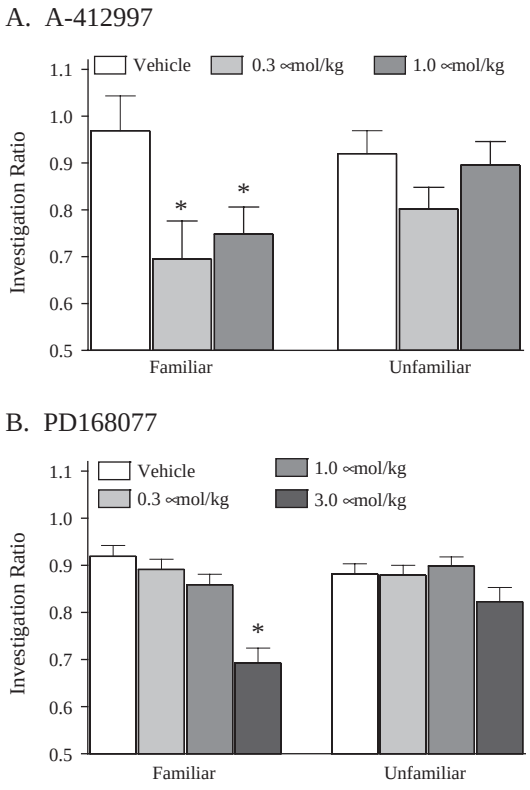

Fig. 3. Effects of A-412997 (A; $N=11-12$/group) and PD168077 (B; $N=15-16$/group) on short-term, social recognition memory. Plotted is the investigation ratio (mean±S.E.M.) for both familiar (investigation time during the second exposure/investigation time for the first exposure) and unfamiliar (investigation time for the third exposure/investigation time for the first exposure) juveniles. There was a significant effect of A-412997 (top panel) on the investigation of the familiar juvenile following the administration of 0.3 and 1.0 μmol/kg, indicating improved memory of the adult for the juvenile over controls. In contrast, there were no non-specific effects, as indicated by a lack of significance in investigation ratios for the unfamiliar juveniles. Similar specific effects were observed following the administration of 3.0 μmol/kg PD168077 (bottom panel). *p <0.05 compared with vehicle-treated controls.

$p<0.001$]. In order to assess whether the observed hyperactivity in habituated rats following the administration of 3 and 10 μmol/kg was specific to rat strain, Wistar and Sprague Dawley rats were tested in the same experiment following the administration of both doses of A-412997 (Table 2). The administration of both 3 and 10 μmol/kg A-412997 increased locomotor activity in both strains [Wistar rats, $F(2,28)=5.62$, $p<0.001$; Sprague Dawley rats, $F(2,29)=11.36$, $p<0.001$] tested side-by-side in the same experiment. Finally, to assess the contribution

Table 2
Effects of A-412997 on locomotor activity in habituated Wistar or Sprague Dawley rats

Dose (μmol/kg)	0	3	10
Wistar	616.89 (±173.17)	1754.00* (±432.29)	2331.30* (±395.34)
Sprague Dawley	147.9 (±89.77)	1237.30 (±188.37)	1575.2* (321.62)

Data are presented as mean (±SEM) collapsed across a 60-minute test session.
* $p<0.05$ compared with same strain vehicle-treated controls.

of the dopamine D4 receptor to the hyperactivity observed following the administration of 10 μmol/kg of A-412997 in habituated Wistar rats, animals were pretreated with clozapine (0, 1, 3 or 10 μmol/kg) 60 min prior to the administration of A-412997 or vehicle (Table 3). A two-way ANOVA conducted on these data indicated a significant main effect of treatment [$F(1,3)=16.744$, $p<0.0001$], no significant effect of clozapine pretreatment [$F(3,3)=0.56$, $p=0.65$], and a trend towards a significant interaction [$F(3,110)=2.29$, $p=0.08$]. To identify the groups specifically differing from rats receiving a vehicle treatment and pretreatment, a one-way ANOVA was conducted [$F(7,117)=3.59$, $p<0.01$] indicating a significant enhancement in rats pretreated with vehicle and treated with A-412997. A dose of 1 μmol/kg of clozapine was not sufficient to block this enhanced response, while 3 and 10 μmol/kg of clozapine attenuated the observed hyperactivity following an acute administration of A-412997.

4. Discussion

These studies evaluated the potential cognitive enhancing properties of the selective dopamine D_4 receptor agonist, A-412997, in rat models of attention/impulsivity (5-trial repeated acquisition inhibitory avoidance in SHR pups) and short-term memory (social recognition), and compared the results obtained with the administration of known (Hrib, 2000), but less selective, dopamine D_4 receptor agonists CP226269 and PD168077. A-412997 is a selective dopamine D_4 receptor full agonist (fluorometric imaging plate reader (FLIPR) functional assay $EC_{50}=28.4\pm1.9$) that binds with high affinity to rat dopamine D_4 and human dopamine $D_{4.4}$ receptors ($K_i=12.1$ and 7.9 nM, respectively; (Moreland et al.,

Table 1
Effects of A-412997 on locomotor activity in habituated or non-habituated Wistar rats

Dose (μmol/kg)	0	0.1	0.3	1.0	3.0	10.0
Habituated	205.5 (±78.6)	410.6 (±130.9)	289.1 (±81.69)	640.7 (±235.5)	1061* (±24.5)	1536* (184.1)
Non-habituated	3820 (±471.7)	3744 (±466.3)	2394 (±371.1)	3474 (±656.1)	3928 (±505.8)	4143 (±597.1)

Data are presented as mean (±SEM) collapsed across a 60-minute test session.
* $p<0.05$ compared with vehicle-treated controls.

Table 3
Effects of pretreatment with clozapine on the locomotor response to A-412997 in habituated Wistar rats

Clozapine dose (μmol/kg)	0	1.0	3.0	10.0
Vehicle	802.07 (±247.47)	658.4 (±199.42)	1022.00 (±231.96)	1012.80 (±370.52)
A-412997 (10 μmol/kg)	2043.64* (±201.89)	2021.73* (±303.38)	1504.80 (±340.98)	1147.97 (±263.97)

Data are presented as mean (±SEM) collapsed across a 60-minute test session.
* $p < 0.05$ compared with vehicle-vehicle treated controls.

2005). A-412997 did not bind to other dopamine receptors or proteins in a panel of seventy different receptors and channels [greater than 300-fold selective over other dopamine receptors, and 125-fold over 5-HT_{1A} receptors; (Moreland et al., 2005)]. Furthermore, while CP226269 and PD1688077 also bind with high affinity to rat D_4 receptors (K_i=1.0 and 6.1, respectively) and act as full agonists (EC_{50} in rat D_4 FLIPR assay 28.9±6.7 and 26.1±3.4, respectively), these compounds are not selective for the dopamine D_4 receptor. CP226269 has additional full agonist properties at the rat dopamine D_{2L} receptor while PD168077 has the potential to interact at α adrenoceptors and 5-HT_{1A} receptor subtypes (Moreland et al., 2005).

The current data expand upon previous reports of efficacy with dopamine D_4 receptor agonists in models of cognition (Nayak and Cassaday, 2003), and clarify the role of the dopamine D_4 receptor by demonstrating significant effects following the administration of the specific and highly selective dopamine D_4 receptor agonist A-412997. In the 5-trial inhibitory avoidance model, A-412997 showed robust dose-dependent improved acquisition compared to vehicle controls. CP226269 also showed significant effects, although the response was not dose-dependent, and the administration of PD168077 to rat pups tested in this model produced a non-significant improvement (two independent studies were conducted with similar results; for clarity only one is presented here). A-412997 and PD168077 were further profiled in an adult rat model of social memory. Administration of A-412997 or PD168077 significantly improved social memory for the familiar juvenile (relative to vehicle controls), although A-412997 was more potent. While all 3 compounds demonstrated positive effects on cognitive function, they did so with apparent differences in efficacy. It can be difficult to dissect the differential contributions of receptor profiles in compounds that have multiple activities, but that A-412997, with the cleanest profile, showed the most robust and consistent results of the compounds tested here, supports the contention that dopamine D_4 receptors play a role in processes involved in cognition relevant to ADHD.

The improved cognitive function observed following the administration of A-412997 in both the 5-trial inhibitory avoidance paradigm and the social recognition model appear to be specific to the cognitive domains tested, as indicated by a lack of effect on locomotor activity in non-habituated rats. It is interesting to note that significant effects on locomotor activity were observed in both Wistar and Sprague Dawley rats habituated to the testing chamber before testing, although this observed hyperactivity was at doses much higher than those required for efficacy in cognition models. The profile of this hyperactivity was an increase in the beginning of the testing session, with values approaching vehicle controls by the end of the hour session (data not shown). The increases in locomotion observed in rats treated with high doses of A-412997 were blocked by the high affinity D_4 antagonist, clozapine (Van Tol et al., 1991). Although clozapine has high affinity for the dopamine D_4 receptor subtype, clozapine binds with high affinity to multiple neurotransmitter receptors (Wilson et al., 1998). The selective antagonist, A-381393 (30 μmol/kg), reported to block the effects of PD89211 on penile erection (Nakane et al., 2005), was not sufficient to block the hyperactivity elicited by the administration of A-412997 (data not presented). Given the relatively high dose of A-412997 needed to elicit hyperactivity (3 and 10 μmol/kg) compared to the low doses of dopamine D_4 receptor agonists required for eliciting penile erections (in the same range as those efficacious in cognition models), it is not clear whether 30 μmol/kg of A-381393 would be sufficient to overcome the effects of A-412997. Thus, while it is not possible to conclusively demonstrate the relative contributions of different receptor subtypes to the observed increases in locomotion, it is clear that the doses required to elicit the observed increases in activity are much greater than those required for efficacy.

Furthermore, in the social recognition model no effects of A-412997 were observed in the investigation ratio for an unfamiliar juvenile. Were the compound having effects on investigation times and not on recognition memory, the time spent investigating the unfamiliar juvenile would be significantly altered relative to vehicle treated rats. In a similar respect, it would be reasonable to expect any non-specific effects of A-412997 in the 5-trial inhibitory avoidance model to be observed on trial one. Thus, the current data support previous research dissociating D_4 receptor-mediated hyperactivity from cognitive effects (Nayak and Cassaday, 2003).

Recent literature reports have supported a role for dopamine D_4 receptors in animal models of ADHD, particularly for the hyperactive component. For example, rats receiving neonatal lesions with the neurotoxin 6-hydroxydopamine (6-OHDA) exhibit a hyperactive phenotype, hypothesized to mimic aspects of human ADHD. Administration of the reported dopamine D_4 antagonists CP-293019 (Zhang et al., 2001), U-101958, L-745870, and S-18126 (Zhang et al., 2002), which exhibit some affinity for potential targets other than the dopamine D_4 receptor, attenuate the hyperactivity normally present in these rats. Data in dopamine D_4 receptor deficient mice receiving a

neonatal 6-OHDA lesion did not demonstrate the hyperactive phenotype observed in wildtype control mice, again supporting the role of dopamine D_4 receptors in hyperactivity (Avale et al., 2004). These data combined have led to the notion that dopamine D_4 antagonists reverse locomotor hyperactivity elicited by a neonatal neurotoxic lesion of the dopaminergic system.

While both knockout mice and 6-OHDA lesion paradigms are intriguing models of ADHD, the studies using these models do not address cognitive aspects of the disorder, and it is not necessarily surprising that different results are generated depending on the tool employed. For example, 6-OHDA lesions affect dopamine projections widely, and are not restricted to the dopamine D_4 receptor subtype. Furthermore, the hyperactivity observed in these studies was transient, as was the ability of the dopamine D_4 receptor antagonists to attenuate the increased behavioral response (Zhang et al., 2002). In addition, the antagonists administered (Zhang et al., 2002) have activity with serotonergic receptors and sigma sites, making it difficult to conclusively demonstrate that effects were due to blockade of the dopamine D_4 receptor. While the null mutant for the dopamine D_4 receptor lacks expression of the dopamine D_4 receptor, the effects of developmental compensation are not clear. Indeed, associated with a lack of dopamine D_4 receptor in null mutant mice was an increase in binding levels of D_1 receptors in the caudate (an area involved in locomotor behavior), implicating compensatory actions of the dopamine D_1 receptors in studies using dopamine D_4 receptor knockout mice (Gan et al., 2004).

There is no one definitive model of ADHD or any other disorder in which cognitive deficits are an important factor. The SHR, however, demonstrates all of the behavioral characteristics of ADHD: hyperactivity, impulsivity, and problems with sustained attention (Sagvolden, 2000). These impairments are genetically based, and are not the result of surgical or pharmacological manipulation. Methylphenidate, one of the treatments of choice for ADHD (Fox et al., 2002) and ABT-418, a nicotinic acetylcholine receptor agonist reported to have positive effects in adult ADHD in a phase II clinical study (Wilens et al., 1999) improve performance of SHR juveniles in the 5-trial inhibitory avoidance model at plasma concentrations similar to those efficacious in the clinic (unpublished observations), providing predictive validity for this model. There are a number of hypotheses for why the SHR demonstrates so many of the components of ADHD, but similar to humans with ADHD, there appear to be deficits in dopamine, glutamate, and norepinephrine neurotransmission. Thus, the cognitive enhancing effects of A-412997, in a model of ADHD, is direct evidence that dopamine D_4 receptor agonism might have a beneficial role in the treatment of this disorder.

The high affinity of clozapine for the dopamine D_4 receptor and the atypical antipsychotic profile of this compound contributed to a hypothesized role of the dopamine D_4 receptor in the pathophysiology of schizophrenia (Hrib, 2000; Wong and Van Tol, 2003a,b). To date, antagonists at this receptor have not been successful in clinical trials (Kramer et al., 1997). It is not clear, however, what agonists at this receptor site would do. Given the improvements in cognitive function observed here, and given the current hypothesis that cognitive disturbances observed in schizophrenics are a component of the disorder, it is tempting to speculate that dopamine D_4 receptor agonists could improve cognitive function in these patients, without the debilitating side effects found with other approaches.

In summary, while a role for the dopamine D_4 receptor has yet to be conclusively linked to a disease state, the dopamine D_4 receptor has been suggested as a candidate gene for ADHD (Faraone et al., 2001; Grady et al., 2003; LaHoste et al., 1996; Swanson et al., 1998). The development of a potent and highly selective agonist, such as A-412997, is an important tool with which to further address these hypotheses. Taken together, these data generated with A-412997 suggest a direct role for dopamine D_4 receptor agonists in ameliorating attention/impulsivity deficits in a model of ADHD. Further, effects in improving baseline social memory suggest that efficacy of dopamine D_4 receptor agonists may extend across additional cognitive domains.

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