Mutation of the α_{2A}-Adrenoceptor Impairs Working Memory Performance and Annuls Cognitive Enhancement by Guanfacine

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Norepinephrine strengthens the working memory, behavioral inhibition, and attentional functions of the prefrontal cortex through actions at postsynaptic α_2-adrenoceptors (α_2-AR). The α_2-AR agonist guanfacine enhances prefrontal cortical functions in rats, monkeys, and human beings and ameliorates prefrontal cortical deficits in patients with attention deficit hyperactivity disorder. The present study examined the subtype of α_2-AR underlying these beneficial effects. Because there are no selective α_{2A}-AR, α_{2B}-AR, or α_{2C}-AR agonists or antagonists, genetically altered mice were used to identify the molecular target of the action of guanfacine. Mice with a point mutation of the α_{2A}-AR, which serves as a functional knock-out, were compared with wild-type animals and with previously published studies of α_{2C}-AR knock-out mice (Tanila et al., 1999). Mice were adapted to handling on a T maze and trained on either a spatial delayed alternation task that is sensitive to prefrontal cortical damage or a spatial discrimination control task with similar motor and motivational demands but no dependence on prefrontal cortex. The effects of guanfacine on performance of the delayed alternation task were assessed in additional groups of wild-type versus α_{2A}-AR mutant mice. We observed that functional loss of the α_{2A}-AR subtype, unlike knock-out of the α_{2C}-AR subtype, weakened performance of the prefrontal cortical task without affecting learning and resulted in loss of the beneficial response to guanfacine. These data demonstrate the importance of α_{2A}-AR subtype stimulation for the cognitive functions of the prefrontal cortex and identify the molecular substrate for guanfacine and novel therapeutic interventions.

Key words: prefrontal cortex; norepinephrine; mice; attention deficit hyperactivity disorder; adrenoceptor subtype; delayed alternation; neuropsychiatric disorder

The prefrontal cortex (PFC) regulates behavior using working memory, guiding both overt behaviors and the contents of attentional focus, inhibiting inappropriate responses and organizing plans for the future (Goldman-Rakic, 1996; Robbins, 1996). Deficits in PFC function are common in many neuropsychiatric disorders (Weinberger et al., 1986; Barkley et al., 1992; Blumberg et al., 1999) and are a fundamental feature of ailments such as attention deficit hyperactivity disorder (ADHD), in which symptoms of impulsivity correlate with reduced size of the right PFC (Casey et al., 1997).

Recent studies have shown that norepinephrine (NE) has a critical beneficial influence on PFC functions through actions at postsynaptic α_2-adrenergic receptors (α_2-ARs) (for review, see Avery et al., 2000). For example, systemic administration of α_2-AR agonists such as guanfacine (GFC) to rats, monkeys, or human beings enhances performance of PFC tasks (Arnsten et al., 1988; Carlson et al., 1992; Jakala et al., 1999). Similarly, direct infusion of α_2-AR agonists into the PFC improves cognitive function, whereas infusions outside the relevant PFC region are ineffective (Tanila et al., 1996; Mao et al., 1999). On the basis of research in animals, guanfacine is currently in use for the treatment of ADHD in children and adults (Scahill et al., 2001; Taylor and Russo, 2001), particularly in patients for whom stimulant medication is contraindicated. However, the α_2-AR subtype underlying these important beneficial effects is not known.

Three α_2-AR subtypes have been cloned in humans: the α_{2A}-AR, α_{2B}-AR, and α_{2C}-AR, with the α_{2A}-AR and α_{2C}-AR, but not the α_{2B}-AR, subtypes localized in the PFC (for review, see Aoki et al., 1994; MacDonald et al., 1997). Previous studies in monkeys used agonists with varying affinities for the α_{2A}-AR, α_{2B}-AR, and α_{2C}-AR to attempt to dissect the subtype or subtypes underlying the cognitive-enhancing, hypotensive, and sedating actions of these agents (Arnsten et al., 1988; Arnsten and Leslie, 1991). These pharmacological analyses indicated that the cognitive-enhancing effects were mediated by the α_{2A}-AR, whereas the hypotensive and sedating actions were mediated by the α_{2B}-AR and α_{2C}-AR. It is now known that these conclusions were erroneous. Studies of mice with genetically altered α_2-AR subtypes demonstrated conclusively that the α_{2A}-AR subtype mediates the hypotensive actions of α_2-AR agonists (Link et al., 1996; MacMillan et al., 1996), and the sedative effects of these agents involve α_{2A}-AR actions as well (for review, see MacDonald et al., 1997). The faulty conclusions from the previous pharmacological studies most likely arose from several factors: (1) there are no highly selective α_{2A}-AR, α_{2B}-AR, or α_{2C}-AR agonists or antagonists to dissociate actions between the subtypes; (2) many

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α_2-AR compounds have nonadrenergic actions as well (e.g., at imidazoline I_1 receptors) that can alter blood pressure, sedation, and cognition function (e.g., the potent hypotensive effects of clonidine most likely arise from a combination of α_2-AR and I_1 receptor actions); and (3) differences in bioavailability can confuse measures of potency (e.g., clonidine enters the brain more quickly than guanfacine). Thus, studies in mice with genetically altered α_2-AR subtypes are needed to rigorously determine the α_2-AR subtype underlying the cognitive-enhancing effects of NE and α_2-AR agonists for the first time. A recent study showed that α_{2C}-AR knock-out mice exhibit normal spatial working memory performance and normal enhancement of performance by an α_2-AR agonist (Tanila et al., 1999). The present study performed analyses in mice with a mutation of the α_{2A}-AR subtype (MacMillan et al., 1996).

MATERIALS AND METHODS

Design

α_{2A}-AR mutant mice were compared with wild-type controls in their ability to habituate to handling in a T maze and subsequently to learn and perform a task dependent on the PFC, spatial delayed alternation in a T maze (Larsen and Divac, 1978). This test of spatial working memory requires behavioral inhibition and attentional regulation, particularly as delays are increased. Wild-type and α_2-AR mutant mice were also compared for their ability to learn a control task, spatial discrimination in the T maze, which has the same motor and motivational demands but does not depend on the PFC (Boyd and Thomas, 1977). Finally, wild-type and α_2-AR mutant mice were assessed on the delayed alternation task for their response to guanfacine, an α_2-AR agonist that *in vitro* prefers the α_{2A}-AR by 10- to 60-fold (Uhlen and Wikberg, 1991; Devedjian et al., 1994), and *in vivo* improves PFC cognitive function (Arnsten et al., 1988; Jakala et al., 1999).

Subjects

The present study performed analyses in mice with a point mutation (D79N) of the α_{2A}-AR subtype (MacMillan et al., 1996), which has been shown to effect a functional knock-out of the receptor (Lakhlani et al., 1997). The α_{2A}-AR mutant mice (C57BL/6D79NTG strain) were created by the laboratory of Dr. Lee Limbird (Vanderbilt University, Nashville, TN) and supplied by Dr. Brian Kobilka (Stanford University, Stanford, CA). These animals were shown previously to lack hypotensive α_{2A}-AR mechanisms, although they demonstrated normal blood pressure under basal conditions (MacMillan et al., 1996). The expression of mutated α_{2A}-AR in these mice results in reduction of receptor density by 80% compared with levels in wild-type mice. Furthermore, the residual α_{2A}-AR cannot activate potassium currents or suppress calcium currents. Thus, the D79N α_{2A}-AR mouse serves as a functional knock-out (Lakhlani et al., 1997). At the time that these behavioral studies were initiated, the D79N α_{2A}-AR mice were the only mice altered at the α_{2A}-AR locus to be backcrossed against C57BL/6 mice >12 generations, permitting direct comparison with C57BL/6 wild-type mice; α_{2A}-AR and α_{2B}-AR knock-out mice were not available for examination.

The D79N α_{2A}-AR mice were bred at Yale and were 3–6 months of age at the start of the research. Wild-type C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were purchased at 2 months of age and lived in the Yale University vivarium for 1–5 months before inclusion in the study to be age-matched to mutant animals. Wild-type and mutant strains (males, 19–27 gm) were housed singly, and their food was regulated to ensure motivation on the tasks. Animals were fed a 4 gm biscuit immediately after cognitive testing; water was available *ad libitum*. Animals showed a normal growth curve. Care of the animals followed the guidelines in the *Guide For the Care and Use of Laboratory Animals* and was approved by the Yale Animal Care and Use Committee.

Behavioral assessment

Adaptation to testing procedures. Animals were tested in a T maze made smaller (15 × 21 × 3 inches) to be appropriate for testing mice. The maze was constructed of wood and painted black, with a guillotine door separating the start box from the main stem of the maze. Testing occurred in a small room near the colony room under normal light conditions. A sink was located on the wall to the left of the maze, and a dustpan and broom on the wall to the right of the maze; the maze was maintained in the same position in the room throughout the duration of the study. All cognitive testing was performed between 8:00 A.M. and 5:00 P.M. during the animals' light cycle; each animal was tested by the same experimenter at the same time of day (e.g., 11:00 A.M.) every day, Monday through Friday. Before cognitive training, all animals were exposed to the food rewards (halved, peeled sunflower seeds) in their home cage. Importantly, all animals were fully habituated to the T maze and to eating food rewards on the maze (criterion of eating 10 rewards in 8 min for two consecutive days) and were subsequently habituated to handling procedures on the maze (criterion of eating 10 food rewards in 5 min while being picked up five times for two consecutive days). This was done to minimize any affective differences between the mutant and wild-type animals that might obscure changes in cognitive performance. It is common for genetically altered mice to exhibit changes in emotional responding; thus, it was particularly important that all mice achieved the same criterion of comfort with each procedure before continuing with the cognitive aspects of the study.

Cognitive assessment. α_{2A}-AR mutant mice subsequently were compared with wild-type mice on the number of days needed to reach a criterion performance of two consecutive days of ≥90% correct under 0 sec delay conditions for the delayed alternation and spatial discrimination tasks. Half of the animals were trained on delayed alternation first, and the other half were trained on spatial discrimination first to control for any possible order effects. In the spatial delayed alternation task, the animal is rewarded for choosing either the left or right arm on the first trial (not counted), but from then on must always choose the arm not entered on the previous trial. In contrast, in the spatial discrimination task, half the animals are trained to always choose the left arm to receive reward, whereas the other half are trained to always choose the right arm. In both tasks, the animal is picked up immediately after eating the reward (or picked up with no reward if he was incorrect) and placed in the start box between trials. The choice point of the maze is wiped with alcohol to prevent olfactory cues from guiding behavior. This process takes ~1–2 sec and is designated a 0 sec delay. Each daily test session consisted of 10 trials.

For the assessment of the effects of guanfacine on delayed alternation performance, delays were increased as needed to maintain performance of ~70% correct, thus allowing room for either improvement or impairment with drug treatment. The delay was increased in 5 sec intervals if a mouse performed at ≥90% for two consecutive testing sessions (rarely, delay was also decreased if baseline deteriorated to <60–70%). Thus, the delay needed to maintain baseline performance at 70% correct can be used as a measure of general performance on the task.

Drug administration

Mice were considered ready for drug administration when they had been at their current delay for at least three test sessions and had performed at between 60 and 70% for two consecutive test sessions. Furthermore, a washout period of at least 7 d was required between drug treatments. Guanfacine was dissolved in saline and was injected (0.15 ml, i.p.) 1 hr before cognitive testing. Animals were extensively adapted to the injection procedure before the experiment. Guanfacine was generously provided by Wyeth-Ayerst (Princeton, NJ). An initial pilot study identified the proper dose range for examination; doses of 0, 0.0001, 0.001, 0.01, 0.1, 1.0, or 10.0 mg/kg were injected in random order in wild-type mice (n = 4–7) and in α_{2A}-AR mutants (see below). Mean percentage correct ± SEM for each dose is shown in Table 1. The 0.01 mg/kg dose tended to impair performance in wild-type mice ($p > 0.1$; $n = 6$), as has been seen occasionally in monkeys (Arnsten et al., 1988; Franowicz and Arnsten, 1998) and is probably a result of presynaptic drug actions reducing endogenous NE release. The 10 mg/kg dose induced sedation that interfered with testing in wild-type and mutant mice. Only the 1.0 mg/kg dose improved the working memory performance of wild-type mice, and this dose is similar to that which reliably improves working memory performance in young adult monkeys as well (0.5–1.0 mg/kg). Thus, the 1.0 mg/kg dose was compared with saline in a larger number of animals (n = 12) and was repeated to ensure reliability of response within subjects. All doses of guanfacine were tested in a large sample of α_{2A}-AR mutants (n = 10) to ensure that no other dose of guanfacine was effective (Table 1).

Table 1. Preliminary data showing mean percent correct on the delayed alternation task after administration of guanfacine

Dose of guanfacine (mg/kg)	0.0	0.0001	0.001	0.01	0.1	1.0
Wild-type mice (n = 4–7) (delays 0–20 sec) (mean ± SEM)	71.4 ± 3.5	70.0 ± 3.5	67.1 ± 6.6	57.5 ± 6.0	74.0 ± 7.6	80.3 ± 2.9
α_{2A} mutant mice (n = 10) (delays 0–5 sec) (mean ± SEM)	70.4 ± 2.1	62.0 ± 4.9	70.0 ± 2.4	72.4 ± 3.3	66.0 ± 3.2	70.0 ± 2.3

Statistics

Simple comparisons between the wild-type and mutant mice were analyzed with an independent t test (T_{ind}). Delay achieved over time was analyzed by ANOVA with a mixed design: a within-subjects factor of time (25th vs 50th test session) and a between-subjects factor of α_{2A}-AR mutation (wild type vs α_{2A}-AR mutation). Statistical analyses were performed with Systat (Evanston, IL) software.

RESULTS

Habituation to test procedures

The mice were initially adapted to the maze, food rewards, and handling procedures to minimize contamination of affective influences on cognitive assessment. Animals were required to reach criterion levels of responding before continuing to the next level of the study. These procedures are particularly important for mice, given their neophobic response to novel environments and procedures. The α_{2A}-AR mutants were not different from wild-type mice in their time to habituate to eating food rewards in the maze (wild-type mice, n = 6, 4.8 ± 0.9 d to reach criterion; α_{2A}-AR mutant mice, n = 6, 3.3 ± 0.7 d to reach criterion; T_{ind}, $p > 0.1$); however, they were markedly slower to habituate to handling procedures in the maze (Fig. 1*A*) (T_{ind}; $p < 0.002$). These results are consistent with recent data suggesting that mice with genetically altered α_{2A}-AR can show heightened signs of anxiety (Schramm et al., 2001), whereas in contrast, those with reduced α_{2C}-AR exhibit decreased response to stress (Sallinen et al., 1999).

Acquisition of the cognitive tasks

Once habituated to the maze and handling procedures, the mice were trained in the T maze on either the spatial working memory task, delayed alternation, or the reference memory task, spatial discrimination. There were no significant differences between the α_{2A}-AR mutants and the wild-type controls in the time needed to learn either the delayed alternation task (0 sec delays; T_{ind}, p = 0.92) (Fig. 1*C*) or the spatial discrimination task (T_{ind}, p = 0.82) (Fig. 1*B*) to criterion levels of responding. Thus, learning was unaffected by the mutation of the α_{2A}-AR.

Delay achieved on the delayed alternation task

A second group of animals was used to evaluate the effects of the α_2-AR agonist guanfacine on the delayed alternation performance of the α_{2A}-AR mutant versus wild-type mice (n = 12). After habituation to the maze as described above, the mice were trained on the delayed alternation task, and a stable baseline of performance was established for assessment of drug effects on working memory performance. Delays were increased as needed (see Materials and Methods) to maintain performance of ~70% correct, thus allowing room for either improvement or impairment with guanfacine treatment. Thus, the delay needed to maintain baseline performance at 70% correct can be used as a measure of general performance on the task.

Wild-type mice were able to withstand significantly greater delays than the mutant mice, as shown in Figure 2, comparing mice at the 25th and 50th testing sessions. Although all mice were able to withstand increasing delays as the study progressed (ANOVA; within-subjects effect of time; $F_{(1,22)}$ = 15.21; p = 0.001), there was a highly significant effect of the α_{2A}-AR mutation on the delay needed to maintain baseline performance at 70% correct (between-subjects effect of the α_{2A}-AR mutation; $F_{(1,22)}$ = 27.23; $p < 0.0001$). The effects of the mutation became increasingly evident over time (significant interaction between the effects of the mutation and time: $F_{(1,22)}$ = 12.57; p = 0.002). Thus, the differences between the mice had already emerged by the 25th test session (mean ± SEM delay needed: wild-type, 5.0 ± 2.2 sec; α_{2A}-AR mutants, 0 ± 0 sec; $p < 0.03$) but were more evident by the 50th test session (mean ± SEM delay needed: wild-type, 13.7 ± 2.1 sec; α_{2A}-AR, 0.4 ± 0.4 sec; $p < 0.00001$). Thus, by the 50th test session, no α_{2A}-AR mutant mouse had experienced a delay >5 sec, whereas the majority of wild-type mice were able to show solid baseline performance with delays of 15–20 sec. These data indicate that the α_{2A}-AR mutant mice have measurably poorer performance when working memory abilities are challenged.

Response to the α_2-AR agonist guanfacine

The effects of the α_2-AR agonist guanfacine were compared in the wild-type and α_{2A}-AR mutant mice after establishment of stable baseline performance. Pilot studies of a wide range of guanfacine doses indicated that the 1.0 mg/kg dose was appropriate for cognitive enhancement in mice. Thus, the 1.0 mg/kg dose was compared with saline in wild-type versus α_{2A}-AR mutant mice. The results are shown in Figure 3. A two-way ANOVA with a between-subjects factor of α_{2A}-AR mutation (wild-type versus α_{2A}-AR mutation) and a within-subjects factor of drug (saline vs guanfacine) found a significant effect of α_{2A}-AR mutation ($F_{(1,19)}$ = 5.73; p = 0.027), no significant overall effect of drug ($F_{(1,19)}$ = 1.39; p = 0.25), and a significant α2A-AR mutation by drug interaction ($F_{(1,19)}$ = 5.021; p = 0.037). Thus, guanfacine significantly improved delayed alternation performance in the wild-type mice (p = 0.04; n = 12) but had no effect on the α_{2A}-AR mutant mice (p = 0.46; n = 12). Wild-type mice were improved by 1.0 mg/kg guanfacine irrespective of whether they received guanfacine early in the study when their delays were short (e.g., 0 sec) or later in the study when their delays were longer. Thus, guanfacine improved performance in WT mice whether their delays were 0 sec (saline 65%; GFC 77%; p = 0.02), 15 sec (saline 74%; GFC 85%; p = 0.02), or 20 sec (saline 68%; GFC 87%; p = 0.05) at the time when they received guanfacine. A similar pattern has been observed in monkeys, in which guanfacine improves spatial working memory performance on trials with either short or long delays (Franowicz and Arnsten, 1998). Other doses of guanfacine were without effect in the α_{2A}-AR mutant mice (Table 1).

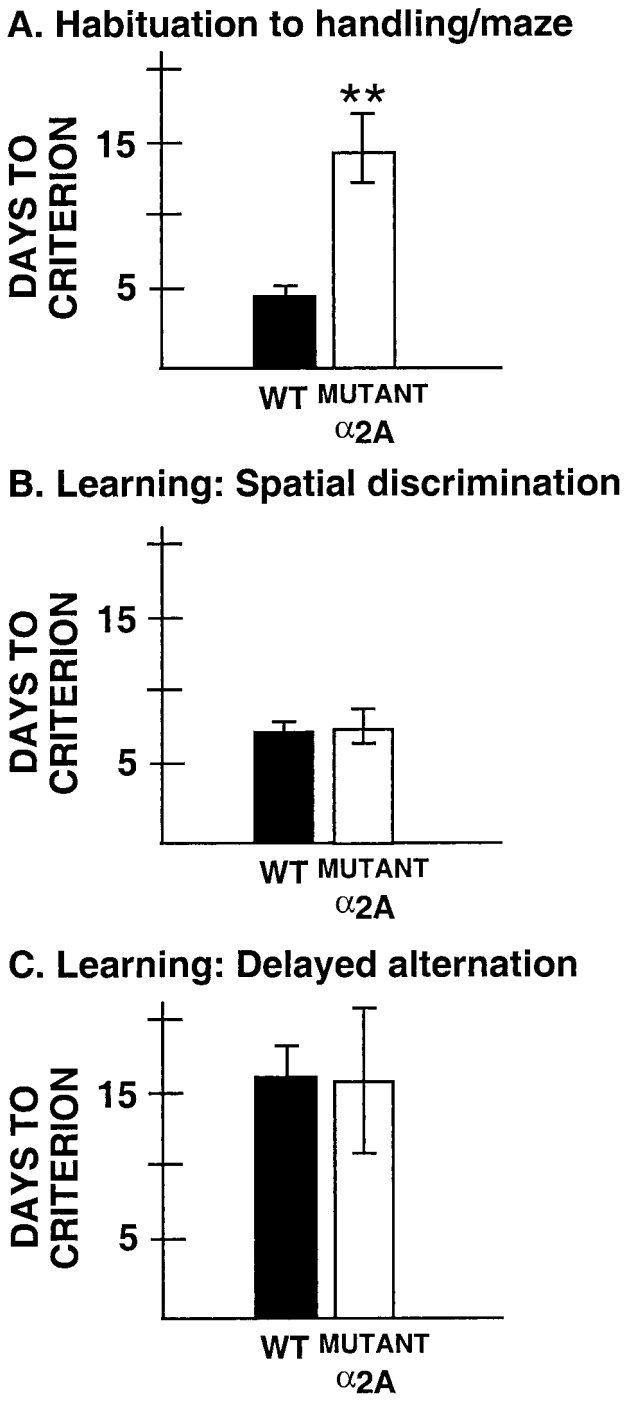

Figure 1. A comparison of the performance of wild-type versus α_{2A}-AR mutant mice in the time needed to habituate to the test procedures and to learn the cognitive tasks. The graph illustrates the mean ± SEM days to criterion for habituation to handling in the T maze (*A*), spatial discrimination performance (reference memory, control task) (*B*), and spatial delayed alternation performance with 0 sec delays (working memory, PFC task) (*C*). Criterion for each condition is described in Materials and Methods. *Solid bars*, Results for wild-type (WT) mice; *open bars*, for α_{2A}-AR mutant mice (α_{2A}). α_{2A}-AR mutant mice took significantly longer to habituate to the testing conditions but subsequently learned both tasks at normal rates. **Significantly different from wild-type animals, $p < 0.002$, $n = 6$.

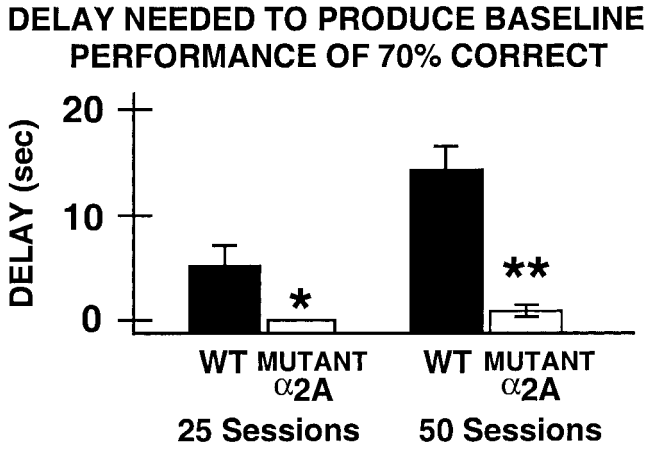

Figure 2. Comparison of the abilities of the wild-type versus α_{2A}-AR mutant mice on the delayed alternation task, a test of working memory, behavioral inhibition, and attention regulation. Results represent the mean ± SEM delay needed to achieve baseline performance of ~70% correct after the 25th and 50th daily test sessions of the delayed alternation task. *Solid bars*, Results for wild-type mice; *open bars*, for α_{2A}-AR mutant mice. α_{2A}-AR mutant mice needed significantly lower delays than wild-type controls to perform at 70% correct. *Significantly different from wild-type animals, $p < 0.03$; **significantly different from wild-type animals, $p < 0.00001$; $n = 12$.

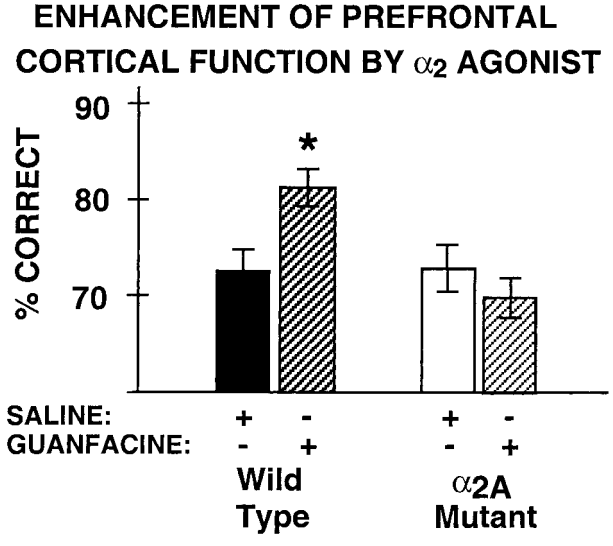

Figure 3. The effects of saline versus the α_2-AR agonist guanfacine (1.0 mg/kg) in wild-type mice (*left*) versus α_{2A}-AR mutant mice (*right*) performing the delayed alternation task, a test of working memory, behavioral inhibition, and attention regulation. Guanfacine improved performance in the wild-type mice but had no effect in the α_{2A}-AR mutant animals. Results represent mean ± SEM percentage correct on the delayed alternation task. *Significantly different from saline, $p < 0.04$; $n = 12$.

DISCUSSION

Studies of rats, monkeys, and human beings have found that α_2-AR agonists, such as guanfacine, improve the cognitive functions of the PFC but have no effect on or impair the cognitive abilities of posterior cortical or subcortical regions. For example, systemic administration of α_2-AR agonists to rats, monkeys, or human beings enhances performance of tasks that require working memory, planning, behavioral inhibition, and attention regulation and depend on the integrity of the PFC (Arnsten and Goldman-Rakic, 1985; Jackson and Buccafusco, 1991; Arnsten

and Contant, 1992; Carlson et al., 1992; Coull et al., 1995; Rama et al., 1996; Steere and Arnsten, 1997; Jakala et al., 1999; O'Neill et al., 2000). These beneficial effects are dissociable from the hypotensive and sedating actions of these compounds (Arnsten et al., 1988) and are consistent with the known role of NE in attention regulation (Carli et al., 1983). In contrast, the learning and memory abilities of medial temporal lobe (Arnsten and Goldman-Rakic, 1990; Sirviö et al., 1991; Genkova-Papazova et al., 1997) and parietal cortex (Witte and Marrocco, 1997) are unaffected or even impaired by α_2-AR stimulation. Thus, the beneficial effects on cognitive function appear to be selective to the PFC.

It is likely that the improved performance induced by guanfacine in the present mouse study similarly reflects enhanced PFC cognitive function. Although guanfacine was administered systemically and thus influenced the entire neuroaxis, studies in rats and monkeys have demonstrated that these beneficial effects of α_2-AR agonists arise from direct actions in the PFC. In monkeys, infusion of the α_2-AR agonist guanfacine directly into the PFC produces a delay-related improvement in working memory performance (Mao et al., 1999), and similar beneficial effects have been seen with agonist infusion into the PFC of aged rats (Tanila et al., 1996). Conversely, infusion of the α_2-AR antagonist yohimbine produces a delay-related impairment in performance (Li and Mei, 1994), emphasizing the importance of endogenous NE stimulation of these receptors. At the cellular level, either systemic or iontophoretic application of an α_2-AR agonist increases the delay-related activity of PFC neurons (Li et al., 1999), the electrophysiological measure of working memory and behavioral inhibition (Funahashi et al., 1993). Conversely, the iontophoretic application of an α_2-AR antagonist decreases the delay-related activity of PFC neurons (Sawaguchi, 1998; Li et al., 1999), indicating that endogenous NE release has a critical effect on PFC neuronal responding. The importance of α_2-AR stimulation to PFC function has been corroborated in imaging studies in which systemic administration of the α_2-AR agonist guanfacine increases regional cerebral blood flow in the PFC of both monkeys and human beings (Avery et al., 2000; Swartz et al., 2000). Given the consistency of these findings and the clinical use of guanfacine to treat PFC cognitive disorders, it was of great interest to determine the α_2-AR subtype underlying these important beneficial effects of NE and α_2-AR agonists on PFC function.

The present study found that mutation of the α_{2A}-AR significantly weakened working memory performance on the delayed alternation task and prevented cognitive enhancement by guanfacine. Mice with mutations of this receptor were not able to achieve the longer delays acquired by wild-type mice, indicating weaker PFC regulation of behavior. Poorer achievement on the delayed alternation task by the mutant mice is unlikely to be accounted for by nonspecific changes in performance or affective variables, because the α_{2A}-AR mutant mice learned the task at a rate similar to that of wild-type mice under 0 sec delay conditions and were able to perform the spatial discrimination task similarly to wild-type animals. These findings are consistent with previous studies in which α_{2A}-AR stimulation did not improve learning (Sirviö et al., 1991). Special care was taken to minimize any affective influences on cognitive assessments, because emotional changes are common in mouse mutants and have been specifically identified in mice with altered α_{2A}-AR (Schramm et al., 2001). Mice were carefully adapted to all potentially stressful procedures to set criteria to ensure that mutants and wild-type animals were equally comfortable with manipulations such as handling. After these adaptive measures, mice were indistinguishable in their behavior in the maze, with the exception that mutant mice were unable to achieve the longer delays acquired by wild-type animals. This profile is consistent with weaker PFC regulation of behavior. Deficits on working memory tasks have been observed in other genetically altered mice, e.g., those with overexpression of the mutant human amyloid precursor (Dodart et al., 1999). However, these working memory deficits occurred in the constellation of broader cognitive deficits, i.e., impaired reference memory and object recognition memory, consistent with widespread cortical and hippocampal plaque deposition (Dodart et al., 1999). In contrast, the present data provide the first evidence of a genetic mutation selectively impairing performance of a working memory task dependent on the PFC and emphasize the importance of α_{2A}-AR stimulation for the strength of working memory function. These findings echo those observed in monkeys with blockade of α_2-AR in PFC by local infusions of yohimbine, who exhibited markedly impaired working memory performance at delays as short as 4 or 6 sec (Li and Mei, 1994) and weakened delay-related neuronal activity in the PFC (Sawaguchi, 1998; Li et al., 1999). The present findings identify the molecule in which endogenous NE acts to increase delay-related firing and thus to strengthen PFC regulation of behavior. These findings represent the first, stringent dissociation of the α_2-AR subtype contributions to higher cortical function. Because the functions of the PFC are fundamental to many of the highest-order cognitive abilities in humans, identification of a molecule critical to the functional integrity of the PFC is of wide-ranging significance.

Weakened spatial working memory performance in the mutant mice may not be solely a result of loss of a beneficial, postsynaptic α_{2A}-AR substrate for NE actions in the PFC but also of a disinhibition of detrimental factors. For example, the mutant mice also have lost much presynaptic regulation of NE release and most likely have excessive NE release (Trendelenburg et al., 2001). We have shown that such conditions during stress exposure impair working memory via stimulation of α_1-AR (Birnbaum et al., 1999). This possibility could be tested in the future by determining whether an α_1-AR antagonist improves the performance of mutant mice but not WT animals under nonstress conditions. However, α_1-AR may have been downregulated as a consequence of sustained stimulation, in which case this factor may not contribute to working memory impairment. An additional possibility is that loss of the α_{2A}-AR leads to an imbalance between α_{2A}-AR and α_{2C}-AR subtypes that is harmful. Cognitive deficits on non-PFC tasks have been observed in mice overexpressing the α_{2C}-AR subtype (Bjorklund et al., 1998, 2000), and an imbalance between these subtypes may similarly alter PFC function. Finally, it is possible that differences in early environmental rearing conditions could contribute to differences in working memory abilities as adults, but changes of this kind probably would have been expressed in our measures of spatial learning as well (Liu et al., 2000).

The second major finding of this study was that administration of the α_2-AR agonist guanfacine significantly improved the working memory performance of wild-type mice but had no effect on the α_{2A}-AR mutant mice. These results contrast with those found in mice with a genetic alteration of the α_{2C}-AR, which showed normal improvement with α_2-AR agonist treatment on a spatial working memory task (Tanila et al., 1999). Together, these data demonstrate that stimulation of the α_{2A}-AR subtype underlies the beneficial effects of α_2-AR agonists on PFC function.

Guanfacine is currently being used to treat adults and children

with ADHD (Chappell et al., 1995; Horrigan and Barnhill, 1995; Hunt et al., 1995; Scahill et al., 2001; Taylor and Russo, 2001), and double-blind, placebo-controlled trials have confirmed that guanfacine treatment enhances performance of PFC tasks in these patients (Scahill et al., 2001; Taylor and Russo, 2001). Preliminary research also suggests that guanfacine may benefit other neuropsychiatric disorders that involve PFC dysfunction, such as posttraumatic stress disorder (Horrigan, 1996) and schizophrenia (Friedman et al., 1999). The data from this study, in combination with previous research in rats, monkeys, and human beings, identifies the α_{2A}-AR as the molecular substrate for the therapeutic actions of guanfacine. However, given that the α_{2A}-AR also mediates many of the hypotensive effects of these agents, it will not be possible to develop α_{2A}-AR agonists devoid of hypotensive side effects.

A rekindling of interest in NE mechanisms underlying neuropsychiatric disorders such as ADHD has begun to emerge in recent years (Biederman and Spencer, 2000). For example, whereas previous research has often emphasized the contribution of dopaminergic mechanisms in the actions of stimulants such as methylphenidate and D-amphetamine, recent data indicate that the lower doses of stimulants used to treat ADHD patients preferentially release NE in rat brain (Kuczenski and Segal, 2001). In concert with this basic finding, selective NE reuptake blockers are now being developed for the treatment of ADHD, and this avenue appears promising (Spencer et al., 1998; Biederman and Spencer, 2000). The data from the present study indicate that the therapeutic actions of these agents probably involve facilitation of endogenous NE stimulation of α_{2A}-AR in the PFC. Thus, either endogenous NE or guanfacine-like compounds stimulate α_{2A}-AR in the PFC, which leads to increased delay-related activity of PFC neurons, which results in stronger PFC regulation of behavior and reduced symptoms of impulsivity, distractibility, and disorganization. These data represent a rare example whereby one can establish a direct connection between highly specified drug actions in the brain and therapeutic efficacy in mental disorders.

REFERENCES

Aoki C, Go C-G, Venkatesan C, Kurose H (1994) Perikaryal and synaptic localization of alpha-2A-adrenergic receptor-like immunoreactivity. Brain Res 650:181–204.

Arnsten AFT, Contant TA (1992) Alpha-2 adrenergic agonists decrease distractability in aged monkeys performing a delayed response task. Psychopharmacology 108:159–169.

Arnsten AFT, Goldman-Rakic PS (1985) Alpha-2 adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. Science 230:1273–1276.

Arnsten AFT, Goldman-Rakic PS (1990) Analysis of alpha-2 adrenergic agonist effects on the delayed nonmatch-to-sample performance of aged rhesus monkeys. Neurobiol Aging 11:583–590.

Arnsten AFT, Leslie FM (1991) Behavioral and receptor binding analysis of the alpha-2 adrenergic agonist, UK-14304 (5 bromo-6 [2-imidazoline-2-yl amino] quinoxaline): evidence for cognitive enhancement at an alpha-2 adrenoceptor subtype. Neuropharmacology 30:1279–1289.

Arnsten AFT, Cai JX, Goldman-Rakic PS (1988) The alpha-2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects. J Neurosci 8:4287–4298.

Avery RA, Franowicz JS, Studholme C, van Dyck CH, Arnsten AFT (2000) The alpha-2A-adrenoceptor agonist, guanfacine, increases regional cerebral blood flow in dorsolateral prefrontal cortex of monkeys performing a spatial working memory task. Neuropsychopharmacology 23:240–249.

Barkley RA, Grodzinsky G, DuPaul GJ (1992) Frontal lobe functions in attention deficit disorder with and without hyperactivity: a review and research report. J Abnorm Child Psychol 20:163–188.

Biederman J, Spencer TJ (2000) Genetics of childhood disorders. XIX. ADHD, Part 3: Is ADHD a noradrenergic disorder? J Am Acad Child Adolesc Psychiatry 39:1330–1333.

Birnbaum SG, Gobeske KT, Auerbach J, Taylor JR, Arnsten AFT (1999) A role for norepinephrine in stress-induced cognitive deficits: alpha-1-adrenoceptor mediation in prefrontal cortex. Biol Psychiatry 46:1266–1274.

Bjorklund M, Sirvio J, Puolivali J, Sallinen J, Jakala P, Scheinin M, Kobilka BK, Riekkinen P (1998) Alpha(2C)-adrenoceptor-overexpressing mice are impaired in executing nonspatial and spatial escape strategies. Mol Pharmacol 54:569–576.

Bjorklund M, Sirvio J, Riekkinen M, Sallinen J, Scheinin M, Riekkinen Jr P (2000) Overexpression of alpha2C-adrenoceptors impairs water maze navigation. Neuroscience 95:481–487.

Blumberg HP, Stern E, Ricketts S, Martinez D, de Asis J, White T, Epstein J, Isenberg N, McBride PA, Kemperman I, Emmerich S, Dhawan V, Eidelberg D, Kocsis JH, Silbersweig DA (1999) Rostral and orbital prefrontal cortex dysfunction in the manic state of bipolar disorder. Am J Psychiatry 156:1986–1988.

Boyd MG, Thomas RK (1977) Posterior association cortex lesions in rats: mazes, pattern discrimination and reversal learning. Physiol Psychol 5:455–461.

Carli M, Robbins TW, Evenden JL, Everitt BJ (1983) Effects of lesions to ascending noradrenergic neurons on performance of a 5-choice serial reaction task in rats: implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behav Brain Res 9:361–380.

Carlson S, Ranila H, Rama P, Mecke E, Pertovaara A (1992) Effects of medetomidine, an alpha-2 adrenoceptor agonist, and atipamezole, an alpha-2 antagonist, on spatial memory performance in adult and aged rats. Behav Neural Biol 58:113–119.

Casey BJ, Castellanos FX, Giedd JN, Marsh WL, Hamburger SD, Schubert AB, Vauss YC, Vaituzis AC, Dickstein DP, Sarfatti SE, Rapoport JL (1997) Implication of right frontostriatal circuitry in response inhibition and attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 36:374–383.

Chappell PB, Riddle MA, Scahill L, Lynch KA, Schultz R, Arnsten A, Leckman JF, Cohen DJ (1995) Guanfacine treatment of comorbid attention deficit hyperactivity disorder and Tourette's syndrome: preliminary clinical experience. J Am Acad Child Adolesc Psychiatry 34:1140–1146.

Coull JT, Middleton HC, Robbins TW, Sahakian BJ (1995) Contrasting effects of clonidine and diazepam on tests of working memory and planning. Psychopharmacology 120:311–321.

Devedjian JC, Esclapez F, Denis-Pouxviel C, Paris H (1994) Further characterization of human alpha-2-adrenoceptor subtypes: [3H]RX821002 binding and definition of additional selective drugs. Eur J Pharmacol 252:43–49.

Dodart JC, Meziane H, Mathis C, Bales KR, Paul SM, Ungerer A (1999) Behavioral disturbances in transgenic mice overexpressing the V717F beta-amyloid precursor protein. Behav Neurosci 113:982–990.

Franowicz JCS, Arnsten AFT (1998) The alpha-2A noradrenergic agonist, guanfacine, improves delayed response performance in young adult rhesus monkeys. Psychopharmacology 136:8–14.

Friedman JI, Adler DN, Davis KL (1999) The role of norepinephrine in the pathophysiology of cognitive disorders: potential applications to the treatment of cognitive dysfunction in schizophrenia and Alzheimer's disease. Biol Psychiatry 46:1243–1252.

Funahashi S, Chafee MV, Goldman-Rakic PS (1993) Prefrontal neuronal activity in rhesus monkeys performing a delayed anti-saccade task. Nature 365:753–756.

Genkova-Papazova M, Petkova BP, Lazarova-Bakarova M, Boyanova A, Staneva-Stoytcheva D (1997) Effects of flunarizine and nitrendipine on electroconvulsive shock- and clonidine-induced amnesia. Pharmacol Biochem Behav 56:583–587.

Goldman-Rakic PS (1996) The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. Philos Trans R Soc Lond 351:1445–1453.

Horrigan JP (1996) Guanfacine for PTSD nightmares. J Am Acad Child Adolesc Psychiatry 35:975–976.

Horrigan JP, Barnhill LJ (1995) Guanfacine for treatment of attention-deficit-hyperactivity disorder in boys. J Child Adolesc Psychopharmacol 5:215–223.

Hunt RD, Arnsten AFT, Asbell MD (1995) An open trial of guanfacine in the treatment of attention deficit hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 34:50–54.

Jackson WJ, Buccafusco JJ (1991) Clonidine enhances delayed matching-to-sample performance by young and aged monkeys. Pharmacol Biochem Behav 39:79–84.

Jakala P, Riekkinen M, Sirvio J, Koivisto E, Kejonen K, Vanhanen M, Riekkinen PJ (1999) Guanfacine, but not clonidine, improves planning and working memory performance in humans. Neuropsychopharmacology 20:460–470.

Kuczenski R, Segal DS (2001) Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine. J Pharmacol Exp Ther 296:876–883.

Lakhlani PP, MacMillan LB, Guo TZ, McCool BA, Lovinger DM, Maze M, Limbird LE (1997) Substitution of a mutant alpha2a-adrenergic

receptor via "hit and run" gene targeting reveals the role of this subtype in sedative, analgesic, and anesthetic-sparing responses in vivo. Proc Natl Acad Sci USA 94:9950–9955.
Larsen JK, Divac I (1978) Selective ablations within the prefrontal cortex of the rat and performance of delayed alternation. Physiol Psychol 6:15–17.
Li B-M, Mei Z-T (1994) Delayed response deficit induced by local injection of the alpha-2 adrenergic antagonist yohimbine into the dorsolateral prefrontal cortex in young adult monkeys. Behav Neural Biol 62:134–139.
Li B-M, Mao Z-M, Wang M, Mei Z-T (1999) Alpha-2 adrenergic modulation of prefrontal cortical neuronal activity related to spatial working memory in monkeys. Neuropsychopharmacology 21:601–610.
Link RE, Desai K, Hein L, Stevens ME, Chruscinski A, Bernstein D, Barsh GS, Kobilka BK (1996) Cardiovascular regulation in mice lacking alpha-2-adrenergic subtypes b and c. Science 273:803–805.
Liu D, Diorio J, Day JC, Francis DD, Meaney MJ (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci 3:799–806.
MacDonald E, Kobilka BK, Scheinin M (1997) Gene targeting: homing in on alpha-2-adrenoceptor subtype function. Trends Pharmacol Sci 18:211–219.
MacMillan LB, Hein L, Smith MS, Piascik MT, Limbird LE (1996) Central hypotensive effects of the alpha-2A-adrenergic receptor subtype. Science 273:801–803.
Mao Z-M, Arnsten AFT, Li B-M (1999) Local infusion of alpha-1 adrenergic agonist into the prefrontal cortex impairs spatial working memory performance in monkeys. Biol Psychiatry 46:1259–1265.
O'Neill J, Fitten LJ, Siembieda DW, Ortiz F, Halgren E (2000) Effects of guanfacine on three forms of distraction in the aging macaque. Life Sci 67:877–885.
Rama P, Linnankoski I, Tanila H, Pertovaara A, Carlson S (1996) Medetomidine, atipamezole, and guanfacine in delayed response performance of aged monkeys. Pharmacol Biochem Behav 54:1–7.
Robbins TW (1996) Dissociating executive functions of the prefrontal cortex. Phil Trans R Soc Lond 351:1463–1471.
Sallinen J, Haapalinna A, MacDonald E, Viitamaa T, Lahdesmaki J, Rybnikova E, Pelto-Huikko M, Kobilka B, Scheinin M (1999) Genetic alteration of the alpha2-adrenoceptor subtype c in mice affects the development of behavioral despair and stress-induced increases in plasma corticosterone levels. Mol Psychiatry 4:443–452.
Sawaguchi T (1998) Attenuation of delay-period activity of monkey prefrontal cortical neurons by an alpha-2 adrenergic antagonist during an oculomotor delayed-response task. J Neurophysiol 80:2200–2205.
Scahill L, Chappell PB, Kim YS, Schultz RT, Katsovich L, Shepherd E, Arnsten AFT, Cohen DJ, Leckman JF (2001) Guanfacine in the treatment of children with tic disorders and ADHD: a placebo-controlled study. Am J Psychiatry 158:1067–1074.
Schramm NL, McDonald MP, Limbird LE (2001) The alpha-2A-adrenergic receptor plays a protective role in mouse behavioral models of depression and anxiety. J Neurosci 21:4875–4882.
Sirviö J, Riekkinen P, Vajanto I, Koivisto E, Riekkinnen PJ (1991) The effects of guanfacine, alpha-2 agonist, on the performance of young and aged rats in spatial navigation task. Behav Neural Biol 56:101–107.
Spencer T, Biederman J, Wilens T, Prince J, Hatch M, Jones J, Harding M, Faraone SV, Seidman L (1998) Effectiveness and tolerability of tomoxetine in adults with attention deficit hyperactivity disorder. Am J Psychiatry 155:693–695.
Steere JC, Arnsten AFT (1997) The alpha-2A noradrenergic agonist, guanfacine, improves visual object discrimination reversal performance in rhesus monkeys. Behav Neurosci 111:1–9.
Swartz BE, Kovalik E, Thomas K, Torgersen D, Mandelkern MA (2000) The effects of an alpha-2 adrenergic agonist, guanfacine, on rCBF in human cortex in normal controls and subjects with focal epilepsy. Neuropsychopharmacology 23:263–275.
Tanila H, Rama P, Carlson S (1996) The effects of prefrontal intracortical microinjections of an alpha-2 agonist, alpha-2 antagonist and lidocaine on the delayed alternation performance of aged rats. Brain Res Bull 40:117–119.
Tanila H, Mustonen K, Sallinen J, Scheinin M, Riekkinen P (1999) Role of alpha-2C-adrenoceptor subtype in spatial working memory as revealed by mice with targeted disruption of the alpha-2C-adrenoceptor gene. Eur J Neurosci 11:599–603.
Taylor FB, Russo J (2001) Comparing guanfacine and dextroamphetamine for the treatment of adult attention deficit-hyperactivity disorder. J Clin Psychopharmacol 21:223–228.
Trendelenburg AU, Klebroff W, Hein L, Starke K (2001) A study of presynaptic alpha2-autoreceptors in alpha2A/D-, alpha2B- and alpha2C-adrenoceptor-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 364:117–130.
Uhlen S, Wikberg JES (1991) Delineation of rat kidney alpha 2A and alpha 2B-adrenoceptors with [3H]RX821002 radioligand binding: computer modeling reveals that guanfacine is an alpha-2A-selective compound. Eur J Pharmacol 202:235–243.
Weinberger DR, Berman KF, Zec RF (1986) Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. I. Regional cerebral blood flow evidence. Arch Gen Psychiatry 43:114–124.
Witte EA, Marrocco RT (1997) Alterations of brain noradrenergic activity in rhesus monkeys affects the alerting component of covert orienting. Psychopharmacology 132:315–323.