Pharmacology Biochemistry and Behavior, Vol. 58, No. 2, pp. 477–483, 1997
© 1997 Elsevier Science Inc.
Printed in the USA. All rights reserved
0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00290-6

D_1 Agonist Dihydrexidine Releases Acetylcholine and Improves Cognitive Performance in Rats

THOMAS D. STEELE,[2] DONALD B. HODGES, JR., TAMARA R. LEVESQUE AND KENNETH W. LOCKE[1]

Interneuron Pharmaceuticals Inc., 99 Hayden Avenue, Suite 340, Lexington, Massachusetts 02173

Received 17 May 1996; Revised 4 December 1996; Accepted 4 December 1996

STEELE, T. D., D. B. HODGES, JR., T. R. LEVESQUE AND K. W. LOCKE. *D_1 agonist dihydrexidine releases acetylcholine and improves cognitive performance in rats*. PHARMACOL BIOCHEM BEHAV **58**(2) 477–483, 1997.—Dihydrexidine is a selective, full-efficacy dopamine D_1 receptor agonist that has displayed therapeutic potential in Parkinson's disease by reversing motor deficits of MPTP-treated monkeys. The present study monitored the effects of dihydrexidine on acetylcholine release in rat brain by using in vivo microdialysis. Moderate doses of dihydrexidine [3 and 10 mg/kg, intraperitoneally (IP)] elevated extracellular concentrations of acetylcholine by 40–60% in rat striatum; higher doses did not significantly alter acetylcholine release. SCH 23390 blocked the dihydrexidine-induced increase, indicating a D_1 receptor-mediated action. A more robust stimulatory effect of dihydrexidine on acetylcholine release was observed in prefrontal cortex (to 300% of basal output) than in striatum. Dihydrexidine was also evaluated in a passive avoidance procedure in rats to determine if its neurochemical effects translated into cognition-enhancing activity; in this assay, dihydrexidine (0.3 mg/kg, IP) significantly improved the scopolamine-induced deficits. The results of these studies suggest that the acetylcholine-releasing properties of dihydrexidine and other D_1 agonists may underlie their cognition-enhancing activity and thus may have clinical value in the treatment of dementia. © 1997 Elsevier Science Inc.

Dihydrexidine D_1 receptors Acetylcholine Cognition

DOPAMINE receptors have classically been divided into two major subclasses, D_1 and D_2, based on pharmacological, biochemical and behavioral data (21). Cloning studies have subdivided the two major classes further based on their distinct molecular forms (37). At the biochemical level, D_1 and D_2 receptors have been distinguished based on radioligand binding profiles and receptor–effector coupling to adenylate cyclase (21), but the contribution of each subtype to different functional dopamine-related activities is complex. Until recently, the significance of the D_1 site was difficult to establish due to the lack of selective D_1 agonists. A major breakthrough in the evaluation of D_1 receptors was the synthesis (5) and pharmacological characterization of dihydrexidine (*trans*-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine; DHX) as a potent, selective, full-efficacy agonist at the D_1 receptor (5,23,25). In radioligand binding assays, dihydrexidine is more potent than the D_1 partial agonist SKF 38393 in displacing [3H]-SCH 23390 from rat (23) or monkey (45) striatal membranes. Ratio estimates of D_1/D_2 selectivity for dihydrexidine have ranged from 12 to 60 (23,25) depending on assay conditions. Dihydrexidine is 70-fold more potent than dopamine in stimulating adenylate cyclase (25), whereas the partial agonist SKF 38393 has only 50% (or less) of the activity of dopamine in this assay in rats (23,25) and monkeys (45). In behavioral studies, dihydrexidine stimulates locomotor activity, grooming and sniffing, which are blocked by the D_1 antagonist SCH 23390, suggesting its utility in the study of D_1-mediated behaviors (13).

Significant therapeutic potential for dihydrexidine has been demonstrated in the MPTP primate model of parkinsonism. Dihydrexidine-ameliorated parkinsonian signs including tremor, motor freezing, abnormal posture, rigidity and bradykinesia and increased eye blink rate (42). The D_1 antagonist SCH 23390 but not the D_2 antagonist remoxipride

[1]To whom requests for reprints should be addressed.
[2] Present address: Food and Drug Administration, Center for Drug Evaluation and Research, 5600 Fishers Lane, HFD-120, Rockville, MD 20857.

blocked the antiparkinsonian activity of dihydrexidine, indicating a D_1 locus of action (40). Animal studies with other D_1 agonists support the notion that the D_1 receptor, like the D_2 subtype, is a potential target for novel antiparkinsonian drugs (15,20), although clinical testing of the partial D_1 agonist SKF 38393 in patients with Parkinson's disease was negative (4).

An interaction between nigrostriatal dopaminergic and cholinergic systems in motor control is well-established and is shown by the clinical utility of both dopamine agonists and cholinergic antagonists in Parkinson's disease. Previous studies have suggested that dopamine exerts a solely inhibitory effect on striatal acetylcholine release (35,41) mediated by what are now recognized as D_2 receptors (3,10,14). In contrast, more recent neurochemical studies have shown that D_1 agonists stimulate striatal acetylcholine release (9,11,12). Thus, dopamine exerts reciprocal control of striatal acetylcholine release, depending on which receptor subtype is activated; the D_1 stimulatory effect may predominate under basal conditions (17). The opposing effects of distinct receptor subtypes present an interesting model to distinguish D_1 vs. D_2 activities of dopamine agonists in vivo using microdialysis techniques. In other brain regions, similar acetylcholine-releasing effects may be significant because of the established connection between this transmitter, learning and memory (16,26) and Alzheimer's disease (2,30,46,48,49). Thus, in the present study, we sought to identify D_1 vs. D_2 activities of dihydrexidine by using striatal acetylcholine release as the model. D_1 stimulatory effects on acetylcholine release were also evaluated in the frontal cortex, an area in which dopamine may be involved in learning and memory in rodents (6,38,39) and primates (1,7,33,34). Subsequently, studies were conducted to determine if the neurochemical effects on acetylcholine release could translate into cognitive improvements in a rodent model of dementia.

METHODS

Animals and Housing

Male Sprague-Dawley rats (Charles River, Kingston, NY) were group housed (three per cage) in a climate-controlled animal facility with food and water available ad libitum. Rats for the microdialysis studies weighed 200–300 g. After surgery, animals were housed individually. Rats for the passive avoidance experiments weighed 125–150 g.

In Vivo Microdialysis

Animals were anesthetized with ketamine/xylazine (87/13 mg/kg) for surgical implantation of a nonpenetrating guide cannula [Bioanalytical Systems (BAS), West Lafayette, IN]. A small hole was drilled through the skull to the dura at the site of guide cannula placement. The site was determined by using the following stereotaxic coordinates with reference to bregma (28): striatum A: 1.5, L: 2.6, V: 5.5; frontal cortex A: 3.2, L: 1.3, V: 1.0. The ventral coordinate was set by the distance the microdialysis probe extended through the cannula. The guide cannula was fixed to the skull with dental acrylic and anchor screws, and a dummy cannula was inserted. Animals were returned to their cages and allowed to recover from surgery for 24–48 h.

At the time of the experiment, dummy cannulae were removed, and the microdialysis probes (model CMA-12, BAS; tip length = 3 mm, diameter = 225 μm) were inserted. The dialysis probes were connected to a microinfusion pump (CMA-100; BAS) equipped with a liquid switch (CMA-110; BAS) via low dead-volume fused-silica tubing (inner diameter = 75 μm, outer diameter = 150 μm; Polymicro Technologies, Phoenix, AZ). The probe was perfused with artificial cerebrospinal fluid (in mM: 121 NaCl, 3.5 KCl, 1.2 $CaCl_2$, 1.2 $MgCl_2$, 1.0 NaH_2PO_4, 25 $NaHCO_3$) at a flow rate of 1.5 μl/min. Inclusion of 1 μM neostigmine bromide in the perfusate was necessary because basal levels of acetylcholine approached the limit of sensitivity of the analysis. A 1-h equilibration period was allotted prior to sample collection. Once initiated, baseline dialysate samples were collected every 20 min. A 20 μl aliquot of the sample (total sample volume = 30 μl) was analyzed immediately. No experimental manipulations were invoked until three consecutive samples were collected, which did not change in composition of acetylcholine by more than 10%.

For analysis of dialysate acetylcholine, instrumentation consisted of components from BAS/CMA including a CMA-200 refrigerated microsampler, a PM-60 pump and an LC-4C amperometric detector equipped with a platinum working electrode and a Ag/AgCl reference electrode. The analytical column and acetylcholinesterase/choline oxidase postcolumn enzyme reactor were also obtained from BAS. The mobile phase was 35 mM Na_2HPO_4 (pH 8.5) containing 0.005% Kathon as an antibacterial agent delivered at a flow rate of 1 ml/min. The H_2O_2 product of the enzyme reactor was detected at a potential of +500 mV. Output was recorded on a chart recorder and electronically with a Hewlett Packard ChemStation. Peak heights were integrated and converted to pmol/20 μl injection by interpolation on a standard curve.

Racemic dihydrexidine HCl was prepared by Interneuron Pharmaceuticals Inc. (Lexington, MA). (−)-Quinpirole HCl, (±)-SKF 81297, R-(−)-SCH 23390 and (−)-eticlopride were purchased from Research Biochemicals Inc. (Natick, MA). All drugs were prepared in saline to deliver the indicated dosages calculated as the free base in a volume of 1 ml/kg. Dihydrexidine and SKF 81297 were administered intraperitoneally (IP) for experiments in the striatum. All other drugs were administrated subcutaneously (SC). Doses for the antagonist experiments were selected by determining the highest dose that by itself did not affect acetylcholine release. The antagonists were administered 20 min prior to agonist (i.e., one sample collection interval). Samples were collected every 20 min for 3 h following agonist administration. Typically, four to six animals were used for each test group. Upon completion of the experiment, animals were killed by decapitation, and their brains were examined grossly to verify probe placement.

The amount of acetylcholine in each sample was normalized with respect to baseline samples, which were the last three samples collected before the initial drug injection. To assess the significance of single drug effects over time relative to the baseline samples, a one-way analysis of variance (ANOVA) with repeated measures was used. For agonist/antagonist experiments, a two-way ANOVA with repeated measures was used. Post-hoc comparisons were made with a Student–Newman–Keuls multiple range test ($p < 0.05$).

Passive Avoidance

Testing was conducted in a darkened room by using standard two-compartment rectangular passive avoidance chambers (San Diego Instruments, San Diego, CA) with black Plexiglas sides and grid floors. The light compartment of the chambers was illuminated by a 20-W lamp; the dark side was shielded from extraneous light sources.

On training (acquisition) day, groups of eight rats were injected with scopolamine (3.0 mg/kg, IP) or vehicle 30 min

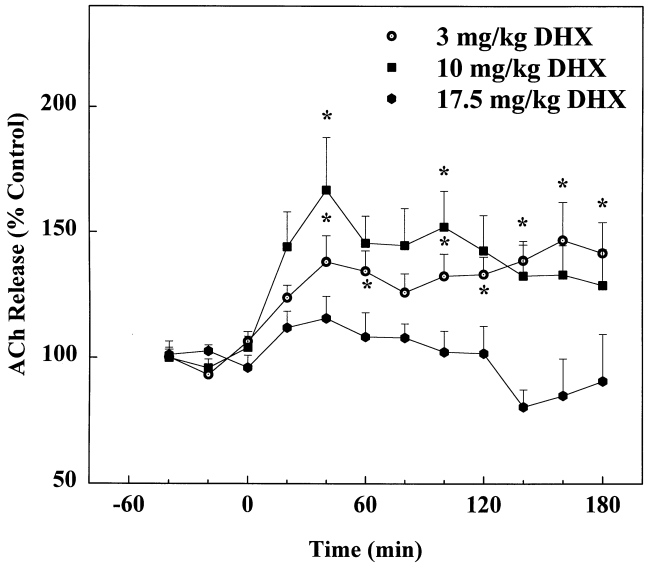

FIG. 1. Dose relationship for dihydrexidine on striatal acetylcholine release. Dihydrexidine (DHX) was injected (IP) at time 0 after the collection of three baseline samples. Dialysate acetylcholine levels were normalized with respect to the baseline samples (basal release ≈2.0 pmol/20 ml). Moderate doses of dihydrexidine (3 and 10 mg/kg, IP) stimulated acetylcholine release, whereas the highest test dose (17.5 mg/kg) had no significant effect. Values are the mean ± SEM for four to six animals per dose group. *Mean is significantly different ($p < 0.05$) from the three baseline values for that dose group.

prior to the training session. Ten minutes prior to the session, animals were injected with either dihydrexidine (0.1, 0.3 or 1.0 mg/kg, IP) or vehicle (0.02% ascorbic acid in 0.9% saline). The sessions were initiated by placing the rats in the light compartment facing away from the opening between the two compartments. The latency for the rat to enter the darkened side of the chamber was measured up to a maximum of 300 s; animals that did not enter the dark side were eliminated from the group. Upon entering the dark side completely, a 1-mA, 3-s scrambled shock was delivered to the entire grid floor. The animal was allowed to remain in the dark compartment or escape to the light compartment during the shock period. Animals were then returned to their home cages.

Twenty-four hours after training, each rat was tested for retention of the task. The procedures on test day were essentially identical to those on the training day except that no injections were given and the rats did not receive a shock upon entering the darkened side. The latency for animals to enter the darkened side (i.e., step-through latency) was recorded up to a maximum of 600 s.

A one-way ANOVA and Student–Newman–Keuls post-hoc comparisons were used to identify significant deficits in passive avoidance responding produced by scopolamine and its reversal by dihydrexidine ($p < 0.05$).

RESULTS

The dose dependence and time course of the effects of dihydrexidine on striatal acetylcholine release are shown in Fig. 1. Doses of 3 and 10 mg/kg dihydrexidine (IP) elevated extracellular concentrations of striatal acetylcholine by 40–60% over the course of the experiment. Statistical analysis showed that the effect of the 3 mg/kg dose was significant at all time points

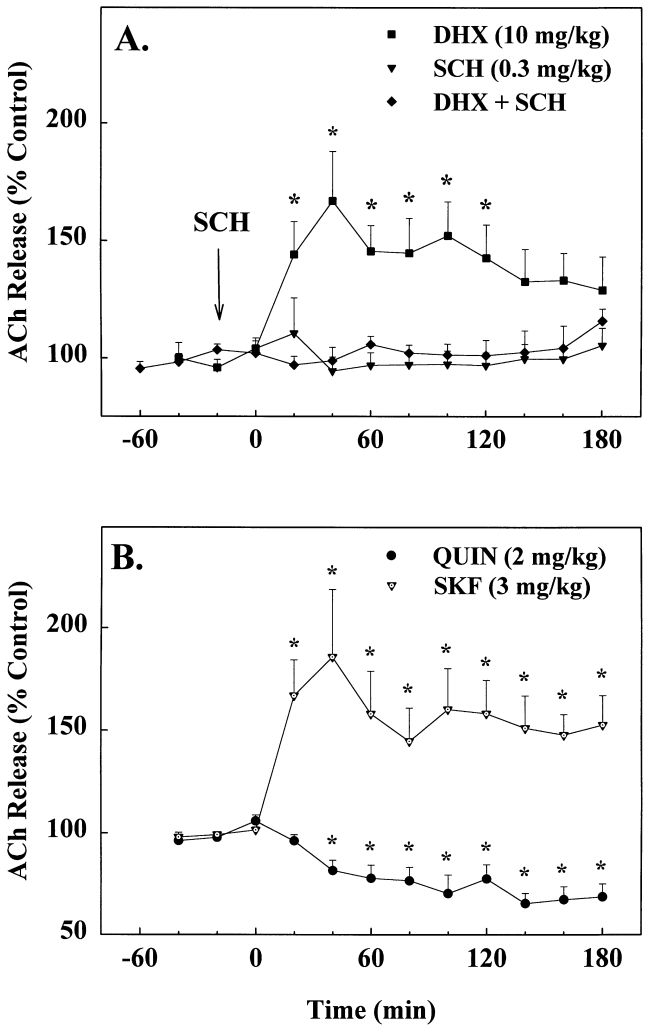

FIG. 2. Determination of receptor subtype responsible for the effects of dihydrexidine on rat striatal acetylcholine release. A: Pretreatment with the D_1 antagonist (−)-SCH 23390 (0.3 mg/kg, SC, SCH) blocks the stimulation of striatal acetylcholine release by 10 mg/kg, IP, dihydrexidine (DHX). SCH (arrow) was administered 20 min prior to dihydrexidine (time 0). B: Demonstration of opposing effects of dopamine receptor subtype stimulation on striatal acetylcholine release. Similar to the 3 and 10 mg/kg doses of dihydrexidine, the D_1 agonist SKF 81297 (SKF) stimulated acetylcholine release. In contrast, the D_2 agonist quinpirole (QUIN) reduced striatal acetylcholine output. Values are the mean ± SEM for four to six animals per treatment group. *Mean is significantly different ($p < 0.05$) from the three baseline values for that treatment group.

except at 20 and 80 min postinjection. The 40- and 100-min samples from the 10 mg/kg dose group differed significantly from baseline levels. The lack of significance of seemingly higher mean acetylcholine values in the 10 mg/kg group was due to a greater amount of variation in both the baseline and drug-treatment samples. Interestingly, the highest dihydrexidine test dose, 17.5 mg/kg, had no significant effect on striatal acetylcholine levels.

To identify the receptor subtypes involved in the acetylcholine-releasing effects of dihydrexidine, experiments with selective agonists and antagonists were conducted. Figure 2A shows that pretreatment of rats with 0.3 mg/kg (−)-SCH

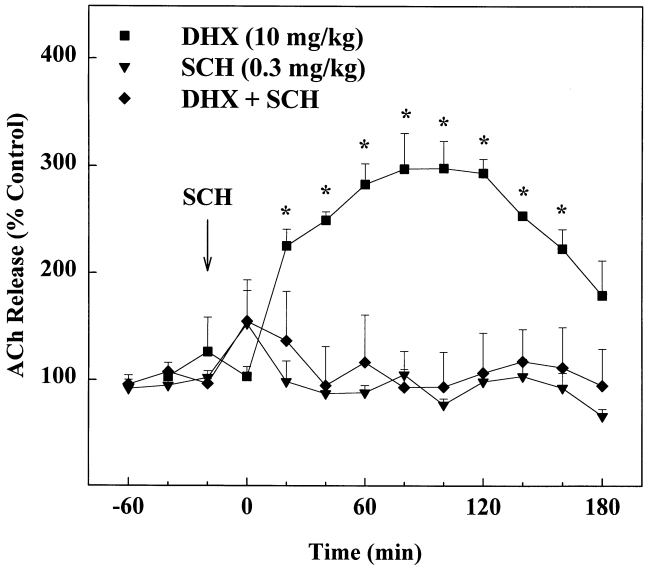

FIG. 3. Effects of dihydrexidine on acetylcholine release in rat frontal cortex. Administration of dihydrexidine (10 mg/kg, SC, DHX) at time 0 produced a dramatic elevation in cortical acetylcholine release (basal release ≈1.0 pmol/20 ml). Pretreatment with (−)-SCH 23390 (0.3 mg/kg, SC, SCH; administered at arrow) completely abolished the stimulatory effect of dihydrexidine. Values are the mean ± SEM for four to six animals per treatment group. *Mean is significantly different ($p < 0.05$) from the three baseline values for that treatment group.

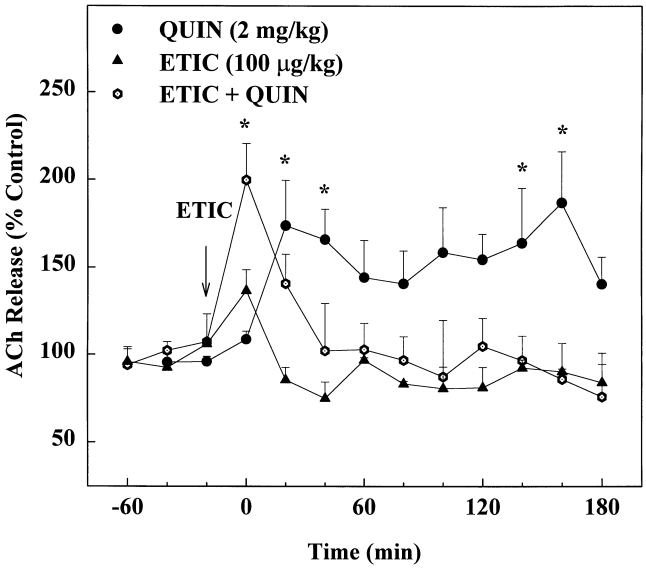

FIG. 4. Effects of a D_2 agonist on cortical acetylcholine release. In contrast to the striatum, D_2 receptor stimulation with quinpirole (2 mg/kg, SC, QUIN; administered at time 0) increased cortical acetylcholine output. Pretreatment with the D_2 antagonist eticlopride (0.1 mg/kg, SC, ETIC; administered at arrow) attenuated the effects of quinpirole. Values are the mean ± SEM for four to seven animals per treatment group. *Mean is significantly different ($p < 0.05$) from the three baseline values for that treatment group.

23390 (SC), a selective D_1 antagonist, completely blocked the dihydrexidine-induced increase in striatal acetylcholine release. This dose of SCH 23390 did not affect basal striatal acetylcholine output. Figure 2B shows the opposing effects of D_1 and D_2 agonists on extracellular striatal acetylcholine concentrations. Administration of 3 mg/kg SKF 81297 (IP), another selective D_1 agonist, produced an increase in striatal acetylcholine release comparable to that of 3 and 10 mg/kg dihydrexidine. The stimulatory effect of D_1 activation was opposite that of the D_2-selective agonist quinpirole (2 mg/kg, SC), which reduced striatal acetylcholine output.

To determine whether dihydrexidine also increased acetylcholine release in brain regions that may be more relevant to cognition, additional microdialysis studies were conducted in the prefrontal cortex. At a dose of 10 mg/kg, dihydrexidine produced a more robust increase in cortical acetylcholine release than was observed in the striatum (Fig. 3). Dialysate acetylcholine levels were raised 200–300% relative to baseline for the duration of the experiment. In these experiments, the subcutaneous route was used because preliminary experiments indicated that the effect of dihydrexidine was prolonged, but of a similar magnitude, by this route (data not shown). The stimulation of cortical acetylcholine release appears to be due solely to actions at D_1 receptors because 0.3 mg/kg (−)-SCH 23390 completely blocked the effect of dihydrexidine. However, in contrast to the striatum, stimulation of D_2 receptors also appeared to facilitate acetylcholine release. Administration of 2 mg/kg quinpirole significantly increased cortical dialysate acetylcholine concentrations, with the peak effects in the range of 150–200% (Fig. 4). Dopamine D_2 receptor specificity for this action was confirmed by the ability of the selective antagonist eticlopride (0.1 mg/kg, SC) to block the stimulatory effect of quinpirole.

In view of the evidence that dihydrexidine is a potent releaser of acetylcholine in the prefrontal cortex, the cognition-improving potential of the drug was assessed in a step-through passive-avoidance paradigm. Doses of 0.1, 0.3 and 1.0 mg/kg of dihydrexidine were tested for their ability to reverse the amnestic effects of scopolamine. Administration of scopolamine (3.0 mg/kg, IP) to rats significantly impaired the acquisition of the passive avoidance task (Fig. 5). Treatment with 0.3 mg/kg of dihydrexidine (IP) significantly improved learning as indicated by a prolonged latency time of scopolamine-amnestic rats to enter the darkened side of a chamber. The effect of dihydrexidine depended on dose; neither lower nor higher test doses produced significant effects.

DISCUSSION

The present studies provide neurochemical evidence that the D_1 receptor agonist dihydrexidine is a potent releaser of acetylcholine in rat striatum and frontal cortex. Experiments in the striatum showed that doses of 3 and 10 mg/kg of dihydrexidine increased striatal acetylcholine release. This effect was blocked by the D_1 antagonist SCH 23390 at a dose comparable to that used in other studies of D_1 receptor-mediated acetylcholine release [e.g., (11,12)]. A D_1 receptor-mediated stimulation of acetylcholine release has been shown in studies with other D_1 agonists (11,12,31) and in studies where the stimulatory effects of the indirect dopamine agonists *d*-amphetamine and cocaine were blocked by D_1 antagonists (9,12,18). The magnitude of the increase produced by 10 mg/kg of dihydrexidine is similar to that reported with relatively high doses of other selective D_1 agonists (11,12) and indirect dopaminer-

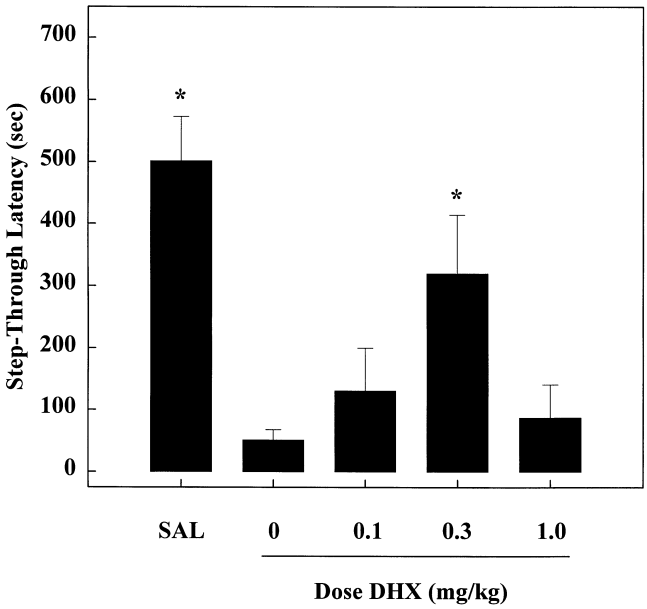

FIG. 5. Reversal of scopolamine-induced deficits in passive avoidance learning by dihydrexidine. Rats were injected with scopolamine (3 mg/kg, IP) and different doses of dihydrexidine (0, 0.1, 0.3, 1.0 mg/kg, IP, DHX) prior to the acquisition phase of the trial. Twenty-four hours later, the step-through latency for animals to enter the darkened compartment of the chamber was measured. Animals that received only saline (SAL) during acquisition did not readily enter the darkened side during retention testing. In contrast, scopolamine-treated animals that were administered either 0, 0.1 or 1.0 mg/kg dihydrexidine (DHX) in the acquisition phase quickly stepped through to the darkened side during retention testing. The intermediate test dose of dihydrexidine (0.3 mg/kg) significantly reversed the scopolamine-induced deficits. Values are the mean ± SEM for eight animals per treatment group. *Mean is significantly different ($p < 0.05$) from the scopolamine treatment group.

gic agonists (9,12,18,19), suggesting that the effects of 10 mg/kg of dihydrexidine approach the maximal limit for striatal acetylcholine release. This conclusion is supported by the observation that the highest test dose of dihydrexidine (17.5 mg/kg, IP) had a negligible effect on striatal acetylcholine release, possibly due to the loss of receptor subtype selectivity in this dose range. The inhibition of acetylcholine release by activation of D_2 receptors in the striatum was demonstrated in this study with the D_2 selective agonist quinpirole, thereby confirming the work of other investigators (3,10,14). In vitro ligand binding experiments have suggested that dihydrexidine is approximately 10-fold selective for D_1 vs. D_2 receptors (25). Therefore, at the highest dose (17.5 mg/kg), significant D_2 activation by dihydrexidine may have counteracted the stimulatory D_1 effect, with a net result of only minor fluctuations in striatal acetylcholine release. Attempts to unveil the stimulatory D_1 effect of 17.5 mg/kg of dihydrexidine by blocking striatal D_2 receptors with the D_2 antagonist eticlopride were confounded by the acetylcholine-releasing effect of low doses (e.g., 0.1 mg/kg) of eticlopride alone (data not shown).

The interaction between dopaminergic and cholinergic systems in the nigrostriatal tract is well established and pharmacologically significant in the therapy for Parkinson's disease. Recent studies have suggested that these transmitter systems also interact in other brain regions, including areas relevant to learning and memory (e.g., frontal cortex). Consistent with this suggestion, dihydrexidine produced a robust increase in extracellular concentrations of cortical acetylcholine to approximately 300% of baseline values. This effect was completely blocked by SCH 23390, thus supporting a D_1 site of action. In contrast to the striatum, D_2 receptors also appear to stimulate cortical acetylcholine release. The selective agonist quinpirole (2 mg/kg, SC) significantly elevated extracellular concentrations of cortical acetylcholine to 150% of baseline; this effect was blocked by the D_2 antagonist eticlopride. The stimulation of acetylcholine release by quinpirole was substantially lower than the peak effect produced by dihydrexidine, perhaps suggesting a more significant role for D_1 receptors on cortical acetylcholine release.

The neurochemical evidence that dihydrexidine is a potent releaser of acetylcholine in frontal cortex suggests a possible novel use of the drug as a cognition-enhancing agent. This contention is based on the wealth of evidence showing that pharmacological methods that elevate central cholinergic transmission improve learning and memory performance in animals (26). The potential clinical relevance is obvious because of the established connection of Alzheimer's disease with degeneration of central cholinergic neuronal systems (29,48, 49). Although acetylcholinesterase inhibitors are beneficial to some patients, significant limitations and toxic effects have been associated with their use (44,50). Therefore, novel mechanisms to promote acetylcholine release, such as through D_1 receptor activation, in brain regions where diminished release underlies cognitive impairments should be explored. This mechanism is particularly intriguing in view of the potential for dihydrexidine to treat both movement and cognitive impairments associated with Parkinson's disease in which neural pathologies similar to those found in Alzheimer's disease have been reported (30,32,43,47).

The improvement of scopolamine-induced impairments of passive avoidance performance, a widely used model of learning and memory, supports the contention that dihydrexidine has cognition-enhancing potential. The improvement by dihydrexidine is consistent with other rodent behavioral studies in which elevation of dopaminergic transmission improved cognitive performance in a variety of tasks. For example, selective D_1 and D_2 agonists facilitate and antagonists impair retention of inhibitory avoidance response in mice (8). Similarly, intracerebral injections of either SKF 38393 or quinpirole improved radial arm maze task performance (27). Others have reported a selective effect of D_1 activation for alleviating choice-accuracy deficits associated with scopolamine amnesia (22). The rodent prefrontal cortex is a candidate for putative D_1-acetylcholine mechanisms in cognition; D_1 agonists stimulate release, as shown in the present study, and dopamine depletion in this region impairs memory performance (39). Cortical D_1 receptors also have been implicated in cognition in primates. Infusions of D_1 antagonists (33,34), but not D_2 or D_3 antagonists (33), directly into prefrontal cortex and the systemic administration of SCH 23390 (1) impair delayed response performance in monkeys. Conversely, the administration of 1 mg/kg of the D_1 agonist dihydrexidine improved working memory performance in young monkeys; this effect was blocked by SCH 23390 (1). In addition, a dose of 0.6 mg/kg of dihydrexidine improved delayed response performance in MPTP-treated monkeys (36). Thus, the cognition-improving effects of D_1 agonists and cognition-impairing effects of dopaminergic depletion or D_1 blockade in both rodents and primates supports further investigation of the role of D_1 receptors in pathways associated with learning and memory.

Different doses of dihydrexidine were used in the passive-avoidance and in vivo microdialysis assays. However, in view of the limitations of the two assays, there are inherent difficulties in obtaining a precise correspondence between neurochemically active and behaviorally active doses. In the neurochemical studies, 10 mg/kg of dihydrexidine produced a dramatic increase (almost 200%) in acetylcholine release in the prefrontal cortex. In view of the magnitude of this increase, lower doses of dihydrexidine should produce increases in synaptic acetylcholine levels, with important physiological consequences. Support for this contention is evident in the experiments in the striatum, where lower doses of dihydrexidine produced significant increases in acetylcholine release; 3.0 mg/kg produced a 40–60% increase in striatal acetylcholine release. Furthermore, in a brain region associated with cognition (prefrontal cortex), 10 mg/kg of dihydrexidine produced approximately four times the increase in acetylcholine release (twice the absolute acetylcholine level) produced in the striatum. In the passive-avoidance study in rats, dihydrexidine showed an inverted-U-shaped dose–response curve common in this and other cognitive function assays. Although the reason doses greater than 0.3 mg/kg of dihydrexidine did not produce improvements in passive avoidance performance is unclear, competing dopaminergic behaviors may have offset any potential beneficial effects of increased acetylcholine release. In cognitive assays in primates (1,36), effective doses of dihydrexidine ranged from 0.01 to 1.0 mg/kg. Thus, although a particular cognitively active dose of dihydrexidine (0.3 mg/kg) was identified in the present study, the cognitive effects of dihydrexidine can be expected to change across a range of doses depending on the species, behavioral assay and specific assay conditions. Therefore, the present findings provide only preliminary evidence for a link between dihydrexidine-induced acetylcholine release and an improvement of cognitive deficits.

In summary, these studies have revealed that the D_1 agonist dihydrexidine is a potent stimulator of acetylcholine release in the striatum and frontal cortex and improves cognitive performance in rats in a passive-avoidance paradigm. These findings suggest that elevation of central cholinergic neurotransmission by dihydrexidine may be responsible for its cognition-improving effects. Evidence that D_1 and D_2 agonists reverse choice-accuracy deficits due to lesions of the medial projection of the basal forebrain to the cortex (24) presents the intriguing possibility of a local dopamine agonist action on acetylcholine release via receptors located on intrinsic cortical cholinergic neurons. This finding raises the possibility that cortical D_1 receptor activation, even under conditions of diminished ascending input from subcortical regions, may evoke acetylcholine release and reverse cognitive deficits associated with cholinergic hypofunction in this region. This novel mechanism, whereby acetylcholine release is stimulated in brain regions receiving diminished ascending cholinergic input, may have therapeutic applications in the treatment of dementia.

ACKNOWLEDGEMENTS

Portions of these studies were presented at the 24th Annual Meeting of the Society for Neuroscience (Miami Beach, 1994 November 13–18) and the 8th Meeting of the International Study Group on the Pharmacology of Memory Disorders Associated with Aging (Zurich, 1995 February 17–19).

REFERENCES

1. Arnsten, A. F. T.; Cai, J. X.; Murphy, B. L; Goldman-Rakic, P. S.: Dopamine D_1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. Psychopharmacology 116:143–151; 1994.
2. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S.: The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–414; 1982.
3. Bertorelli, R.; Consolo, S.: D_1 and D_2 dopaminergic regulation of acetylcholine release from striata of freely moving rats. J. Neurochem. 54:2145–2148; 1990.
4. Braun, A.; Fabbrini, G.; Mouradian, M. M.; Serrati C.; Barone P.; Chase, T. N.: Selective D-1 dopamine receptor agonist treatment of Parkinson's disease. J. Neural. Transm. 68:41–50; 1987.
5. Brewster, W. K.; Nichols, D. E.; Riggs, R. M.; Mottola, D. M.; Lovenberg, T. W.; Lewis, M. H.; Mailman, R. B.: *Trans*-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo-[a]phenanthridine: a highly potent, selective dopamine D_1 full agonist. J. Med. Chem. 33:1756–1764; 1990.
6. Broersen, L. M.; Heinsbroek, R. P. W.; de Bruin, J. P. C.; Joosten, R. N. J. M. A.; van Hest, A.; Olivier, B.: Effects of local application of dopaminergic drugs into the dorsal part of the medial prefrontal cortex of rats in a delayed matching to position task: comparison with local cholinergic blockade. Brain Res. 645:113–122; 1994.
7. Brozoski, T. J.; Brown, R. M.; Rosvold, H. E.; Goldman, P. S.: Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkeys. Science 205:929–932; 1979.
8. Castellano, C.; Cestari, V.; Cabib, S.; Puglisi-Allegra, S.: Post-training dopamine receptor agonists and antagonists affect memory storage in mice irrespective of their selectivity for D1 or D2 receptors. Behav. Neural Biol. 56:283–291; 1991.
9. Consolo, S.; Girotti, P.; Russi, G.; Di Chiara, G.: Endogenous dopamine facilitates striatal in vivo acetylcholine release by acting on D1 receptors localized in the striatum. J. Neurochem. 59:1555–1557; 1992.
10. Damsma, G.; DeBoer, P.; Westerink, B. H.; Fibiger, H. C.: Dopaminergic regulation of striatal acetylcholine release: an *in vivo* microdialysis study. Naunyn-Schmiedeberg Arch. Pharmacol. 342:523–527; 1990.
11. Damsma, G.; Robertson, G. S.; Tham, C.-S.; Fibiger, H. C.: Dopamine D_1 receptor stimulation increases striatal acetylcholine release in the rat. Eur. J. Pharmacol. 186:335–338; 1990b.
12. Damsma, G.; Robertson, G. S.; Tham, C.-S.; Fibiger, H. C.: Dopaminergic regulation of striatal acetylcholine release: importance of D1 and N-methyl-D-aspartate receptors. J. Pharmacol. Exp. Ther. 259:1064–1072; 1991.
13. Darney, K. J., Jr.; Lewis, M. H.; Brewster, W. K.; Nichols, D. E.; Mailman, R. B.: Behavioral effects in the rat of dihydrexidine, a high-potency, full-efficacy D_1 dopamine receptor agonist. Neuropsychopharmacology 5:187–195; 1991.
14. DeBoer, P.; Damsma, G.; Schram, Q.; Stoof, J. C.; Zaagsma, J.; Westerink, B. H. C.: The effect of intrastriatal application of directly and indirectly acting dopamine agonists and antagonists on the *in vivo* release of acetylcholine measured by brain microdialysis: the importance of post-surgery interval. Naunyn-Schmiedeberg Arch. Pharmacol. 345:144–152; 1992.
15. Domino, E. F.; Sheng, J.: Relative potency and efficacy of some dopamine agonists with varying selectivities for D_1 and D_2 receptors in MPTP-induced hemiparkinsonian monkeys. J. Pharmacol. Exp. Ther. 265:1387–1391; 1993.
16. Fibiger, H. C.; Damsma, G.; Day, J. C.: Behavioral pharmacology and biochemistry of central cholinergic neurotransmission. Adv. Exp. Biol. Med. 295:399–414; 1991.
17. Imperato, A.; Obinu, M. C.; Dazzi, L.; Gessa, G. L.: Does dopamine exert a tonic inhibitory control on the release of striatal acetylcholine in vivo? Eur. J. Pharmacol. 251:271–279; 1994.
18. Imperato, A.; Obinu, M. C.; Demontis, M. V.; Gessa, G. L.:

Cocaine releases limbic acetylcholine through endogenous dopamine action on D1 receptors. Eur. J. Pharmacol. 229:265–267; 1992.
19. Imperato, A.; Obinu, M. C.; Gessa, G. L.: Stimulation of both dopamine D_1 and D_2 receptors facilitates in vivo acetylcholine release in the hippocampus. Brain Res. 618:341–345; 1993a.
20. Kebabian, J. W.; Britton, D. R.; DeNinno, M. P.; Perner, R.; Smith, L.; Jenner, P.; Schoenleber, R.; Williams, M.: A-77636: a potent and selective dopamine D1 receptor agonist with antiparkinsonian activity in marmosets. Eur. J. Pharmacol. 229:203–209; 1992.
21. Kebabian, J. W.; Calne, D. B.: Multiple receptors for dopamine. Nature 277:93–96; 1979.
22. Levin, E. D.; Rose, J. E.: Interactive effects of D_1 and D_2 agonists with scopolamine on radial-arm maze performance. Pharmacol. Biochem. Behav. 38:243–246; 1991.
23. Lovenberg, T. W.; Brewster, W. K.; Mottola, D. M.; Lee, R. C.; Riggs, R. M.; Nichols, D. E.; Lewis, M. H.; Mailman, R. B.: Dihydrexidine, a novel selective, high potency full dopamine D-1 agonist. Eur. J. Pharmacol. 166:111–113; 1989.
24. McGurk, S. R.; Levin, E. D.; Butcher, L. L.: Dopaminergic drugs reverse the impairment of radial-arm maze performance caused by lesions involving the cholinergic medial pathway. Neuroscience 50:129–135; 1992.
25. Mottola, D. M.; Brewster, W. K.; Cook, L. L.; Nichols, D. E.; Mailman, R. B.: Dihydrexidine, a novel full efficacy D_1 dopamine receptor agonist. J. Pharmacol. Exp. Ther. 262:383–393; 1992.
26. Olton, D. S.; Wenk, G. L.: Dementia: animal models of the cognitive impairments produced by degeneration of the basal forebrain cholinergic system. In: Meltzer, H. Y., ed. Psychopharmacology: the third generation of progress. New York: Raven Press; 1987: 941–953.
27. Packard, M. G.; White, N. M.: Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. Behav. Neurosci. 105:295–306; 1991.
28. Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1982.
29. Perry, E. K.; Perry, R. H.; Blessed, G.; Tomlinson, B. E.: Necropsy evidence of central cholinergic deficits in senile dementia. Lancet i:189; 1977.
30. Perry, R. H.; Tomlinson, B. E.; Candy, J. M.; Blessed, G.; Foster, J. F.; Bloxham, C. A.; Perry, E. R.: Cortical cholinergic deficit in mentally impaired parkinsonian patients. Lancet ii:789–790; 1983.
31. Robertson, G. S.; Hubert, G. W.; Tham, C. S.; Fibiger, H. C.: Lesions of the mesotelencephalic dopamine system enhance the effects of selective dopamine D1 and D2 receptor agonists on striatal acetylcholine release. Eur. J. Pharmacol. 219:323–325; 1992.
32. Ruberg, M.; Ploska, A.; Javoy-Agid, F.; Agid, Y.: Muscarinic binding and choline acetyltransferase activity in parkinsonian subjects with reference to dementia. Brain Res. 232:129–139; 1982.
33. Sawaguchi, T.; Goldman-Rakic, P. S.: The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. J. Neurophysiol. 71:515–528; 1994.
34. Sawaguchi, T.; Goldman-Rakic, P. S.: D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251: 947–950; 1991.
35. Scatton, B.: Further evidence for the involvement of D_2 but not D_1 dopamine receptors in dopaminergic control of striatal cholinergic neurotransmission. Life Sci. 31:2883–2890; 1982.
36. Schneider, J. S.; Sun, Z.-Q.; Roeltgen, D. P.: Effects of dihydrexidine, a full dopamine D-1 agonist, on delayed response performance in chronic low dose MPTP-treated monkeys. Brain Res. 663:140–144; 1994.
37. Sibley, D. R.; Monsma, F. J.: Molecular biology of dopamine receptors. Trends Pharmacol. Sci. 13:61–69; 1992.
38. Simon, H.; Scatton, B.; Le Moal, M.: Dopaminergic A10 neurones are involved in cognitive functions. Nature 286:150–151; 1980.
39. Simon, H.; Taghzouti, K.; LeMoal, M.: Deficits in spatial memory tasks following lesions of the septal dopaminergic terminals in the rat. Behav. Brain Res. 19:7–16; 1986.
40. Spicer, M. A.; Elsworth, J. D.; Lawrence, M. S.; Roth, R. H.; Taylor, J. R.; Mailman, R. B.; Nichols, D. E.; Redmond, D. E., Jr.: Dihydrexidine in MPTP monkeys. Paper presented at the 1st International Meeting on Dopamine Agonists in Parkinson's Disease; 1993.
41. Stoof, J. C.; Kebabian, J. W.: Independent *in vitro* regulation by the D-2 dopamine receptor of dopamine-stimulated efflux of cyclic AMP and K^+-stimulated release of acetylcholine from rat neostriatum. Brain Res. 250:263–270; 1982.
42. Taylor, J. R.; Lawrence, M. S.; Redmond, D. E.; Elsworth, J. D.; Roth, R. H.; Nichols, D. E.; Mailman, R. B.: Dihydrexidine, a full dopamine D_1 agonist, reduces MPTP-induced parkinsonism in monkeys. Eur. J. Pharmacol. 199:389–391; 1991.
43. Vermersch, P.; Delacourte, A.; Javoy-Agid, F.; Hauw, J.-J.; Agid, Y.: Dementia in Parkinson's disease: biochemical evidence for cortical involvement using the immunodetection of abnormal tau proteins. Ann. Neurol. 33:445–450; 1993.
44. Watkins, P. B.; Zimmerman, H. J.; Knapp, M. J.; Gracon, S. I.; Lewis, K. W.: Hepatotoxic effects of tacrine in patients with Alzheimer's disease. JAMA 271:992–998; 1994.
45. Watts, V. J.; Lawler, C. P.; Gilmore, J. H.; Southerland, S. B.; Nichols, D. E.; Mailman, R. B.: Dopamine D_1 receptors: efficacy of full (dihydrexidine) vs. partial (SKF 38393) agonists in primates vs. rodents. Eur. J. Pharmacol. 242:165–172; 1993.
46. Whitehouse, P. J.: Pathology in the cholinergic basal forebrain: implications for treatment. Adv. Exp. Biol. Med. 295:447–452; 1991.
47. Whitehouse, P. J.; Price, D. L.; Clark, A. W.; Coyle J. T.; DeLong, M. R.: Alzheimer's disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann. Neurol. 10: 122–126; 1981.
48. Whitehouse, P. J.; Price, D. L.; Struble, R. G.; Clark, A. W.; Coyle J. T.; DeLong, M. R.: Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science 215:1237–1239; 1982.
49. Whitehouse, P. J.; Hedreen, J. C.; White, C. L. I.; Price, D. L.: Basal forebrain neurons in the dementia of Parkinson's disease. Ann. Neurol. 13:243–248; 1983.
50. Winkler, M. A.: Tacrine for Alzheimer's disease. Which patient, what dose? JAMA 271:1023–1024; 1994.