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Scopolamine Reversal of Nicotine Enhanced Delayed Matching-to-Sample Performance in Monkeys

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TERRY, A. V., JR., J. J. BUCCAFUSCO AND W. J. JACKSON. *Scopolamine reversal of nicotine enhanced delayed matching-to-sample performance in monkeys.* PHARMACOL BIOCHEM BEHAV **45**(4) 925–929, 1993.—The basis for the memory enhancing action of nicotine was evaluated in five adult monkeys (*Macaca fascicularis*) well trained in the performance of a delayed matching-to-sample (DTMS) paradigm. Nicotine (1.25–20 μg/kg, IM) produced a dose-dependent improvement in performance of the task. The optimal dose of nicotine for each monkey also improved performance when the animals were tested 24 h later in the no-drug situation. In the same animals, low doses of scopolamine produced a dose-dependent decrement in DTMS performance. A subthreshold dose (defined by DMTS performance decrement) of scopolamine was administered 20 min prior to the optimal dose of nicotine. Scopolamine pretreatment completely blocked the enhanced performance observed earlier with nicotine. The results of this study are consistent with the hypothesis that the enhanced cognitive performance associated with nicotine is due to central acetylcholine release and subsequent muscarinic receptor stimulation.

Nicotine Scopolamine Monkey Learning and memory Matching-to-sample
Cholinergic Alzheimer's disease

A GROWING body of evidence is consistent with the hypothesis that nicotinic receptors in the mammalian central nervous system are associated with presynaptic cholinergic nerve terminals and that these receptors may participate in the regulation of acetylcholine release (7,20,23). As early as 30 years ago, Day and Vane (9) hypothesized that part of the stimulatory activity of nicotine was due to its ability to enhance acetylcholine release. In addition, studies in human and nonhuman primates have demonstrated a significant facilitory effect of nicotine in certain tasks involving memory and cognition (5, 26). Because it is well established that brain acetylcholine plays an important role in learning and memory, the mechanism underlying this beneficial action of nicotine may be due to a central facilitation of cholinergic neuronal activity. Some evidence for a role of muscarinic receptors in mediating the memory enhancing actions of nicotine in animals and humans has already been presented (18,26). Thus, acetylcholine release stimulated by nicotine may, in turn, stimulate muscarinic receptors via a presynaptic positive feedback mechanism as described in a number of studies (2,3,7,8,23). Because one of the neurochemical abnormalities demonstrated to exist in the postmortem brains of Alzheimer's patients is a loss of cholinergic nicotinic receptor density (12,22,28); and selective blockade of central nicotinic receptors has been shown to produce memory impairment in rodent animal models (1,10,13,16) nonhuman primates (10) and in humans (25) it is of interest to further study the mechanisms involved in nicotinic receptor stimulation related to cognition.

Recently, as observed in this laboratory, performance of a delayed matching-to-sample (DMTS) task in monkeys was enhanced after administration of low (μg/kg) doses of nicotine (5). This dose range is consistent with that in humans, which provides improvement of learning and memory (26). In the present study, the basis for this memory enhancing action of nicotine was evaluated in five additional young monkeys (*Macaca nemestrina*) in this DMTS paradigm. Based on the hypothesis that this performance enhancing property of nicotine is due to acetylcholine release and subsequent stimulation of muscarinic receptors, and that scopolamine is a muscarinic antagonist and well-known amnestic, experiments were designed to determine whether scopolamine inhibits the facilitory action of nicotine on DMTS performance.

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METHOD

Subjects

Five young adult monkeys (*Macaca nemestrina*, 10–15 years) 4 male, 1 female, served as subjects. The monkeys were colony-reared at the Washington Regional Primate Center and are currently housed at the Animal Behavior Center of the Medical College of Georgia. The facilities of the Animal Behavior Center exceed current Federal standards for nonhuman primate housing. Monkeys are housed within individual stainless steel cages, which were composed of 50 × 28 × 26 inch units. Two units were connected for smaller animals (up to 6 kg) and four units were connected for larger animals (up to 14 kg). Toys are provided routinely and the monkeys are allowed to observe television programs each afternoon as a means of promoting psychological well-being. During periods when animals are not tested routinely (e.g., during quarantine after arrival, or during washout from drug studies) they are allowed access to an enclosed outdoor exercise facility on an individual or selected-group basis. Both cages and exercise facilities contain perch bars and play objects.

Animals were generally maintained on unlimited water, standard laboratory monkey chow, and fresh fruits and vegetables. During the work-week, the animals were maintained on a feeding schedule that allowed approximately 15% of their normal daily food intake to be derived from the food pellets (commercial composition of standard monkey chow and banana flakes, P.J. Noyes, Inc., Lancaster, NH), which served as rewards during experimental sessions. The remainder of the food was made available following each test session. On holidays, Friday evenings, Saturday morning and evening, and Sunday mornings the animals were fed extra quantities.

Behavioral Testing: Delayed Matching-to-Sample

Behavioral testing was conducted essentially as described previously (5). At the start of DMTS sessions test panels were attached to the home cage, and testing was conducted in a dimly illuminated room. Up to four animals per room were tested simultaneously using a computer-automated training and test system, which not only presented the DMTS task, but also measured and categorized a number of parameters for each DMTS trial. DMTS stimuli were 25- cm diameter colored disks (red, green, and yellow) presented via light-emitting diodes located behind clear push-keys. Sessions consisted of 96 trials/day and were conducted 5 days per week.

A trial began with illumination of the sample key by one of the colored stimuli. The sample remained illuminated until the animal responded to the sample key. A key press by the animal extinguished the sample light and initiated the delay interval, during which no keys were illuminated. Following a preprogrammed delay interval, two choice lights located below the sample key were then illuminated. One of the choice stimuli always matched the previously presented sample light, while the nonmatching choice was one of the other two colors. The choice stimuli, but not the sample stimulus, remained illuminated until the animal depressed one of the choice keys. Responses to the choice key illuminated by the color that matched the previously presented sample panel were rewarded by a 300 mg banana-flavored pellet. Responses to the nonmatching choice key were neither rewarded nor punished, but simply followed by the next trial. A noncorrective procedure was used throughout the study; therefore, the next trial involved a different stimulus configuration. Four possible delay intervals between a monkey's response to the sample stimulus and the presentation of the two choice stimuli were used: zero delay and three longer delay intervals, which hereafter will be referred to as short, medium, and long delays. The animals were trained until performance using zero delay trials averaged approximately 85–100% correct. Short, medium, and long delays were adjusted in length to produce stable performance levels that approximated the following performance levels: Short delay (75–85% correct); Medium delay (65–75% correct); and Long delay (55–65% correct). Following this procedure, the length of delays for each animal varied according to skill level. The overall mean for all five monkeys for short delays was 7.00 ± 2.42 s, for medium delays 36.60 ± 12.32 s, and for long delays, 69.00 ± 24.72 s. Drug administration did not begin until each animal's matching performance was at the apparent limit of that individual's ability. The delays ranged from as little as 0–25 s to a maximum of 0–160.

Drug Administration

Drugs or vehicle (sterile, normal saline) were administered IM (gastrocnemius muscle) in a volume of 0.3 ml Drug effects were calculated as the absolute change from each individual's matched baseline. Baseline data were obtained following administration of vehicle (sterile, normal saline), and each monkey served as its own control. Test sessions began 10 min after nicotine or vehicle injection. In the case of experiments involving scopolamine pretreatment, two consecutive vehicle injections spaced 20 min apart were made and testing begun 10 min after the second injection. A minimum drug "wash out" period of 2 days was allowed between sessions in which nicotine was administered and two weeks between nicotine and the regimen including scopolamine. During this period a return to baseline DMTS performance was established in each animal before again administering drug. Each monkey received the following doses of nicotine: 1.25, 2.5, 5.0, 10.0, 15.0, and 20.0 μg/kg. Each dose was replicated at least once and the optimal dose (producing the greatest enhancement over baseline) was also determined for each animal. In addition, a dose-response study was also performed for scopolamine. Doses of 1.25, 2.5, 5, 10, and 25 μg/kg were evaluated. A low dose of scopolamine (which did not significantly affect baseline performance) was later used for the scopolamine-nicotine regimen. All doses are expressed as the respective salts: nicotine bitartrate and scopolamine hydrobromide.

For statistical analysis the performance during vehicle sessions preceded by drug administration were compared to the values obtained during the previous day's testing and to the following session 24 h later using a two-way analysis of variance (ANOVA) with repeated measures and Student's *t*-test for paired data.

RESULTS

Baseline Data

The monkeys used in this study were trained to meet the performance criteria for each of the delays in the DMTS task as described in the Method section. Analysis of baseline data yielded the following results: zero delay trials averaged 98.8

± 0.37% correct; short delays averaged 83.8 ± 2.8% correct; medium delays averaged 75.1 ± 3.4% correct; and long delays averaged 59.0 ± 1.4% correct.

Nicotine

The results confirm previous findings (5,10) in which DMTS performance was enhanced in the presence of nicotine. Low, μg/kg doses of nicotine produced a dose-dependent improvement in the animals' overall (all delays combined) performance of the DMTS task (Fig. 1). The maximal effect, on average, occurred at the 15-μg/kg dose. The improvement observed with the 20-μg/kg dose was essentially due to enhanced DMTS performance by only two of the animals. While no visible behavioral side effects were noted to be associated with the 20-μg/kg dose, this dose apparently was limiting for three of the monkeys who did poorly, or failed to complete all 96 trials of their sessions.

The "optimal dose" (the dose that elicited the highest improvement for each test subject) of nicotine was 5 μg/kg for three of the animals and 15 μg/kg for the remaining two. The improvement in DMTS performance associated with each optimal dose of nicotine in the five animals was averaged and expressed according to delay intervals. As indicated in Fig. 2, the greatest improvement was found at the longer, presumably more difficult delay intervals. In fact, nicotine enhanced performance at the long delay intervals by 15.14% points/session above baseline at 10 min after injection. This improvement was slightly greater than observed in our previous study in which a 10.50% point change in long-delay performance was obtained (5). Another interesting finding that was also observed in the previous study was that when the animals were tested on the day following nicotine administration (24 h later), significant enhancement was still observed for the longer delay intervals (11.50% points/session, Fig. 2).

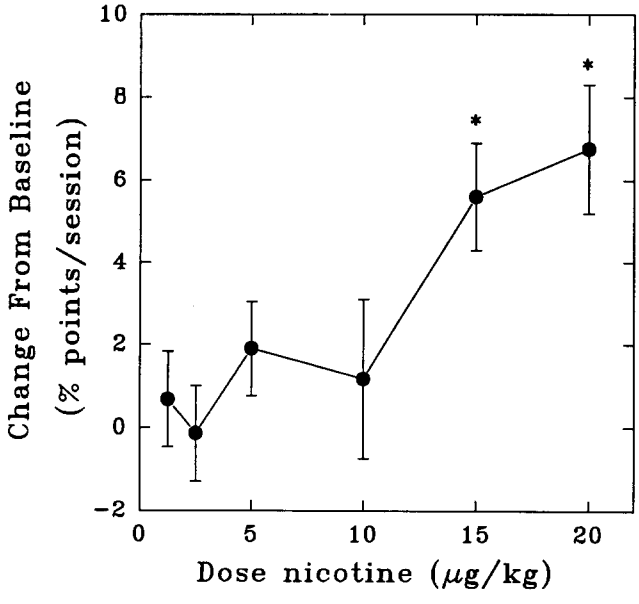

FIG. 1. The effect of several IM doses of nicotine administered to monkeys on their overall performance of the delayed-matching-to-sample (DMTS) task. Each point represents the mean of 2-3 replicates performed in each of the five animals ± SEM except for the 20 μg/kg dose, which could only be tolerated by two of the animals. * = significantly ($p < 0.05$) from baseline (saline) performance.

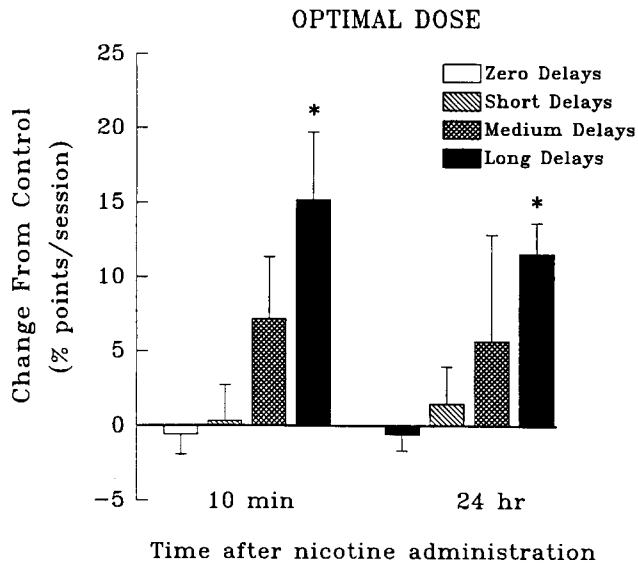

FIG. 2. Change in performance for each delay interval of the DMTS task by five monkeys following IM injection of the individualized optimal dose of nicotine. The 10 min time point refers to testing 10 min after drug administration and the 24 h time point refers to testing with no drug on the following day. Error bars refer to the SEM. * = significantly ($p < 0.05$) from baseline (saline) performance.

Scopolamine Pretreatment

In a separate group of experiments in the same animals, scopolamine produced a dose-dependent decrement in the DMTS performance over the dose range 1.25-25.0 μg/kg (Fig. 3). Higher doses were not used because we were interested in defining a subthreshold dose for producing the decrement in performance. A subthreshold dose of scopolamine was used to obviate the possibility that any inhibition of nicotine-induced enhancement of DMTS performance was due to nonspecific physiological antagonism. The dose that was selected, 2.5 μg/kg, did not significantly affect baseline performance. This dose was then administered 20 min prior to nicotine (optimal dose) and found to completely block any improvement in DMTS performance in response to nicotine irrespective of delay (Fig. 4). Scopolamine pretreatment also inhibited improvement remaining 24 h after nicotine injection. At the long delays, DMTS performance in the presence of the combination of scopolamine and nicotine actually dropped below baseline although this effect did not reach statistical significance.

DISCUSSION

A credible body of evidence has accumulated in the past several years indicating the importance of central nicotinic receptors in the process of learning and memory. While the central muscarinic receptor system has been studied quite extensively with respect to learning, memory and cognition, less is known about central nicotinic receptors in this regard. The evidence supporting a nicotinic-muscarinic interaction underlying the expression of behavioral and physiological responses to cholinergic stimulation is even less understood.

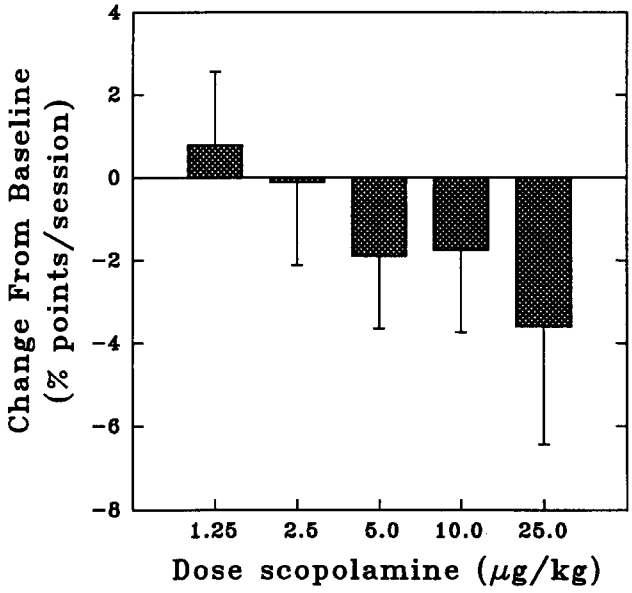

FIG. 3. The effect on overall performance of several IM doses of scopolamine hydrobromide in monkeys administered 30 min prior to testing in the DMTS task. Each bar represents the mean of 2-3 replicates performed in each of the five animals ± SEM. $p = 0.214$.

The findings of this investigation confirm results from an earlier study (5,10) in a different group of monkeys that nicotine produces a significant enhancement of performance in the DMTS task. Moreover, pretreatment with the nicotinic antagonist, mecamylamine (but not the peripherally selective antagonist, hexamethonium) had been found to block the nicotine-induced DMTS enhancement. At higher doses mecamylamine itself produced a deleterious effect on memory performance in nonhuman primates (10) and rodents (11). In the latter study, mecamylamine-induced inhibition of a delayed avoidance task was correlated with the ability of the drug to inhibit the biosynthesis of brain acetylcholine. Thus, nicotinic antagonists may decrease acetylcholine release by interfering with a nicotinic receptor-mediated positive feedback system (11).

As observed previously in this laboratory (5,10) nicotine was most effective in enhancing the longest delay intervals, which are presumably the most difficult with respect to recall for the monkeys. In addition, this delay-specific effect may be related to a potential ceiling effect at the zero and short delays. The basis for the improvement in performance that is still evident after 24 h is unknown, but similar long-lasting effects of nicotine have been observed by other investigators (17). This effect may be may be due to a circulating metabolite of nicotine. Several metabolites of nicotine have been demonstrated to possess considerable pharmacological activity (4). Alternatively, nicotine has been demonstrated to induce a trophic action in several neuronal systems (14,19,24), an effect that could lead to more long-term alterations in memory function.

The beneficial mnemonic action of nicotine produced in our subjects is particularly encouraging because subape primates represent the best available models for human memory and cognitive function, with the exception of higher apes. Moreover, the complete reversal of this improvement in the presence of a low dose of scopolamine provides additional evidence to support the hypothesis that the memory enhancing properties of nicotine is expressed through muscarinic receptor activation, subsequent to acetylcholine release. An earlier, related study in humans demonstrated that nicotine-induced enhancement of state-dependent learning can also be blocked by scopolamine (27). In addition, scopolamine has also been shown to block nicotinic-induced enhancement of memory function in rats (18). The ability of a muscarinic antagonist to reverse a response to nicotine has also been observed in cardiovascular studies. The hypertensive response to central injection of nicotine in unanesthetized rats was found to be inhibited in animals pretreated with atropine (6).

It should also be pointed out, however, that while we suggest a nicotinic-muscarinic interaction as the basis for the cognitive effects in the DMTS paradigm, nicotine has been shown to produce a host of pharmacological actions that may involve learning and memory. In addition to the effects on acetylcholine release, evidence has been presented that nicotine, either directly or through acetylcholine release, is capable

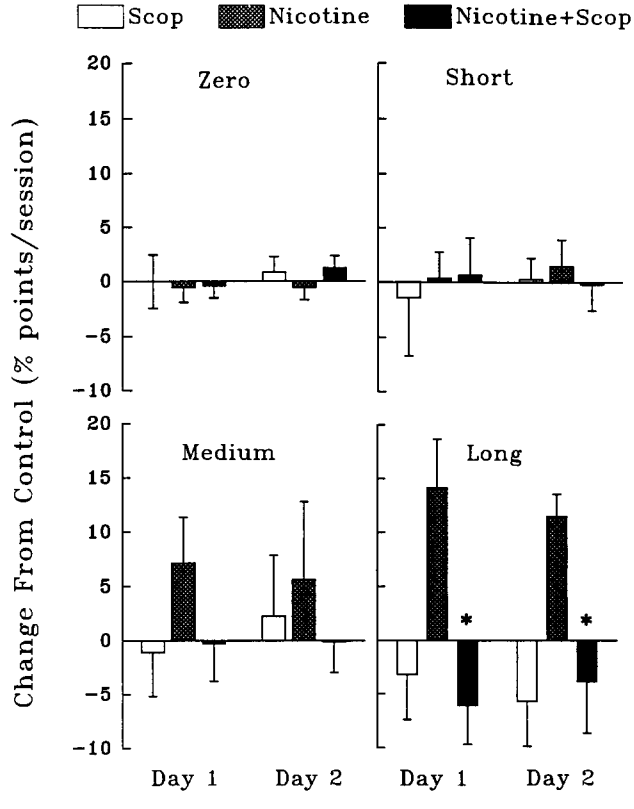

FIG. 4. The effect of pretreatment with a subthreshold (in terms of decrement in the DMTS performance) dose of scopolamine (2.5 μg/kg) administered 20 min prior to nicotine (optimal dose) on DMTS performance. Open bars indicate average performance after scopolamine administered alone. Hatched bars indicate average performance of animals after nicotine administered alone. Filled bars indicate response to nicotine after scopolamine pretreatment. Day 1 refers to testing the same day after drug administration. Day 2 refers to testing 24 h after drug administration. * = significantly different from nicotine (alone) response.

of releasing norepinephrine, dopamine, serotonin and GABA (15), all of which may participate in the memory process. It is likely that nicotine's actions, which affect learning and memory processes, involve a cascade of neurotransmitter interactions. However, because it is well known that cortical nicotinic and M_2 receptors are diminished in Alzheimer's disease (12,21,29,30) and that there may be a significant interaction between these receptors in the process of learning and memory, further studies providing a better understanding of these processes are certainly warranted. New therapeutic strategies that target these receptor interactions may, thus, prove beneficial.

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