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Improvement in performance of a delayed matching-to-sample task by monkeys following ABT-418: a novel cholinergic channel activator for memory enhancement

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Abstract ABT-418, a newly characterized centrally acting cholinergic channel activator (ChCA), was evaluated for its ability to improve performance in a delayed matching-to-sample (DMTS) task by mature macaques well trained in the task. Previous studies in rodents have indicated that ABT-418 shares the memory/cognitive enhancing actions of nicotine, but without many of nicotine's dose-limiting side effects. As DMTS provides a measure both of general cognitive function (the matching concept) and of recent memory, it was hypothesized that some doses of ABT-418 would enhance the monkeys' ability to correctly perform the DMTS task. Intramuscular administration of ABT-418 significantly enhanced DMTS performance at low (2–32.4 nmol/kg) doses. In fact, the drug was slightly more potent that nicotine in this regard, and all eight animals tested in this study exhibited enhanced performance at one or more doses. ABT-418 produced the greatest improvement in DMTS performance at the longest delay interval. In animals repeatedly tested with their individualized "Best Dose", DMTS performance increased on average by 10.1 ± 3.5 percentage points correct, which was equivalent to an increase of 16.2% over baseline performance. ABT-418 did not significantly affect response times, i.e., latencies to make a choice between stimuli, or latencies to initiate new trials. Whereas nicotine enhanced DMTS performance both on the day of administration and on the following day (in the absence of drug), ABT-418-induced enhanced performance was detected only on the day of administration. Finally, single daily administration of the individualized best dose in three monkeys over a period of 8 days generally maintained enhancement of DMTS performance. Thus, the data were not consistent with the development of significant tolerance to the drug's mnemonic actions. In contrast to nicotine, no overt toxicity or side effects to acute or repeated administration of the drug were noted. Thus, ABT-418 represents a prototype of a new class of nicotinic agonists designed for the potential treatment of human dementias having a low profile of toxicity.

Key words Nicotine · Nicotinic acetylcholine receptors (nAChRs) · Learning and memory · Cognition · Monkey · Delayed-matching

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Introduction

Over the last few years the knowledge concerning the brain nicotinic acetylcholine receptor (nAChR) system has increased dramatically. Progress has been made on many fronts, including neurochemistry, ligand binding sites, molecular biology and pharmacology (see Levin 1992; Arneric et al. (1994). Of recent interest has been the role of central nAChRs in normal and pathologic cognitive processing. Postmortem brain tissue receptor binding studies in Alzheimer's disease have demonstrated significant abnormalities of the nicotinic cholinergic system (Flynn and Mash 1986; Nordberg and Winblad 1986; Quirion et al. 1986; Whitehouse et al. 1986; Schroder et al. 1991). Ligand binding studies with normal human and rat brain tissue have revealed that the highest levels of nAChR binding are found in the nucleus basalis of Meynert (Shimohama et al. 1985) and thalamus (Adem et al. 1987). The nucleus basalis

is part of the basal forebrain cholinergic system which provides the major source of extrinsic cholinergic input to the frontal cortex, and is known to be involved in neuronal processes mediating cognitive function including memory function (Butcher and Wolf 1986). Interestingly, this region is known to undergo extensive degeneration in Alzheimer's disease (Whitehouse et al. 1981; Etienne et al. 1986; Younkin et al. 1986). The possibility exists, therefore, that nAChRs may be associated with, or located on, cholinergic fibers projecting to the cortical regions which are involved in Alzheimer's disease.

While the functional role of such nAChRs is largely unexplored, several possibilities are that they may serve as a positive feedback mechanism for neurotransmitter release (Chiou 1973; Balfour 1982; Briggs and Cooper 1982; Beani et al. 1985; Wessler and Kilbinger 1986; Wessler et al. 1986), enhancement of central neurogenic pathways regulating cortical cerebral circulation (Linville and Arneric 1991; Linville et al. 1993), or activation/enabling of neural systems that integrate incoming sensory/motor information (McCormick 1990). Nicotine enhances cognitive function in normal rats (Levin et al. 1990; Levin 1992) and attenuates memory deficits produced by destruction of cholinergic input to the cortex and hippocampus (Tilson et al. 1988; Decker et al. 1992; Hodges et al.1992), an effect shared by some other nAChR agonists (Decker et al. 1993; Meyer et al. 1994). In addition, nicotine improves short term memory performance in both young and aged monkeys (Elrod et al. 1988; Buccafusco and Jackson 1991). The involvement of nicotinic neurotransmission in cognitive function processes is further substantiated by observed deficits in cognitive performance following administration of mecamylamine, an nAChR channel blocker, to rodents (Oliverio 1966; Levin et al. 1987; Riekkinen et al. 1990; Decker and Majchrzak 1992), monkeys (Elrod et al. 1988), and humans (Newhouse et al. 1992). Perhaps the strongest evidence for the potential of central nicotinic stimulation to enhance cognitive performance in Alzheimer's disease patients derives from several recent clinical trials. In one study, nicotine was infused intravenously in six patients (Newhouse et al. 1988). The (total) dose range employed, 7.5–30 μg/kg, was similar to that which was effective in our earlier studies in primates (Elrod et al. 1988; Jackson et al. 1989). They reported a significant decrease in intrusion errors in cognitive tests and concluded that "...central nicotinic cholinergic stimulation deserves further investigation as a treatment in Alzheimer's disease and that nicotine may also be a useful investigative tool in other populations as a probe of central cholinergic function...". Similar conclusions were reached after a similar study by Sahakian and coworkers (1989) following subcutaneous administration.

Nicotine, however, has limited utility as a therapeutic agent in Alzheimer's disease because of its dose-limiting side-effects in humans, that are primarily gastrointestinal (e.g. nausea, abdominal pain) and cardiovascular (e.g. increased catecholamine release, tachycardia, peripheral vasoconstriction and elevated blood pressure) in nature. In an aged patient population such side effects may result in more serious complications (Benowitz 1992), especially in patients with pre-existcling arrhythmias or angina pectoris. Compounds that selectively interact with subtypes of nAChRs might be expected to elicit a greater degree of selectivity of action than nicotine.

ABT-418 [(*S*)-3-methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole] is a novel bioisotere of (–)-nicotine that selectively activates mammalian brain nAChRs (Arneric' et al. 1994; Garvey et al. 1994) and is in clinical development for the treatment of Alzheimer's disease. Preclinically, this compound shares many of the positive CNS attributes (e.g. memory enhancing action) of nicotine, but exhibits a reduced propensity to elicit the side effects that limit the potential of nicotine for the safe treatment of Alzheimer's disease (Decker et al. 1994a, b; Garvey et al. 1994). The purpose of this study was to determine whether ABT-418 can enhance performance of the DMTS task in mature monkeys, to establish a relative range of effective plasma concentrations, and to compare this action with that of nicotine.

Materials and methods

General procedures

Non-human primate housing facilities at the Medical College of Georgia are located in a secure and remote site. 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 periods from drug studies) they are allowed access to an enclosed outdoor exercise facility (floor area of 1260 ft^2; 30 ft high at the apex) on an individual or selected-group basis. Both cages and exercise facilities contain perch bars and play objects.

During this test series, the monkeys were maintained on unlimited water, standard laboratory monkey chow, fresh fruits and fresh vegetables. During the test-week, the animals were maintained on a feeding schedule which 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), 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.

Subjects and drug administration

The subjects for this study were mature (ages 10–20 years) animals, four males and two female *Macaca nemestrina* (pig-tailed monkeys)

and two male *Macaca mulatta* (rhesus monkeys). All pigtail monkeys were colony reared at the Washington Regional Primate Center and the rhesus monkeys were colony reared at the University of Sourthwestern Louisiana, New Iberia Research Center. All animals performed the DMTS task for at least 1 year prior to this study. They had previously participated in one or more short-term studies of memory enhancing agents. Prior drug experience had produced no untoward effects in the animals, and they were allowed at least a 1-month washout period and re-establishment of typical baseline performance prior to the onset of this study.

Doses of ABT-418 and (–)-nicotine were administered in the gastrocnemius muscle within a volume of 0.1 ml saline/kg. Control vehicle sessions involved the administration of 0.1 ml saline/kg only. After drug or control vehicle injection, test panels were attached to the front of the animal's home cage, and the session began 10 min following the injection. A minimum drug "wash-out" period of 2 days was maintained between various ABT-418 administrations. In general, saline was administered each Monday, drug was administered on Tuesdays and Thursdays (except for chronic studies), and no drug was administered on the remaining days of the week. Since the animals were overtrained in the DMTS task, there was never any effect of day of the week on performance levels. For repeated ABT-418 administration, the drug was given IM each day at about the same time of day for 8 days.

Behavioral testing: delayed matching-to-sample

For DMTS sessions the test panels were attached to the home cages, and while testing was in progress, the room lights were dimmed to a low level. Up to four animals per room were tested simultaneously using a computer-automated training and test system which has been described in more detail elsewhere (Buccafusco and Jackson 1991). Various categories of performance data were collected and stored in a data file, and then imported into a spreadsheet (Microsoft Excel, Bothell, Wa.). Included were: the percent correct performance at each of four delay intervals, the latency of response at each step of individual trials (both for correct and incorrect trials) and the percent correct for left and right choice position. Stimuli were 2.54-cm diameter colored disks (red, yellow and green) presented via light-emitting diodes located behind clear push keys. Sessions consisted of 96 trials. 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 a pre-programmed delay interval, during which no keys were illuminated. Following the delay interval, two choice lights located below the sample key were then illuminated. One of the choice stimuli always matched the hue of the previously presented sample light, while the non-matching choice was one of the other two colors. The choice stimuli remained illuminated until the animal depressed one of the choice keys. Responses to the choice key illuminated by the color matching the color of the previously presented sample key were rewarded by a 300-mg banana-flavored pellet. Non-matching choices were neither rewarded nor punished, but simply followed by the next trial. A non-corrective procedure was used throughout the study; therefore, the next trial involved a different stimulus configuration. There are four possible delay intervals between a monkey's response to the sample stimulus and the presentation of the two choice stimuli: zero delay and three longer delay intervals, which hereafter will be referred to as short, medium, and long delays. Each stimulus color configuration occurred in conjunction with each delay interval an equal number of times. The animals were trained until performance for zero delay trials averaged approximately 85–100% correct. Short, medium and long delays were adjusted in length to produce stable performance levels which 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 delays ranged from as little as 0–25 to a maximum of 0–160 s (Table 1). The rational for this procedure is to normalize DMTS performance, since the capabilities of monkeys to perform such problems vary greatly. The side of the correct response (either left or right) followed controlled sequences (Gellerman 1933) to ensure exactly 50% correct (chance) reward ratios for spatial strategies – including position habits and double/single alternation. In addition, the position of each color was counterbalanced so that each color in relation to each of the other colors was presented an equal number of times. Each color was paired an equal number of times with each of the other colors – both as correct stimulus and incorrect stimulus. This counterbalanced arrangement of stimulus configuration prevents the success of such strategies as the animal positioning in front of the left choice following a "Red" stimulus, etc. Each color combination was presented an equal number of times in conjunction with each of the four delays to comprise 24 combinations of stimulus configuration and delay interval. These 24 combinations were repeated four times/session to comprise 96 trials/session.

Table1 DMTS delay intervals employed for each animal in the study

Primate subject		Delay intervals (s)[a]		
		Short	Medium	Long
#12	Female pigtail	20	40	80
#146	Male pigtail	3	15	30
#242	Male pigtail	12	60	120
#270	Male pigtail	20	80	160
#284	Male pigtail	8	40	80
#13	Female pigtail	12	60	120
#215	Male rhesus	5	10	25
#446	Female rhesus	5	10	20
Average		10.6	39.4	79.4

[a] A 0-s delay interval was also employed for each animal

Pharmacokinetic studies

The pharmacokinetic behavior of ABT-418 and (–)- nicotine following intramuscular injection was evaluated in groups of female *Macaca fascicularis* (long-tailed macaque) 2.6–4 kg in weight. The animals were selected from the established Drug Analysis colony which is maintained within the primate facility at Abbott Laboratories. Each monkey was housed within individual stainless steel cages containing perch bars and play objects in accordance with the specifications of the Institutional Animal Care and Use Committee (IACUC); paired housing between amicable animals was utilized between study periods. The animals were fasted overnight prior to dosing but were permitted water ad libitum; food was returned to each animal approximately 3 h after drug administration. Solutions of ABT-418 (as the hydrochloride salt) or (–)-nicotine (as the bitartrate salt) were prepared in normal saline at concentrations appropriate for a 0.1 ml/kg dose volume in each monkey. In a series of sequential studies, groups of monkey (n = 3–5) received single intramuscular injections of ABT-418 (20, 100 or 500 nmol/kg doses) or (–)-nicotine (100 or 500 nmol/kg doses). Heparinized blood samples were obtained from a femoral artery or vein of each monkey prior to dosing and 0.08, 0.16, 0.33, 0.5, 1, 1.5, 2, 3, 4 and 6 h after drug administration. A washout/recovery period of at least 6 weeks separated each dose.

The heparinized blood samples obtained from each monkey were immediately chilled in an ice bath. Plasma was separated from the red cells by centrifugation (2500 rpm, 10 min, 4°C) and frozen (–30°C) until analysis. ABT-418 and (–)-nicotine were selectively removed from monkey plasma using liquid-liquid extraction under alkaline conditions. A plasma aliquot (1.0 ml) was combined with

an aliquot of internal standard (0.1 ml), 0.5 ml 0.5 M Na_2CO_3 and 6 ml ethyl acetate: hexane (1:1, by volume). The samples were vortexed vigorously for 20 s followed by centrifugation at 2500 rpm for 10 min (4°C). The upper organic layer was transferred to glass centrifuge tubes containing 0.3 ml 0.01 N HCl. The samples were vortexed for 20 s and centrifuged as described above. The upper organic layer was aspirated to waste. The aqueous layer was transferred to auto sampler vials for HPLC analysis. The compounds of interest and respective internal standards were separated from plasma contaminants on a 15 cm × 4.6 mm 5 μm Spherisorb ODS-AQ column (YMC) with an acetonitrile: methanol; buffer mobile phase at an isocratic flow rate of 1.0 ml/min. The mobile phase buffer consisted of 0.05 M phosphate buffer in 0.01 M tetramethylammonium hydroxide; the buffer was adjusted to pH 6.9 prior to addition of the organic mobile phase components. Quantitation of the analytes was accomplished with an electrochemical detector (ESA) run in the oxidative mode (+ 0.8 V). The drug concentration in each plasma sample was calculated by least-squares linear regression (unweighted) analysis of the peak area ratio (ABT-418 or nicotine/internal standard) of the spiked plasma standards versus concentration. The spiked plasma standards were assayed simultaneously with the samples. All plasma concentrations are reported in terms of the base. The assays for each compound were linear(correlation coefficient > 0.99) over the concentration range 0–150 ng/ml, with a mean percent standard deviation < 6% for the analysis of triplicate standards at six separate concentrations and an estimated limit of quantitation of ~ 0.05 ng/ml (ABT-418) or ~ 1 ng/ml (nicotine). The peak plasma concentrations (C_{max}) and time to peak plasma concentration (T_{max}) were observed experimental values. Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. The plasma elimination half-life was estimated from the log linear regression of the terminal plasma concentrations as a function of time.

Statistics

Data derived from more than two treatment groups were anlayzed using one-or two-way analysis of variance (ANOVA) with repeated measures. In cases where significant differences were found or to compare only two treatment groups, a paired Student's *t*-test was employed to determine which group means differed. Means were considered to be statistically significant at the $P < 0.05$ level. Statistical comparisons for all behavioral studies were made using the baseline data (not change from baseline). All data is presented at the mean ± SEM.

Drugs

(–)-Nicotine hydrogen tartrate was purchased from Sigma (St Louis, Mo.), and ABT-418 [(*S*)-3-methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole hydrochloride] was prepared as described previously (Garvey et al. 1994). When doses are expressed in μg, this refers to the respective salt. Solutions of nicotine or ABT-418 were prepared daily in sterile normal saline.

Results

The eight animals employed in this study exhibited DMTS baseline performance according to the criteria mentioned above prior to saline or drug administration. Performance for the group in the presence of saline administration averaged 97.3 ± 1.1, 82.8 ± 2.2, 68.6 ± 2.7 and 58.0 ± 2.5% correct, respectively for 0, short, medium and long delay intervals (see inset, Fig. 4). To obtain these values, saline runs for each animal over the course of the repeat "Best Dose" series (see below) were averaged.

ABT-418 individualized Best Dose

Five animals received doses of ABT-418 in an ascending series as follows: 2.0, 4.1, 8.1, 16.2, and 32.4 nmol/kg. The animals employed in the series included one female and four male pigtail monkeys. An ascending dose series was employed initially, since there was no prior experience with the effect of the drug on cognitive performance in monkeys. In such cases we elect not to begin with high doses (which could be encountered using a purely random series), since any unexpected side effect might preclude further testing, even with lower doses. The performance of the DMTS task by the five monkeys was significantly improved following administration of ABT-418. The Best Dose was selected as being that which provided the greatest benefit across all delay intervals. The dose eliciting the greatest enhancement (Best Dose) ranged from 2.0 to 32.4 nmol/kg for the five animals, with the average Best Dose for the group being 15.0 nmol/kg (Table 2).

Figure 1 presents the best dose data separated for performance at each of the delay intervals. ABT-418 produced an increase in DMTS performance only at long delay intervals. Performance was significantly increased by 17.7% points correct for the 96-trial session. The improvement was only observed on the day of drug administration as no significant improvement in DMTS performance was noted 24 h after drug administration.

After a minimum 2-week washout period, (–)-nicotine was administered to the same animals in an increasing dose series as follows: 2.7, 5.4, 10.8, 21.6, 32.4 and 43.2 nmol/kg. The selected best dose ranged from 10.8 to 32.4 nmol/kg for the five animals, with the average best dose for the group being 19.4 nmol/kg (Table 2). Figure 2 presents the data derived from the best dose analysis following (–)-nicotine administration. Comparison of Fig. 1 versus Fig. 2 illustrates the similar behavioral effects of the two compounds measured on the day of administration. Improvement at long

Table 2 Summary of the doses of nicotine or ABT-418 selected from an ascending dose series. The best dose was that dose which provided the greatest enhancement of overall performance in the DMTS task

Animal ID	Best dose nicotine	Best dose ABT-418
# 12 (female)	5 μg/kg (10.8 nmol/kg)	1.6 μg/kg (8.1 nmol/kg)
# 146 (male)	15 μg/kg (32.4 nmol/kg)	6.6 μg/kg (32.4 nmol/kg)
# 242 (male)	5 μg/kg (10.8 nmol/kg)	0.4 μg/kg (2.0 nmol/kg)
# 270 (male)	5 μg/kg (10.8 nmol/kg)	3.3 μg/kg (16.2 nmol/kg)
#284 (male)	15 μg/kg (32.4 nmol/kg)	3.3 μg/kg (16.2 nmol/kg)
Averages	9 μg/kg (19.4 nmol/kg)	3 μg/kg (15.0 nmol/kg)

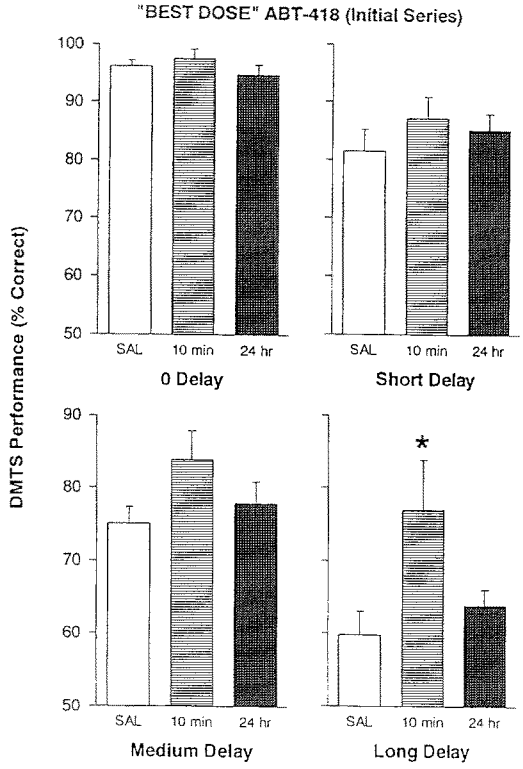

Fig. 1 Effect of ABT-418 on delayed matching-to-sample (*DMTS*) performance in 5 monkeys as a function of delay interval. The best dose was that which was selected from an ascending series of five doses which provided the greatest enhancement of overall performance in the DMTS task. *SAL* refers to DMTS performance after injection of the saline vehicle, *10 min* refers to data obtained when the testing began 10 min after compound administration. *24 hr* refers to testing 24 h after the administration of compound. Values are means ± SEM. * Significantly different from baseline performance ($P < 0.05$)

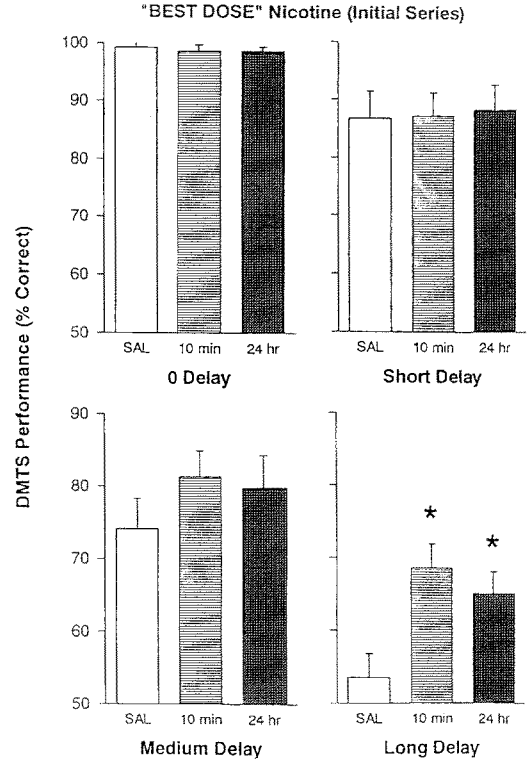

Fig. 2 Effect of (–)-nicotine on delayed matching-to-sample (*DMTS*) performance in five monkeys as a function fo delay interval. These animals were the same subjects employed in experiment presented in Fig. 1. The Best Dose was that which was selected from an ascending series of six doses which provided the greatest enhancement of overall performance in the DMTS task. Values are means ± SEM. * Significantly different from baseline performance ($P < 0.05$)

delay intervals was similar to that for ABT-418, that is, an increase of 15.1% points correct for the session. One marked difference between the effects of ABT-418 and nicotine is that nicotine produced a significant improvement in DMTS performance in animals tested 24 h later. In fact, in the absence of drug, performance at long delay intervals for the 24-h point was significantly greater than baseline DMTS performance by 11.5% points correct for the session.

Repeat of the ABT-418 best dose

Since the best dose is essentially selected from the series, it is possible that the predicted level of performance may occur as an artifact (although similar anlaysis with saline runs do not yield significant increases in DMTS performance). In order to preclude this possibility, each animal from the first series again received its best dose as indicated in Table 2. The best dose was repeated at least two additional times, providing an average level of performance for each animal at its best dose. These data are illustrated in Fig. 3. Note that the profile of the improvement was maintained. There was no significant difference ($P > 0.05$) in the magnitude of improvement between the initial run and the repeated run. In fact, DMTS performance after ABT-418 was similar in the two runs at long delay (77 versus 72% correct, respectively, for the initial and repeat studies). In this series, long delays provided an increase of 10.1% points correct for the session, which was equivalent to an increase of 16.2% over baseline performance. Again, there was no significant improvement in performance observed at the 24-h time point after drug administration.

ABT-418 dose-response

Since one of the animals (#148, Table 2) in the initial ABT-418 series responded best to the highest dose employed, we sought to provide an extended dose-response relationship. For this experiment we included three animals from the first series and three additional animals. Two of the new animals were rhesus and one was an additional pigtail monkey. None of these three had prior experience with ABT-418. The animals from the earlier series were allowed to washout for at least 2 weeks prior to initiating this experiment. In this series

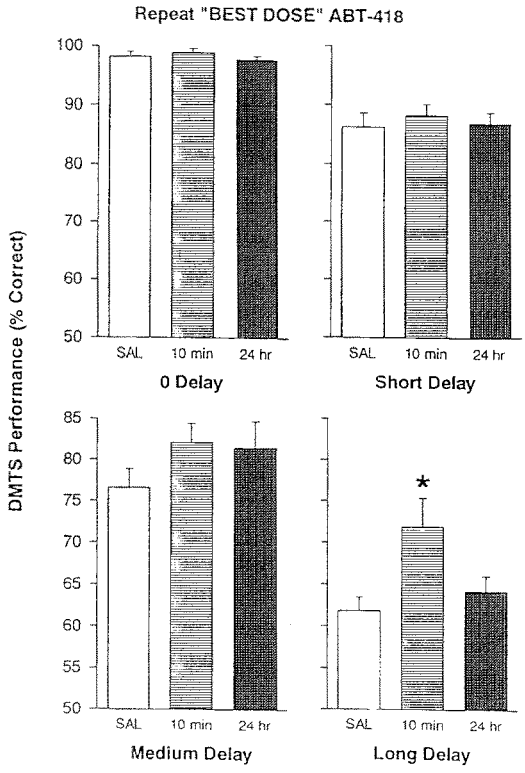

Fig. 3 Effect of ABT-418 on delayed matching-to-sample (*DMTS*) performance in five monkeys as a function of delay interval. Repeat Best Dose indicates that each animal received its best dose (see Table 2) at least three additional times over a 2-week period. The data derived from each of at least four sessions per animal were averaged. The averaged baseline values for the five monkeys after ABT-418 were compared statistically to respective saline performance averages and the resulting changes in performance are presented. There was a significant main treatment effect by ANOVA [$F(1, 4) = 47.5$, $P < 0.003$]. Values are means ± SEM. * Significantly different from baseline performance (paired t-test $P < 0.05$)

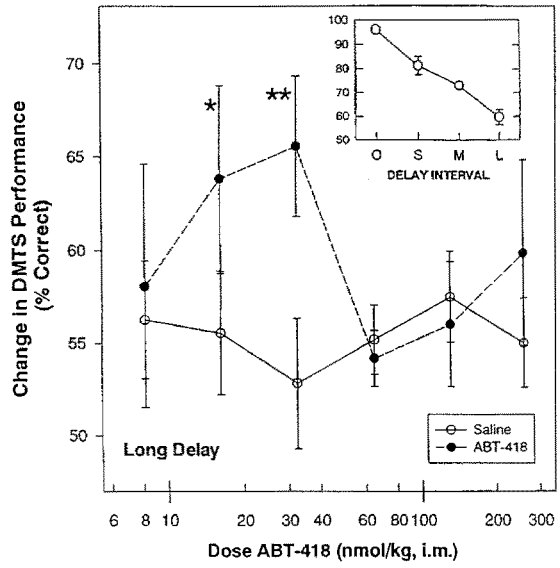

Fig. 4 Effect of ABT-418 on DMTS performance as a function of dose. Data are presented for long delay intervals. Each value represents the mean ± SEM derived from six monkeys, except for the first two doses which are derived from five monkeys. *Filled circles* indicate data derived from sessions begun 10 min after ABT-418. *Open circles* indicate data derived after saline administration employed as controls for each week of testing for the entire dose-response study. There was a significant main treatment effect by ANOVA [$F(1, 10) = 5.0$, $P < 0.05$]. * Trend toward significance with respect to baseline performance ($P = 0.054$). ** Significantly different from baseline performance ($P = 0.011$). *Inset*: DMTS performance in saline-treated monkeys over the course of the study as a function of delay interval, *O* zero, *S* short, *M* medium, *L* long. Absence of error bars indicates that the bars were smaller than the diameter of the symbol

the doses employed were as follows: 8.1, 16.2, 32.4, 64.8, 129.6 and 259.2 nmol/kg. Where the data overlapped for the three animals employed in both series, the values were averaged. Because of the variability and smaller magnitude of ABT-418-induced increases in DMTS performance for the first three delay intervals, no significant dose-response relationships were discernable. Thus, only long delay intervals are presented in the dose-response relationship illustrated in Fig. 4. As expected, there was considerable variability associated with the responses averaged for the six animals. However, it is clear that DMTS performance was significantly enhanced in the range of 16.2–32.4 nmol/kg. In fact, doses higher than 32.4 nmol/kg were not associated with improvement in performance. The exception was the 259.2 nmol/kg dose which was, in fact, the best dose for one animal (one of the original pigtails) whose performance increased by 16.7% points correct for the session. Although performance fell off at the higher doses tested, the animals exhibited no overt signs of toxicity, nor did they fail to finish sessions.

Repeated administration of best dose over 8 days

Several behavioral and autonomic actions of nicotine are subject to rapid tolerance. The purpose of this study was to determine whether the improvement in DMTS performance provided by ABT-418 could be maintained on a repeated basis. Thus, three animals from the original group of pigtails were allowed a washout period of at least 2 weeks prior to initiating this experiment. Initially, saline was administered once per day over an 8-day period and DMTS performance was assessed 10 min after each saline injection. For this series we employed the combined performance across medium and long delay intervals to reduce the variability inherent in using the data derived from only one delay. Although performance associated with medium delay intervals was not significantly different from control levels (Fig. 3), individual animals sometimes performed similarly at medium delay and long delays. Thus, for these three animals, measurement of DMTS performance combined for both delays was subject to less variability than that for either delay alone.

As indicated in the upper panel of Fig. 5, chronic administration of saline produced a rather stable level of DMTS performance of about 70–74% correct for the session over the 8-day period. After this series was

Table 3 Sample latencies, choice latencies and position preferences in animals pretreated with saline or ABT-418

Drug	Sample latency[a] correct trials	Sample latency incorrect trials	Choice latency[b] correct trials	Choice latency incorrect trials
Saline	6.34 ± 0.84	7.46 ± 1.09	3.40 ± 0.89	5.64 ± 1.21*
ABT-418	7.16 ± 0.71	7.68 ± 1.06	3.18 ± 0.69	4.68 ± 1.10
	% Correct left side[c]		% Correct right side	
Saline	79.9 ± 1.71		81.6 ± 1.59	
ABT-418	85.8 ± 1.20†		85.1 ± 2.17†	

* Significantly different ($P = 0.01$) from the mean for choice latency correct trials
† Significantly different ($P < 0.01$) from the respective saline means. Each value represents the mean ± SEM for five monkeys
[a] The time interval (s) between initiation of the new trial (with illumination of the stimulus light below the sample key) and the animal's pressing the sample key
[b] The time interval (s) between presentation of the two choice stimuli and the animal's keying in its choice.
[c] The number of trials in which the animal made a correct choice expressed as the percentage of the total number of trials presented in which the correct choice appeared on the left side

completed, the best dose of ABT-418 for each individual was injected once daily over 8 consecutive days, and DMTS sessions undertaken after each injection as indicated for the acute studies. The results, which are shown in the lower panel of Fig. 5 indicate that on day 1 of ABT-418 administration, the improvement again averaged about what it did for the acute best dose studies (Figs 1 and 3), confirming the beneficial response. Although, performance over the remaining 7 days was somewhat inconsistent, most average levels of improvement were numerically greater than baseline performance. These increases reached significance on days 1, 3 and 8.

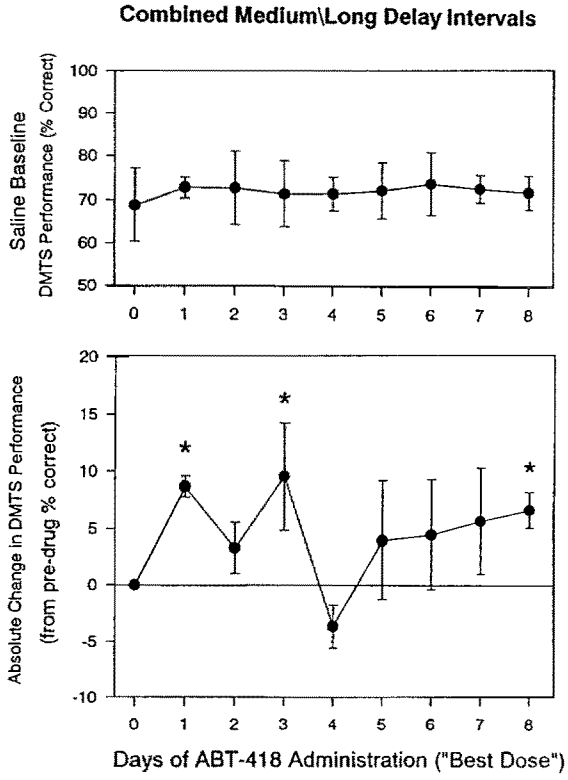

Fig. 5 Effect of repeated once daily saline administration (*upper panel*) on baseline DMTS performance by three monkeys over 8 consecutive days. The effect of ABT-418 on the same three monkeys is presented as the change from saline-baseline performance (*lower panel*). The ABT-418 experiment was conducted immediately after the saline experiment. * Significantly different from baseline performance ($P < 0.05$)

Other components of DMTS performance in the presence of ABT-418

Additional information regarding trial latencies and potential position preferences was obtained for the five animals performing the first experimental series. Two animal-controlled latencies are incorporated in this version of the DMTS task. One is the choice latency, i.e., the time interval between presentation of the two choice stimuli and the animal's keying in its choice. The other is the sample latency; the time interval between initiation of the new trial (with illumination of the stimulus light below the sample key) and the animal's pressing the sample key. Both latencies reflect the animal's attention to the task, but the choice latency probably partially reflects the time involved in considering which illuminated key to press. The data for latencies have been broken down further into latencies associated with trials in which correct choices were made and latencies associated with trials in which incorrect choices were made. As indicated in Table 3, animals took 6–8 s to initiate new trials. There was no difference between these sample latencies for trials associated with correct or incorrect choices when the animals were pretreated with saline, nor was there any significant effect of ABT-418 (best dose) pretreatment. In contrast, when animals were pretreated with saline, choice latencies for trials associated with incorrect selections were significantly longer by 66%. In the presence of ABT-418 this difference between latencies for correct and incorrect choices was rendered non-significant. ABT-418 did not reduce choice latencies for all error trials, since the overall mean response rate for choice latencies in saline-treated animals (3.9 ± 0.97 s) was not significantly different from that for ABT-418-treated animals (3.4 ± 0.74 s).

Table 4 Pharmacokinetic comparison of ABT-418 and (–)-nicotine following intramuscular administration in cynomolgus monkeys[a]

Compound	Dose (nmol/kg)	C_{max} (ng/ml)	C_{max}[b]	T_{max} (h)	$t_{1/2}$ (h)	AUC(0–6 h) (ng · h/ml)	AUC[c]	*n*
ABT-418	20	2.3 (0.4)	116.2 (17.6)	0.25 (0.0)	ND	1.65(0.66)	82.5 (32.9)	3
	100	11.5 (1.9)	114.5 (19.0)	0.25 (0.0)	ND	10.43(2.05)	104.3 (20.5)	3
	500	66.0 (11.3)	131.9 (22.6)	0.10 (0.02)	0.66	45.97(3.55)	91.9 (7.1)	4
(–)-nicotine	100	27.4 (13.7)	274.3 (151.0)	0.11 (0.03)	ND	4.78(1.32)	7.8 (13.2)	3
	500	77.6 (25.5)	155.1 (51.0)	0.16 (0.05)	0.62	48.65(6.44)	97.3 (12.9)	5

[a] Data provided as the mean (SEM) for replicate animals; all plasma values are reported in terms of free base (parent drug) concentrations
[b] Dose normalized peak plasma concentration – pg/ml per nmol/kg
[c] Dose normalized area under the curve – pg · h/ml per nmol/kg
ND Unable to calculate plasma elimination half-life

In previous studies we have noted that some animals exhibit marked position preferences when solving problems. That is, when a correct choice is presented on a particular side (left or right with respect to the geometry of the testing panel) the probability will be greater that a correct choice will be made on one side or the other – termed a position preference. In animals pretreated with saline, we observed no position preference; as the level of overall performance was similar whether the correct choice appeared on the right or left side of the panel. ABT-418 pretreatment significantly improved overall performance, but did not alter this relationship (Table 3).

Pharmacokinetic studies

In a series of sequential studies, groups of female *Macaca fascicularis* (n = 3–5 animals per dose-group) received single intramuscular injections of either ABT-418 (20, 100, 500 nmol/kg doses) or (–) nicotine (100 and 500 nmol/kg). The plasma concentrations of parent drug, as a function of time after administration of the dose, were determined by reverse phase HPLC with electrochemical detection. Both compounds were rapidly absorbed, with peak plasma concentrations of parent drug recorded primarily in the first sampling time point (Fig. 6, Table 4). The peak plasma concentrations of ABT-418 increased in a manner roughly proportionally to the increase in dose, ranging from a low of 2.3 ± 0.4 ng/ml (mean ± SEM) in the 20 nmol/kg dose group to 11.5 ± 1.9 and 66.0 ± 11.3 ng/ml in the 100 and 500 nmol/kg dose groups, respectively. The peak plasma concentrations of nicotine were similar to those recorded for the same doses of ABT-418, averaging 27.4 ± 13.7 and 77.6±25.5 ng/ml in the 100 and 500 nmol/kg dose-groups (Table 4). A higher degree of animal to animal variability was noted in monkeys dosed with (–)nicotine compared to the monkeys receiving ABT-418. Plasma concentrations of parent drug declined with very similar eliminations half-lives (0.6 h).

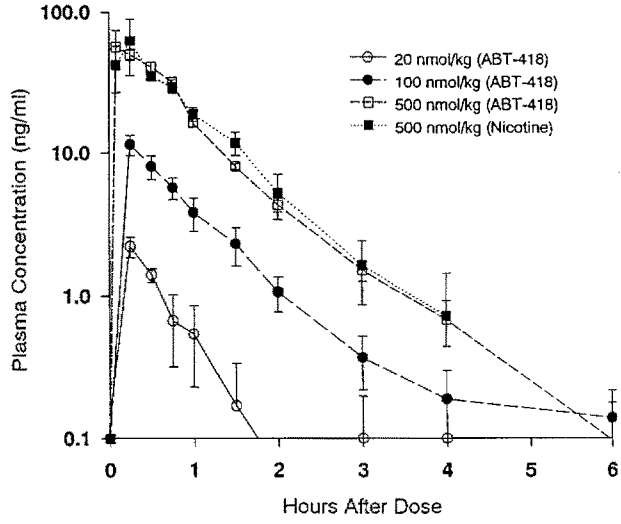

Fig. 6 Plasma levels of ABT-418 and (–)-nicotine in monkeys following intramuscular administration. Values are means ± SEM

Discussion

ABT-418 produced a significant enhancement of DMTS performance in mature macaques of different species and gender. Overall, on inspection of the individual data, no overt differences existed between the species or between the male and female subjects. Improvement in DMTS performance produced by ABT-418 was uniform even across the fairly wide age range of the subjects (10 years). The only differences in how individual animals responded to ABT-418 was with respect to the most effective dose (best dose) which enhanced DMTS performance. It is interesting to note that for the five monkeys who received both ABT-418 and nicotine, the animals whose best doses were among the two lowest ABT-418 best doses exhibited the lower of the two nicotine best doses; whereas the animal who exhibited the highest best dose for ABT-418 exhibited the higher of the nicotine best doses. In fact, ABT-418 has been well characterized pharmacologically, and many of its actions are attributable to nAChR activation (Arneric et al. 1994; Brioni et al. 1994; Decker

et al. 1984b), since these responses to ABT-418 are uniformally antagonized by classical nicotinic blocking agents. This is also true for the ability of the drug to enhance retention in the mouse inhibitory avoidance task (Decker et al. 1994b).

Despite this similarity in profile of action to nicotine, ABT-418 differs from the former in many respects. For example, ABT-418 has a much reduced liability in the production of side effects. The drug is significantly less potent than nicotine in evoking hypothermia, in reducing locomotor activity, in evoking seizure activity and in its lethality in mice. ABT-418 also exhibits reduced emetic liability compared with nicotine in dogs (Decker et al. 1994b). The reduced incidence in this profile of side effects compared to nicotine may be related to the relative selectivity of the drug for certain subtypes of nAChRs. For example, ABT-418 is not an agonist for the nAChR at the neuromuscular junction. The compound is as potent as nicotine for nAChR subtypes expressing the α_4 subunit, but is less potent than nicotine for nAChRs expressing the α_3 subunit (Arneric et al. 1994b), a finding which could be related to its reduced cardiotoxic liability compared with nicotine.

In the present study, ABT-418 was equally effective and as potent as nicotine in its enhancement of DMTS performance in monkeys. But perhaps the greatest difference between the two compounds to emerge from this study was the apparent duration of action. As indicated in our previous studies, nicotine's enhancement of DMTS performance extends to at least 24 h after drug administration (Elrod et al. 1988; Buccafusco and Jackson 1991; Terry et al. 1993). Clearly, the beneficial action of ABT-418 was limited to the day of administration. This difference could be due to a shorter (by approximately 50%) plasma $t_{1/2}$ for ABT-418 compared with nicotine as measured after IV administration of the drugs to rats (Decker et al. 1994b). However, as demonstrated in this study, the plasma $t_{1/2}$ for ABT-418 and nicotine were nearly equivalent following IM administration in monkeys. For this reason it is more likely that nicotine has an unusual property, not shared by ABT-418, which allows for improvement in performance some time after the compound has cleared from plasma. This property of nicotine also has been observed after prolonged administration to rats (Levin 1992). Thus, future experiments may take advantage of the neurochemical differences between the two drugs to develop strategies to better understand this interesting property of nicotine. Some of these differences include different selectivity and sensitivity to subtypes of nAChRs, different distribution to brain regions (Anderson et al. 1995), and differences in mode of action at the receptor complex itself (Arneric et al. 1994).

ABT-418 exhibited improved DMTS performance with repeated administration to monkeys. At the most effective doses, improvement in performance ranged from 10 to 20% of control, baseline performance. Even when animals were administered a single dose over 8 days, improved performance was generally maintained. It should be pointed out that in this particular experiment, variability was greater owing to the small number of subjects. Also, the timing of ABT-418 administration (once daily) was not necessarily optimized for a chronic dosing schedule. The results, however, were inconsistent with the development of significant tolerance to the DMTS performance enhancing action of ABT-418. Although a comparable study in monkeys has not been performed with nicotine, nicotine has been shown to enhance performance in rodents following 14 days administration (Levin 1992). ABT-418 also maintains a robust enhancement of performance of the inhibitory avoidance task in rodents following 11 days of continuous subcutaneous infusion (Decker et al. 1994b). Since most of this improvement in the monkeys occurred over trials associated with the long delay intervals, the drug might be considered to be most effective under circumstances when recall is mot taxed. In this context, the drug might be expected to be more useful as a memory aid to individuals with impaired memory or cognitive performance, as in Alzheimer's disease.

All of the animals in this study were well trained in the DMTS task; hence response latencies were short for both sample and choice latencies. Under conditions of saline pretreatment, choice latencies for trials associated with incorrect choices were significantly longer than those associated with correct choices. ABT-418 did not significantly shorten latencies, but appeared to alter the distribution of fast and slow responses to choice. While this effect may seem subtle, it could reflect the improved performance observed with respect to the increased number of correct responses after ABT-418 administration. That is, with improved memory performance, the time involved in making choices, particularly for the most difficult problems (even when an incorrect response is given), may become shorter. That the drug did not simply increase response rates is suggested by the fact that sample latencies were not altered in the presence of ABT-418. An enhanced responsiveness or stimulant type action should have been revealed in shorter sample latencies as well.

The ability of ABT-418 to enhance DMTS performance to the same extent when correct choices appeared on either side of the panel (position preference) is consistent with our earlier studies with nicotine (Buccafusco and Jackson 1991). In aged animals, position preferences play a more important role in the overall DMTS performance, where nicotine more effectively enhances performance on the lesser preferred side. Additional experiments in aged animals will be required to determine whether this is the case for ABT-418.

In summary, ABT-418 is a novel nicotinic agonist which appears to offer significant improvement in DMTS performance by young monkeys, particularly

at medium to long delay intervals. ABT-418 is at least as efficacious as nicotine, but is a shorter-acting compound, with the beneficial action limited to the day of testing. In contrast to that previously seen with nicotine (Decker et al. 1994b), no untoward side effects were noted in the animals with ABT-418. Also, under the conditions of the protocols employed, there exists no evidence for significant tolerance to the memory enhancing actions of ABT-418. In view of the significantly reduced potential for serious side effects compared with nicotine in preclinical studies, ABT-418 has much greater potential for use in patients with Alzheimer's disease and related dementias.

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