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Functional Characterization of the Novel Neuronal Nicotinic Acetylcholine Receptor Ligand GTS-21 In Vitro and In Vivo

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BRIGGS, C. A., D. J. ANDERSON, J. D. BRIONI, J. J. BUCCAFUSCO, M. J. BUCKLEY, J. E. CAMPBELL, M. W. DECKER, D. DONNELLY–ROBERTS, R. L. ELLIOTT, M. GOPALAKRISHNAN, M. W. HOLLADAY, Y.-H. HUI, W. J. JACKSON, D. J. B. KIM, K. C. MARSH, A. O'NEILL, M. A. PRENDERGAST, K. B. RYTHER, J. P. SULLIVAN AND S. P. ARNERIC. *Functional characterization of the novel neuronal nicotinic acetylcholine receptor ligand GTS-21 in vitro and in vivo.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 231–241, 1997.—(2,4)-Dimethoxybenzylidene anabaseine dihydrochloride (GTS-21), a compound that interacts with rat neuronal nicotinic acetylcholine receptors (nAChRs), was evaluated using human recombinant nAChRs in vitro and various pharmacokinetic and behavioral models in rodents, dogs and monkeys. GTS-21 bound to human α4β2 nAChR (K_i = 20 nM) 100-fold more potently than to human α7 nAChR, and was 18- and 2-fold less potent than (−)-nicotine at human α4β2 and α7 nAChR, respectively. Functionally, GTS-21 stimulated [^{3}H]dopamine release from rat striatal slices with an EC_{50} of 10 ± 2 μM (250-fold less potent and 70% as efficacious as (−)-nicotine), an effect blocked by the nAChR antagonist dihydro-β-erythroidine. However, GTS-21 did not stimulate human α4β2 nor human ganglionic nAChRs significantly. In vivo, GTS-21 had no adverse effect on dog blood pressure (≤2.5 μmol/kg i.v. bolus infusion), in marked contrast with (−)-nicotine. GTS-21 (≤62 μmol/kg, s.c.) also did not cross-discriminate significantly with (−)-nicotine in rats and did not reduce temperature or locomotion in mice. Neither was it active in the elevated plus maze anxiety model (0.19–6.2 μmol/kg, IP) in normal mice. However, GTS-21 did improve learning performance of monkeys in the delayed matching-to-sample task (32–130 nmol/kg, i.m.). © 1997 Elsevier Science Inc.

GTS-21 Anabaseine Nicotine Nicotinic receptors Rat Mouse Monkey Human Learning Anxiety Toxicity Pharmacokinetics

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ALZHEIMER'S Disease (AD) is recognized as one of the major central nervous system (CNS) health risks in our aging population. A consistent abnormality thought to contribute to the dementia associated with AD is the progressive loss of cholinergic neurons (18,25,49). Thus, one approach towards a palliative therapeutic has focused upon enhancing cholinergic transmission in the CNS. Tacrine, which has been approved for the treatment of AD, enhances cholinergic transmission at least in part by inhibiting the degradative enzyme acetylcholinesterase (19,21,30). However, tacrine is effective in only a minority of patients and its utility is limited by adverse reactions including hepatotoxicity. Muscarinic agonists selective for CNS receptors also have been targeted, but CNS selectivity has been difficult to achieve and peripheral side effects have been a problem. Nevertheless, compounds such as xanomeline may have improved receptor selectivity and are under clinical investigation for use in AD (14,20,44).

More recently, an approach targeting CNS nicotinic acetylcholine receptors (nAChRs) has been undertaken. The effects of (−)-nicotine that are beneficial in Alzheimer's Disease include enhanced cognitive and attentional performance, reduced anxiety, and cytoprotection (5,46). Recent advances in the molecular and functional identification of neuronal nAChR subtypes has supported the possibility of developing ligands that are selective for CNS receptors. nAChR in skeletal muscle are well characterized and are known to be pentameric complexes composed of the subunits α1, β1, δ and γ or ϵ. Neuronal nAChR, however, are composed from distinct, homologous subunits (36,39,43). In vertebrates, eleven nAChR subunits have been cloned (α2-α9 and β2-β4) and at least nine different neuronal nAChR, distinguishable pharmacologically and functionally, can be formed homomerically from the α7, α8 or α9 subunits or as complexes containing one type of α subunit and one type of β subunit. Combining two different types of β subunit or two different types of α subunit in such complexes may generate additional distinct nAChR subtypes, allowing for a considerable variety of nAChRs that may vary not only among brain regions, but also with ontogeny or pathology. In rat CNS, α4β2 nAChR accounts for about 90% of the high affinity nicotine binding, yet mammalian peripheral neurons appear to express little or no α4 subunit (36,43). The α7-containing nAChR, which binds α-bungarotoxin with high affinity, is another major class in the CNS and is a predominant subtype in cultured hippocampal neurons (1,17,24,47). Although α7 mRNA is also present in sympathetic neurons, α7-containing nAChR do not appear to contribute significantly to synaptic transmission through adult sympathetic ganglia (32,36,41). Thus, the variety and distribution of neuronal nAChRs suggests the possibility of developing nAChR ligands that not only are selective for CNS nAChR, as opposed to those in peripheral neurons and muscle, but also may be selective for different nAChR systems within the CNS.

(2,4)-Dimethoxybenzylidene anabaseine dihydrochloride (GTS-21; DMXB) is an anabaseine derivative (51) that has been found to bind to rat brain [^{3}H]cytisine sites (thought to represent α4β2 nAChR) with an affinity about 24-fold less than (−)-nicotine and [^{125}I]α-bungarotoxin sites (thought to represent α7 nAChR) with an affinity about 4-fold higher than (−)-nicotine (22). Functionally, GTS-21 appeared to be selective for α7 nAChR in that it was a partial agonist (28% relative to acetylcholine) at rat α7 homomeric nAChR and essentially inactive as an agonist at rat α4β2 nAChR expressed in *Xenopus oocytes* (22). However, GTS-21 may be less effective at the human α7 nAChR (8) than that of the rat. In animal models, GTS-21 has been found to enhance classical conditioning in rabbits (50), to enhance learning performance in aged or lesioned rats (3,37) and to enhance long-term potentiation in rat hippocampal slices (31). Additionally, GTS-21 has been reported to be cytoprotective (35). However, there is little published information on the toxicity and pharmacokinetics of GTS-21, and there has been no prior publication on the activity of GTS-21 in any primate model. In this report, the effects of GTS-21 in various in vitro and in vivo models are described. This includes the first published descriptions of GTS-21 action at human ganglionic and cloned α4β2 nAChR in vitro, and of GTS-21 effects on performance in monkeys.

METHODS

Male CD-1 mice (30 g or about 60 days old) and male Wistar rats (⩾ 200 g or ⩾ 60 days old) from Charles River (Portage, MI), male beagle dogs (10.2–11.4 kg) from Marshall Farms (North Rose, NY), and cynomolgus monkeys (*macacca fascicularis*) from the colony established at Abbott Laboratories were treated according to the guidelines of the American Association for the Accreditation of Laboratory Animal Care (AAALAC) as approved by Abbott Laboratories Institutional Animal Care and Use Committee. Mature pigtail monkeys (*macacca nemestrina*, 6 to 12.4 years old) were colony reared at the Washington Regional Primate Center and were housed at the Animal Behavior Center of the Medical College of Georgia. The facilities of the Animal Behavior Center meet or exceed current Federal standards for non-human primate housing. Rodents and primates were allowed free access to food and water except during experimental measurements, except for partial food restrictions when animals were trained to a food reward as detailed below, and except for being fasted overnight prior to pharmacokinetic measurements. Dogs were fed once per day and allowed ad lib access to water.

Membrane Preparations

Cerebral cortex membranes were prepared from male Sprague-Dawley rats as described by Enna and Snyder (26) with some modifications. Cerebral cortices were homogenized in 15 volumes of 0.32 M sucrose and centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged at 20,000 × *g* for 20 min, and the resulting pellet was re-homogenized and centrifuged at 8000 × *g* for 20 min. This supernatant was centrifuged at 40,000 × *g* for 20 min and the membrane pellet was used in binding assays.

Confluent K177 cells stably expressing the human α4β2 subunit combination (29) were rinsed with ice-cold buffer (120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 50 mM Tris HCl buffer, pH 7.4 at 4°C), mechanically disaggregated and homogenized using a Polytron for 10 s. The homogenate was centrifuged at 45,000 × *g* for 20 min at 4°C and the pellet resuspended in ice-cold buffer at a concentration of 40–50 mg protein/ml.

Confluent K28 cells stably expressing the human α7 subunit (28) were rinsed with ice-cold buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 20 mM Na-HEPES buffer, pH 7.5), mechanically disaggregated and homogenized using a Polytron for 10 s. The homogenate was centrifuged at 45,000 × g for 20 min at 4°C and the pellet resuspended in ice-cold buffer at a concentration of 40–50 μg protein/ml.

[^{3}H](−)-Cytisine Binding

[^{3}H](−)-Cytisine binding to membranes was carried out as described by Pabreza et al. (38) with some modifications (2).

Incubations were carried out for 75 min at 4°C in a final volume of 500 μl with [^{3}H](−)-cytisine (0.5 nM) and unlabeled compounds at various concentrations. Specific binding was determined using unlabeled (−)-nicotine (10 μM) added to a duplicate set of tubes. Incubations were terminated by rapid vacuum filtration over GF/B glass fiber filters presoaked in 0.5% polyethyleneimine, and filters washed three times with 1.5 ml of ice-cold binding buffer. Radioactivity was quantified by liquid scintillation spectroscopy at an efficiency of 45% (Beckman LS 5000 TD, Beckman Instruments, Somerset, NJ).

The drug concentration producing 50% inhibition of radioligand binding (IC_{50}) and the Hill coefficient (n_H) were determined using a four parameter logistics program in RS/1 (Bolt Beranek and Newman Inc. Cambridge, MA) from plots of log((B_0–B)/B) versus log([D]) where B_0 and B represent specific binding in the absence and presence of competitor, respectively, and [D] is the concentration of drug. Inhibition constants (K_i) were derived from the IC_{50} values using the Cheng-Prusoff equation (16).

[^{125}I]α-Bungarotoxin Binding

[^{125}I]α-Bungarotoxin ([^{125}I]α-BgT) binding to membranes prepared from rat whole brain or from K28 cells stably expressing the human α7 nAChR subunit was determined using a modification of the method of Marks et al. (34). Rat brain and K28 cell membranes were resuspended in 15 volumes of buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 20 mM Na-HEPES buffer, pH 7.5). Aliquots containing 40 μg (K28 cells)–200 μg (rat brain) of tissue were added to triplicate reaction mixtures containing 1.9 nM [^{125}I]α-BgT (106 Ci/mmol) and the indicated concentrations of the test compounds. Non-specific binding was determined using 1 μM unlabeled α-BgT. Binding was conducted at 37°C for 3 h. Bound radioactivity was isolated by rapid vacuum filtration onto #32 glass fiber filters (Schleicher and Scheull) using a Skatron filtration apparatus.

[^{125}I]α-BgT (106 Ci/mmol) binding to the muscle-type nAChR in *Torpedo californica* electroplax was determined using a solid phase binding assay as described by Chaturvedi et al. (15). Microtiter plates (96-well; Immulon Removawells Strips, Dynatech, Chantilly, VA) were coated with 0.5 μg of *Torpedo* membranes (ABS Inc., Wilmington, DE) in 50 mM $NaHCO_3$ buffer, pH 9.6, for 12 h at 4°C. The wells then were washed twice with phosphate-buffered saline (PBS) and quenched for 1 h with 5% bovine serum albumin. [^{125}I]α-BgT (1.9 nM / 100 μl 10 mM phosphate buffer, pH 7.4 / 0.2% bovine serum albumin) then was added to the wells for 1 h. Competition for of [^{125}I]α-BgT binding was determined in triplicate by adding various concentrations of the compounds (50 μl) to the wells immediately prior to the [^{125}I]α-BgT. Nonspecific binding was determined in the presence of 1 μM α-BgT. After incubation, each well was washed 5 times with PBS and then placed in a vial for determination of bound radioactivity.

[^{3}H]Oxotremorine-M Binding

Binding to muscarinic receptors was measured using [^{3}H]oxotremorine-M (87 Ci/mmol) as described previously (4) and modified after Birdsall et al. (7). The assay was performed in 20 mM sodium phosphate buffer (pH 7.4) at 25°C for 45 min with 100 μg rat brain membrane per tube and 2 nM [^{3}H]oxotremorine-M. Nonspecific binding was determined in the presence of 10 μM atropine. Bound radioactivity was measured as described above.

Cation ($^{86}Rb^+$) Efflux from K177 and IMR 32 Cells

K177 cells stably expressing the human α4β2 nAChR subunit combination were maintained as previously described (29). IMR-32 human neuroblastoma clonal cells (ATCC, Rockville, MD) were maintained in a log phase of growth according to established procedures (33). Cells were seeded at a density of 500,000 cells/ml into a 24-well tissue culture dish and allowed to proliferate for at least 48 h before loading with 2 mCi/ml of $^{86}Rb^+$ (35 Ci/mmol) overnight at 37°C. The $^{86}Rb^+$ efflux assays were performed according to previously published procedures (33) except that serum-free Dulbecco's Modified Eagle's Medium was used during the $^{86}Rb^+$ loading, rinsing, and agonist-induced efflux steps.

Striatal [^{3}H]Dopamine Release

The evoked release of [ring-2,5,6-^{3}H]dopamine (24.4 Ci/mmol) was measured in superfused rat striatal slices as described previously (4). Striata were dissected from male Sprague–Dawley rats (two per experiment) and sliced to 0.35 × 0.25 mm using a McIlwain Tissue Chopper (Brinkman Instrument Co., Westbury, NY). After two washes with Krebs-HEPES buffer (137 mM NaCl, 4.7 mM KCl, 1 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 10 mM glucose, and 15 mM Na-HEPES buffer, pH 7.4) containing 10 μM pargyline and 10 μM ascorbic acid, slices were preincubated for 10 min at 37°C under 95% O_2, 5% CO_2. After refreshing the buffer, slices were incubated with 100 nM [^{3}H]dopamine for 25 min at 37°C. Aliquots of the slices were divided among the 18 chambers of a Brandel SP2000 superfusion apparatus (Brandel, Gaithersberg, MD). Following wash for 47 min, the slices were exposed to agonist for 4 min. Antagonists, when present, were introduced 4 min prior to and during agonist exposure. Superfusate fractions were counted in 5 ml of Ecolume. Tissue recovered from each superfusion chamber was solubilized in 1 ml of Solvable (DuPont-NEN) and counted in 15 ml of Ecolume.

The fractional evoked release of [^{3}H]dopamine was calculated from the agonist-induced increase in [^{3}H] efflux, above the projected baseline efflux, as a fraction of total radioactivity present in the tissue. Agonist efficacy was calculated relative to the release evoked by 1 μM (−)-nicotine. EC_{50} values were determined by non-linear least squares regression analysis of the concentration-response relationships (Inplot, San Diego, CA).

Elevated Plus-Maze

Anxiolytic-like activity was evaluated using the elevated plus-maze, a pharmacologically validated model (11, 40), using procedures previously described (11). The elevated plus-maze was constructed from gray Plexiglas and consisted of two open arms (17 × 8 cm) and two enclosed arms (17 × 8 × 15 cm) extending from a central platform (8 × 8 cm) raised 39 cm above the floor. Light levels on the open and enclosed arms were similar. Each animal was placed on the central platform and allowed to explore the maze for 5 min. The time on the open arms and total distance traveled were assessed using a video camera mounted on the ceiling above the apparatus and an automated tracking system (Videomex, Columbus Instruments). All animals used were naive to the apparatus. Rodent behavioral data were analyzed using analysis of variance, with

post-hoc pairwise comparisons evaluated using Fisher's protected least significant difference test.

Drug Discrimination

Because food reward was used in training and testing, male Wistar rats were given restricted amounts of food. Their body weights were maintained at about 80 ± 5 % of those given free access to the food. Food availability was restricted to food pellets available during experimental sessions and supplemental laboratory rat chow sufficient to maintain stable body weights. Water was continuously available except during experimental sessions. Training began with rats 60 days old and these animals were used for up to one year.

Eight standard experimental chambers (Coulbourn Instruments, Lehigh Valley, PA) contained within sound-attenuated enclosures were used. Each chamber contained two levers separated by a food receptacle in which 45 mg pellets (P. J. Noyes Co., Lancaster, NH) could be presented by the dispenser. The chambers were controlled by a computerized system using OPN software (45).

The training procedure has been described previously (9,10). Briefly, rats were trained to press the levers to obtain food reinforcement under an autoshaping program. The animals were assigned randomly to one of the eight experimental chambers. In four chambers the right lever was designated as the saline-appropriate lever and the left lever as the drug one. A double alternation sequence of pretreatments followed the autoshaping routine. A pair of saline pretreatment sessions were followed by a pair of (−)-nicotine (1.9 μmol/kg, IP) pretreatment sessions. Animals followed a specific individual schedule of injections during training and testing such that no overall drug or saline days were established in order to avoid having olfactory cues used to solve the discrimination task. Saline and drug injections were given immediately before a 20-min session. The rats were placed inside the operant chambers for a 10-min (lights-off) time-out period during which no reinforcement was delivered, followed by a 10-min testing period (lights-on). To satisfy the criteria for stimulus control, in at least 7 out of 8 consecutive sessions the rats lever-press responses had to be at least 80% appropriate before the first reinforcement and at least 90% appropriate during the entire session. At that point, the discrimination was considered acquired and testing began.

The testing sequence consisted of saline (S), nicotine (N) and test-drug (T) injection sessions in the following sequence: S, N, T, N, S, T, repeat. The percent of the total responses that occurred on the nicotine-appropriate lever and the rate of responding on both were recorded for each rat. To avoid extinction of the discrimination during the test sessions, rats were rewarded after ten responses on either lever. Comparisons between the different groups were analyzed by the Kruskal-Wallis and Mann-Whitney "U" tests because the proportion responses on the (−)-nicotine lever did not satisfy the Barlett's test of homogeneity of variance for parametric analysis.

Locomotor Activity and Body Temperature

Both body temperature and open field locomotor activity were measured in the same mice. Beginning 4 min after an IP injection of GTS-21, horizontal and vertical activity counts were recorded for 15 min in an open field (41 × 41 cm) using photobeam activity monitors (San Diego Instruments, San Diego, CA). Body temperature was measured immediately after the mice were removed from the open field (approximately 20 min after drug injection) using a rectal probe inserted 3 cm (YSI TeleThermometer, Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Lethality

Lethality was assessed using a variation of the approximate lethal dose procedure. After initial range-finding experiments, approximate lethal dose values were determined in two groups of five mice, each mouse in a group receiving one of five doses separated by 10 μmol/kg increments. In each group of five mice, the lowest dose at which an animal died within 24 h of injection was designated as the approximate lethal dose for that group. Two such groups were used to determine the mean approximate lethal dose for GTS-21.

Monkey Delayed Matching-to-Sample (DMTS)

Three male and three female mature pigtail monkeys (*macaca nemestrina*), ages 6-12.4 years, served as subjects. They were housed in individual stainless steel cages composed of 50 × 28 × 26 inch units. Toys were provided routinely and monkeys were allowed to observe television programs each afternoon as a means of promoting psychological well-being. During periods when animals were not tested routinely, they were allowed access to an enclosed outdoor exercise facility on an individual or selected-group basis. During the week, monkeys were allowed ad lib access to water and maintained on a feeding schedule that allowed approximately 15% of their normal daily food intake to be derived from 300 mg food pellets which served as rewards during experimental sessions (standard monkey chow and banana flakes, P. J. Noyes, Inc., Lancaster, NH) and the remainder to be obtained from standard laboratory monkey chow following completion of a test session. On days that the animals were not performing the DMTS task (eg. weekends, holidays), the daily allotment of solid food was obtained from standard laboratory monkey chow and supplemented with fruits and vegetables.

At the start of a DMTS session, test panels were attached to the front of home cages. DMTS stimuli were 25 cm diameter colored disks (red, green, and yellow) presented via light-emitting diodes located behind clear push-keys. For five of the monkeys, sessions consisted of 96 trials each day. For one monkey, sessions consisted of 48 trials each day because of the considerable length of this monkey's individualized delay intervals.

A trial began with illumination of the sample key by one of the colored stimuli. The sample remained illuminated until the animal depressed the key. A key press also 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. Four possible delay intervals were employed between a monkey's response to the sample stimulus and the presentation of the two choice stimuli: zero delay and three longer delay intervals, 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 monkeys were trained until performance for zero delay trials averaged 85–100% correct.

Short, medium, and long delays were adjusted in duration to produce stable performance levels which approximated the following levels of accuracy: short delay, 75–85%; medium delay, 65–75%; and long delay, 55–65%. The length of delays for each animal was adjusted according to individual skills level. Individual delays ranged from 0–15 s to a maximum of 0–240 s. The rationale for this procedure was to normalize DMTS performance for all monkeys given that monkeys exhibit considerable variability in baseline matching ability (for further details of this DMTS procedure, see references (13,48).

GTS-21 was administered in an ascending series of doses (8.1, 16.2, 32.4, 64.8, and 129.6 nmol/kg in 0.1 ml saline) in the gastrocnemius muscle. Control data were obtained following administration of vehicle on the first day of each week of testing with each monkey serving as its own control. Test sessions began 10 min following vehicle or GTS-21 administration. A minimum "drug washout" period of 2 days was allowed between sessions. During this period, a return to baseline DMTS performance was established in each monkey.

The DMTS performance after GTS-21 injection was compared to the baseline (saline-injected) performance using one-way repeated measures analyses of variance and the Neuman-Keuls test of post-hoc multiple comparisons when appropriate. Latencies to respond to sample and choice stimuli on correct and incorrect response trials were compared using one-way repeated measures analyses of variance.

Cardiovascular Effects

Male beagle dogs (10.2–11.4 kg) were anesthetized with pentobarbital (35.0 mg/kg, i.v., followed by 5.0 mg/kg/hr constant infusion, i.v.). Once anesthetized, the dog was intubated with a cuffer endotracheal tube and ventilated with room air by a mechanical respiration pump (Model 613, Harvard Instrumnets, South Natick, MA). Expiratory CO_2 was monitored with a Beckman LB-2 Medical Gas Analyzer (Beckman Instruments, Sommerset, NJ) and maintained at 4–5% CO_2. The electrocardiogram (EKG) leads were attached the limbs and lead II EKG was recorded. A dual tip micromanometer catheter (Model SPC-770, 7F, Millar, Houston, TX) was advanced into the left ventricle of the heart, via the right carotid artery, for measurement of diastolic blood pressure. A polyethylene catheter was inserted into the right femoral vein for injection of test compounds.

After the instrumentation was completed, the dog was covered and body temperature was monitored and maintained at 36–38°C throughout the experiment. The primary hemodynamic variables were computed using XYZ Real Time Spreadsheet software on a signal processing workstation (Modular Instruments, Inc., Southeastern, PA). Sixty minutes were allowed following surgery to achieve steady-state baselines for diastolic pressure. GTS-21 was administered as a bolus infusion at increasing doses of 100, 500 and 2500 nmol/kg. Diastolic blood pressure was monitored for 10 min after each infusion of GTS-21. (-)-Nicotine (20, 100 and 500 nmo/kg, i.v.) was administered similarly beginning 90 min after administration of the highest dose of GTS-21.

Data were analyzed by a 2-way analysis of variance (ANOVA) using StatView II (Abacus Concepts, Inc., Berkeley, CA).

Pharmacokinetics

In a series of parallel studies, groups of three beagle dogs (male/female) or three *cynomolgus* monkeys (female) received either a single 100 nmol/kg intravenous dose or a 500 $\leqslant$ nmol/kg oral dose of GTS-21 prepared as an aqueous solution in normal saline. All animals were fasted overnight prior to dosing and throughout the duration of the study; water was provided ad lib. Sequential blood samples (2.0 to 2.5 ml) were obtained from each animal at selected time points for 8 h after dosing. Plasma was separated from the red cells by centrifugation (4°C) and frozen until analysis.

GTS-21 in plasma was determined following alkalinization (0.5 ml 0.5 M Na_2CO_3 added to 1.0 ml plasma) and extraction into 6.0 ml ethylacetate: hexane (1:1 v:v) from alkaline plasma. The upper organic layer was transferred to a conical centrifuge tube, evaporated to dryness with a gentle stream of dry air at room temperature, and reconstituted in 0.2 ml of methanol: water (1:1 v:v). The samples were protected from the light at all times. GTS-21 was measured using reverse phase HPLC on a 10 cm $\times$ 4.6 mm 5 μm YMC basic column with an acetonitrile: 0.05 M phosphate buffer (pH 3.0) mobile phase and UV detection of the 100 μl injection at 395 nm. The assay method was linear (correlation coefficient $>$ 0.99) over the concentration range 0–200 ng/ml (0–650 nM) with a mean standard deviation $<$ 6% (triplicate standards at five concentrations) and a lower limit of detectability of near 4 ng/ml (13 nM). Internal standards were added to plasma and assayed simultaneously with the samples.

The pharmacokinetic parameters for GTS-21 were estimated using non-compartmental analysis. Area under the curve (AUC) values for plasma GTS-21 content as a function of time after administration were calculated by the trapezoidal method over the time course of the study. The bioavailability (F%) was estimated by dividing the dose-normalized AUC obtained following oral administration by the dose-normalized AUC obtained following intravenous administration in a separate group of animals. The plasma elimination rate constant (β) was estimated from the log linear regression of the terminal plasma concentrations as a function of time. The following additional parameters were calculated from the plasma concentration data: plasma clearance (CLp, dose/AUC), and the volumes of distribution Vc (dose/C_0; C_0 = concentration at zero time) and V_β (CLp/β).

Materials

Bovine serum albumin, mecamylamine and (−)-nicotine were purchased from Sigma Chemical Co. (St. Louis, MO). Unlabelled α-bungarotoxin, diazepam and dihydro-β-erythroidine (DHβE) were purchased from Research Biochemicals International (Natick, MA). [^{125}I]α-Bungarotoxin (10–20 μCi/μg), [3,5-^{3}H(N)](−)-cytisine hydrochloride (15–40 Ci/mmol), 3,4-[ring-2,5,6-^{3}H]dihydroxyphenylethylamine ([^{3}H]dopamine; 30–60 Ci/mmol), [methyl-^{3}H]oxotremorine-M acetate (70–90 Ci/mmol) and ^{86}rubidium chloride ($^{86}Rb^+$, $>$ 1 Ci/g) were purchased from DuPont-NEN (Boston, MA). (2,4)-Dimethoxybenzylidene anabaseine dihydrochloride (GTS-21) was synthesized at Abbott Laboratories according to the methods described by Zoltewicz et al. (51). GTS-21 was prepared as the dihydrochloride and was analyzed by proton NMR, mass spectrometry, and elemental analysis.

RESULTS

Receptor Binding

[^{3}H](−)-Cytisine has been shown to bind with high affinity to α4β2-like nAChRs, a major subtype in rodent and human brain (27, 29). GTS-21 displaced [^{3}H](−)-cytisine binding to the human α4β2 subunit combination stably expressed in the

TABLE 1

CHOLINERGIC BINDING PROPERTIES OF GTS-21 AND (−)-NICOTINE

Radioligand Binding (K_i; nM)	GTS-21	(−)-Nicotine
Human α4β2	20 ± 3	1.1 ± 0.2
Rat brain cytisine site	19 ± 4	1.0 ± 0.1†
Human α7	2000 ± 140	1000 ± 160
Rat brain α-BgT site	650 ± 34	4000 ± 890†
Torpedo α1β1δγ	1100 ± 300	> 10,000

Values represent mean ± SEM (n = 3–5).

†Values for (−)-nicotine in rat brain are from Arneric et al. (4).

K177 cell line (29) in a concentration-dependent manner with a K_i value of 20 ± 3 nM (n = 3; Table 1). (−)-Nicotine was 18-fold more potent in displacing binding (K_i = 1.1 ± 0.2 nM; n = 5). Similarly, in rat brain membrane GTS-21 (K_i = 19 ± 4 nM) was 19-fold less potent than (−)-nicotine (K_i = 1.0 ± 0.1 nM) to displace [^{3}H](−)-cytisine binding.

GTS-21 (K_i = 2000 ± 140 nM, n = 3) was 100-fold less potent in displacing [^{125}I]α-BgT from the human α7 subtype stably expressed in K28 cells than in displacing [^{3}H](−)-cytisine binding to the human α4β2 subtype in K177 cells (Table 1). However, GTS-21 was only 2-fold less potent than (−)-nicotine in displacing [^{125}I]α-BgT binding. This differed from the rat brain [^{125}I]α-BgT site, where GTS-21 (K_i = 650 ± 34 nM) was 3-fold more potent than at human α7 subtype in K28 cells and where GTS-21 was 6-fold more potent than (−)-nicotine in displacing [^{125}I]α-BgT binding from the rat brain site.

At the α1β1δγ nAChR subtype found in *Torpedo* electroplax membranes, GTS-21 displaced the binding of [^{125}I] α-BgT with a K_i value of 1100 ± 300 nM, at least 9-fold more potent than (−)-nicotine (Table 1). At [^{125}I]α-BgT sites in various tissues, GTS-21 appeared to bind most potently to rat brain, followed by *Torpedo* electroplax and then by human α7 homomeric nAChR. GTS-21 exhibited little or no affinity for muscarinic oxotremorine-M sites (K_i > value > 10 μM).

Ion Flux Studies

In K177 cells expressing the human α4β2 subtype, GTS-21 at concentrations up to 300 μM did not stimulate $^{86}Rb^+$ efflux (Fig. 1). (−)-Nicotine, however, did stimulate $^{86}Rb^+$ efflux (EC_{50} = 4.6 ± 0.7 μM; n = 5) and this was inhibited by GTS-21 (IC_{50} = 17 ± 5 μM, n = 3).

In IMR-32 cells, where nAChR stimulated ion flux is thought to be mediated principally by ganglionic receptors, possibly α3β4 (33), GTS-21 again had little agonist activity in comparison to (−)-nicotine (EC_{50} = 21 ± 4 μM) (Fig. 1). However, GTS-21 inhibited (−)-nicotine (100 μM) mediated cation flux (IC_{50} = 2.5 μM).

[^{3}H]Dopamine Release from Rat Striatal Slices

In contrast to the low efficacy of GTS-21 at human ganglionic nAChR, human α4β2 nAChR (above), and human α7 nAChR (DMXB; reference (8)), GTS-21 stimulated [^{3}H]dopamine release from rat striatal slices (Fig. 2) with an efficacy of 70% compared to (−)-nicotine and a potency (EC_{50} = 10 ± 2 μM, n = 3) approximately 250-fold less than (−)-nicotine (EC_{50} = 0.04 μM; reference (4). The nAChR antagonist DHβE (10 μM) inhibited these stimulatory effects of GTS-21 (100 μM) and (−)-nicotine (1 μM) by 96 ± 6 % and 93 ± 5 %, respectively (n = 3).

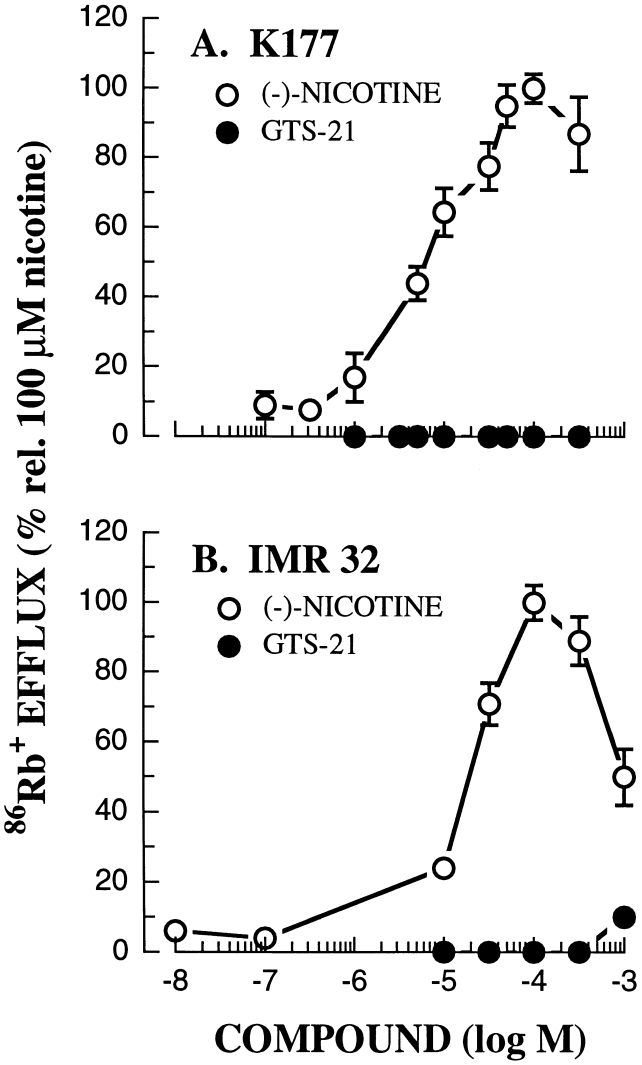

FIG. 1. Ion flux in K177 cells (human α4β2) and IMR 32 cells (human ganglionic nAChR). Cells were preloaded with $^{86}Rb^+$ and then exposed to various concentrations of (−)-nicotine or GTS-21 for 5 min to stimulate cation ($^{86}Rb^+$) efflux as a measure of nAChR activation. The stimulated efflux was normalized to the response elicited by 100 μM (−)-nicotine. In K177 cells (A) transfected to express human α4β2 nAChR and in IMR 32 cells (B) which constitutively express human ganglionic-like nAChR, GTS-21 (solid circles) was inactive as an agonist compared to (−)-nicotine (open circles). Data (mean ± SEM) are from three experiments in (A) and in (B).

Rodent Behavior

Anxiolytic-like activity was evaluated by measuring the time spent in the open arms of the elevated plus maze during 5-min test sessions. GTS-21 (0.19 to 6.2 μmol/kg IP) did not significantly increase the time mice spent in the open arms F(4, 35) = 1.60, n = 8), as shown in Table 2. In the same experiment, a group of mice injected with diazepam (3.5 μmol/kg) as a positive control exhibited a significant increase in the

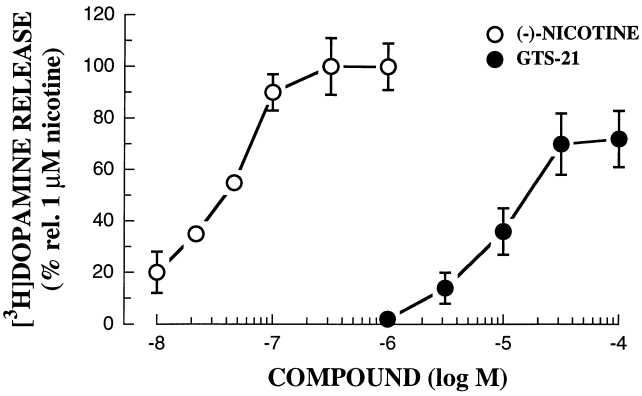

FIG 2. [^{3}H]Dopamine release from rat striatal slices. After preincubation in [^{3}H]dopamine, rat striatal slices were superfused and exposed to various concentrations of (−)-nicotine or GTS-21 for a period of 4 min to stimulate neurotransmitter release. The evoked release of [^{3}H] was expressed relative to the tissue content and was normalized to the response to 1 μM (−)-nicotine. GTS-21 (solid circles) was two orders of magnitude less potent than (−)-nicotine (open circles), and was about 70% efficacious relative to (−)-nicotine. Data are from three experiments.

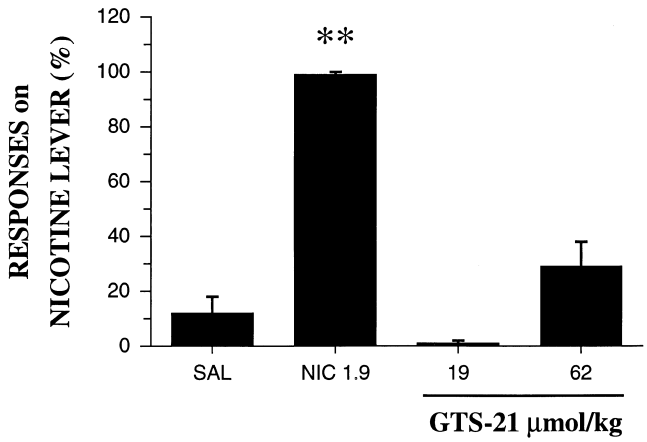

FIG. 3. Effect of GTS-21 in rats trained to discriminate (−)-nicotine from saline. Data represent the proportion of responses (per cent) on the (−)-nicotine lever by 4–5 rats trained to discriminate (−)-nicotine from saline injection (IP) and subsequently challenged with saline (SAL), 1.9 μmol/kg (−)-nicotine (NIC 1.9), or 19 or 62 μmol/kg GTS-21. **$P < 0.01$ compared to the saline-injected group.

time spent in the open arms ($P < 0.05$). General locomotor activity was not affected by GTS-21 at these doses. GTS-21 also had no apparent anxiolytic-like activity in rats (0.62–62 mol/kg IP, $F_{5,39} = 1.82$, n = 6–8, data not shown).

In rats trained to discriminate (−)-nicotine (1.9 μmol/kg IP) from saline injection, GTS-21 (19 and 62 μmol/kg) did not appear to elicit a (−)-nicotine-like cue, as animals responded mainly on the saline lever (Fig. 3). Injections of (−)-nicotine elicited a full generalization as expected ($H = 23.0, P < 0.001$).

Monkey Delayed Matching-to-Sample

GTS-21 did have some efficacy in monkeys, improving memory performance in the delayed matching-to-sample task under some conditions. The 6 monkeys employed in this study exhibited the following baseline DMTS performance following vehicle administration: zero delay = 98.9 ± 0.4 % correct; short delay = 86.5 ± 1.6 % correct; medium delay = 65.0 ± 1.5 % correct; and long delay = 52.0 ± 1.1 % correct. The performance at each of these delay intervals was significantly different from that at the other delay intervals (one-way analysis of variance followed by Neuman-Keuls post-hoc multiple comparisons analysis; $F(3, 119) = 292.8$, $p < 0.0001$). The average short, medium, and long delay intervals in seconds were as follows: short = 11 ± 2; medium = 53 ± 9; and long = 105 ± 17.

TABLE 2

LACK OF EFFECT OF GTS-21
IN THE ELEVATED PLUS MAZE TASK

Compound	Dose μmol/kg IP	EPM Time In Open Arms seconds
Saline Control		69 ± 4
GTS-21	0.19	60 ± 3
	0.62	55 ± 6
	1.9	70 ± 8
	6.2	54 ± 7
Diazepam	3.5	82 ± 4*

Data (mean ± SEM) are from 8 mice per group.
*$P < 0.05$ compared to saline (unpaired t-test).

Two measures of response latencies were recorded during DMTS testing: (a) choice latency, the time interval between presentation of the two choice stimuli and depression of one of the choice keys, and (b) sample latency, the time interval between initiation of a new trial (illumination of the stimulus light behind the sample key) and depression of the sample key by the animal. The choice latency prior to incorrect choices was significantly greater than that prior to correct choices ($F(1, 70) = 9.21$, $P < 0.05$); this pattern was not affected by GTS-21. In addition, there were no significant differences between sample latency responses on correct and incorrect trials.

GTS-21 (8.1, 16.2, 32.4, 64.8, or 129.6 nmol/kg) did not significantly affect DMTS performance for zero, short, and medium delay trials completed 10 min or 24 h after drug administration. Performance on long delay trials, however, improved significantly following administration of 64.8 nmol/kg GTS-21 [$F(2, 10) = 5.57$, $P < 0.05$]. Multiple comparisons using the Neuman-Keuls method indicated that this significant improvement was limited to long delay trials completed 24 h after administration of this dose (Fig. 4). Although DMTS performance appeared to be elevated 10 min after administration of 64.8 nmol/kg (9.05% above baseline), the amount of improvement was highly variable and not statistically significant. A similar trend toward improved DMTS performance accompanied by considerable variance was observed 10 min and 24 h following administration of 32.4 nmol/kg GTS-21 (8.9% and 12% above baseline, respectively). The specificity of GTS-21 in elevating performance on long delay trials, those which are theoretically the most dependent on information storage and retrieval processes, is illustrated in Fig. 5.

Adverse Effects

The toxicity of GTS-21 was relatively low. In mice, body temperature and locomotor activity were not significantly af-

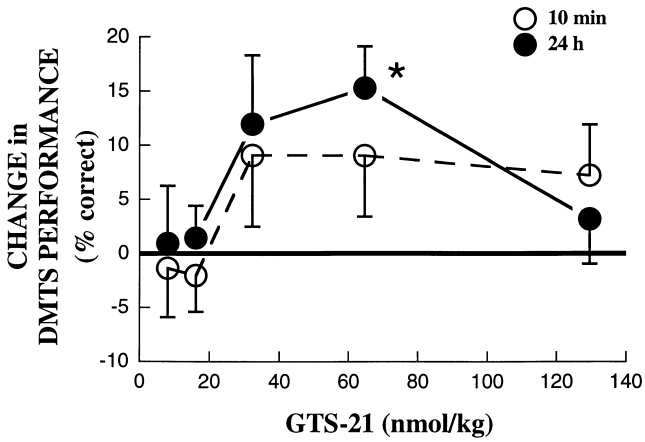

FIG. 4. Monkey delayed matching-to-sample performance at long delay interval. Data (mean ± SEM) represent the change in performance accuracy compared to baseline (saline-injected) scores for each of six monkeys and were obtained 10 min (open circles) and 24 h (solid circles) after intramuscular injection. Data in Figures 4 and 5 are from the same experiments. *$P < 0.05$ compared to baseline performance.

fected until the dose was increased to 62 μmol/kg IP ($n = 8$). At that dose, temperature was reduced by 1.2°C (38.7° ± 0.1° to 37.5° ± 0.4°) and vertical and horizontal activity counts each were reduced by 38% (from 370 ± 36 to 230 ± 52 and

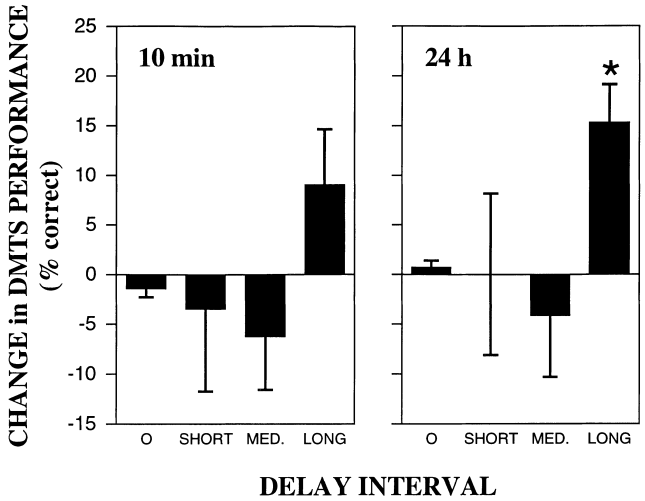

FIG. 5. Selective effect of GTS-21 on long-delay trials in the monkey delayed matching-to-sample (DMTS) task. Data in Figures 4 and 5 are from the same experiments. The changes from control baseline performance (increase or decrease in % correct choices) are shown for 0, short, medium and long delay trials. Left panel: Ten min after administration of GTS-21 (64.8 nmol/kg i.m.) performance levels on zero, short, and medium delay trials tended to be decreased slightly compared to baseline performance. Only on long delay trials was there a trend towards elevated performance. However, these apparent effects were not statistically significant. Right panel: Twenty-four hours after administration of GTS-21 (64.8 nmol/kg i.m.) performance on zero, short, and medium delay trials was little affected. However, performance on long delay trials was significantly elevated [$F(2, 10) = 5.57$, $P < 0.05$].

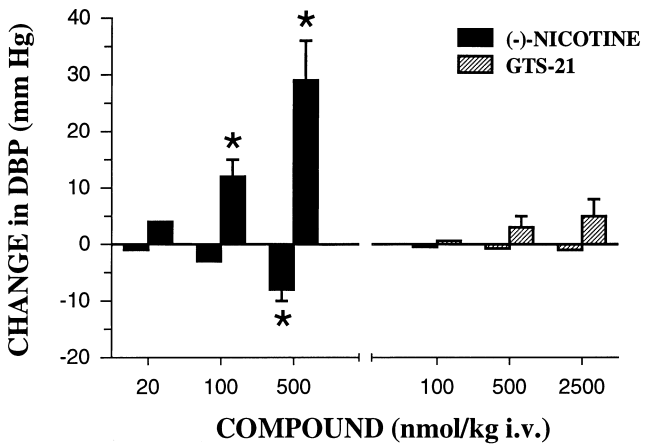

FIG. 6. Lack of effect of GTS-21 on diastolic blood pressure. Diastolic blood pressure (DBP) was measured in the left ventricle of anesthetized dogs ($n = 3$) following intravenous infusion of GTS-21 (100–2500 nmol/kg, hatched bars) followed 90 min later by (−)-nicotine (20–500 nmol/kg, solid bars). GTS-21 had little or no effect on blood pressure while in the same animals 100 nmol/kg (−)-nicotine significantly increased DBP and 500 nmol/kg (−)-nicotine elicited a biphasic effect (decreased DBP followed by increased DBP). *$P < 0.05$.

from 1800 ± 150 to 1110 ± 230, respectively). The approximate lethal dose was 185 μmol/kg (IP).

In dogs, GTS-21 produced negligible effects (< 5 mm Hg) on diastolic blood pressure when administered at bolus doses up to 2500 nmol/kg, i.v. (Fig. 6). In contrast, (−)-nicotine administered to the same animals caused a bi- phasic cardiovascular response consisting of an initial reduction in diastolic blood pressure lasting approximately 45 s, followed by an increase in blood pressure lasting approximately 3 min. The hypotensive effect of (−)-nicotine was statistically significant at the 500 nmol/kg, i.v. dose ($P < 0.05$). The hypertensive effect of (−)-nicotine was statistically significant ($P < 0.05$) at the doses of 100 and 500 nmol/kg i.v. with a change of 12.0 and 29.7 mm Hg, respectively.

Although the experiments in monkeys were not designed to test for adverse effects, none were apparent at the doses used.

Pharmacokinetics

The pharmacokinetic behavior of GTS-21 was estimated in three beagle dogs and three *cynomolgus* monkeys. Parent compound was eliminated with an apparent half life less than 30 min following a bolus intravenous dose and was characterized by plasma clearance rates of about 3-6 l/kg/hr (Table 3). Following oral administration of 0.154 mg/kg (500 nmol/kg), parent GTS-21 was not detectable in the plasma of two monkeys while a plasma concentration of 4.1 ng/ml (13 nM) was measured in one monkey (Table 4). Higher plasma concentrations were observed in dogs with peak values ranging from 6.6 to 18.0 ng/ml (21 to 58 nM). These plasma concentrations indicate ∼27% bioavailability in the dog and ∼1% bioavailability in the monkey.

DISCUSSION

GTS-21, also abbreviated DMXB in some reports, is one of several anabaseine derivatives synthesized by Zoltewicz et al. (51). It has been reported to bind to cytisine and α-bunga-

TABLE 3

PHARMACOKINETIC PARAMETERS OF GTS-21 FOLLOWING INTRAVENOUS ADMINISTRATION IN DOG AND MONKEY

Species	$t_{1/2}$ (h)	AUC(0–∞) (ng·h/ml)	V_c (l/kg)	V_β (l/kg)	CL_p (l/kg/h)
Monkey	0.49	9.8 ± 5.6	3.3 ± 0.2	4.1 ± 0.3	6.1 ± 2.9
Dog	0.27	11 ± 2	0.9 ± 0.06	1.2 ± 0.3	2.9 ± 0.5

The intravenous dose was 0.031 mg/kg (100 nmol/kg). Values (mean ± SEM, $n = 3$) represent the plasma elimination half-life ($t_{1/2}$) of GTS-21, the area under the curve (AUC) of plasma concentration as a function of time, volumes of distribution (V_c and V_β), and the plasma clearance (CL_p).

rotoxin sites, thought to represent α4β2 and α7-containing nAChRs, respectively, in rat brain (22). Although there are some differences in the individual K_i values, the present study confirms the binding of GTS-21 to these sites in rat brain, and, further, characterizes the binding to clonal human α4β2 and α7 nAChR allowing a direct comparison of binding potencies for GTS-21 and (−)-nicotine. In vivo, GTS-21 did not exhibit anxiolytic-like properties in rodent elevated plus maze, but did enhance learning performance in mature, normal monkeys. The adverse effects of GTS-21 were low compared to (−)-nicotine in rodent temperature, locomotion and lethality, and the cardiovascular effects of GTS-21 were negligible compared to (−)-nicotine in dog. GTS-21 was two orders of magnitude less potent than (−)-nicotine to stimulate [^{3}H]dopamine release from rat striatal slices in vitro, and in vivo GTS-21 did not cross-discriminate significantly with (−)-nicotine in rats trained to a (−)-nicotine cue.

GTS-21 bound to human α4β2 nAChR [^{3}H]cytisine sites with a K_i of 20 nM and to human α7 nAChR [^{125}I]α-BgT sites with a K_i of 2,000 nM, indicating a 100-fold selectivity for α4β2 binding compared to α7 binding. However, (−)-nicotine was 1,000-fold selective for human α4β2 nAChR (K_i = 1 nM) compared to human α7 nAChR (K_i = 1,000 nM). In rat brain membranes, GTS-21 and (−)-nicotine bound to cytisine sites, thought to represent α4β2 nAChR (43), with potencies similar to those for human clonal α4β2. In contrast, there were notable differences in displacing α-BgT binding. The binding of GTS-21 to human α7 nAChR α-BgT sites was 3-fold less potent than to rat brain α-BgT sites, while the opposite was found for (−)-nicotine, which bound to human α7 nAChR α-BgT sites about 4-fold more potently than to rat brain α-BgT sites. While GTS-21 was 6-fold more potent than (−)-nicotine at rat brain α-BgT sites, it was 2-fold less potent than (−)-nicotine at human α7 nAChR α-BgT sites. Thus, comparison of these compounds suggests the possibility of a human versus rat species difference in α-BgT displacement at the α7 nAChR subunit. Additionally, GTS-21 (DMXB) appears to be a less efficacious partial agonist at human α7 nAChR (12% of (−)-nicotine (8) than at rat α7 nAChR [28% relative to ACh (22)] expressed in *Xenopus oocytes*.

These results differ somewhat from those of de Fiebre et al. (22) who found GTS-21 to be 4-fold less potent at rat brain cytisine sites and 3-fold more potent at rat brain α-BgT sites than in the present study. However, both studies indicate that, in rat brain, GTS-21 binds more potently than (−)-nicotine to α-BgT sites while it binds less potently than (−)-nicotine to cytisine sites. Additionally, the present report suggests that GTS-21 is less selective than (−)-nicotine for these neuronal nAChR compared to the muscle-type nAChR in Torpedo electroplax, and binds to this muscle-type nAChR about as potently as to the neuronal α7 nAChR. The function of GTS-21 at the muscle-type receptor is not known, but a related anabaseine derivative (DMAC) was reported to be very weak (1% efficacy) at the muscle-type receptor (22).

Although GTS-21 bound to human α4β2 nAChR, it had little efficacy at human α4β2 nAChR or at human ganglionic receptors expressed in IMR-32 cells. GTS-21 was most efficacious (70%) in stimulating [^{3}H]dopamine release from rat striatal slices. One possibility is that the efficacy of GTS-21 is greater at rat striatal nAChR than at human α4β2 or ganglionic nAChR, in analogy with rat versus human α7 nAChR expressed in oocytes (see above). Alternatively, a spare receptor effect or an ability to excite striatal neurons through another mechanism may be involved in the stimulation of [^{3}H]dopamine release.

In human nAChR expression systems, GTS-21 elicited little or no ion flux response in K177 cells transfected with α4β2 nAChR subunits or in IMR-32 cells expressing ganglionic α3-containing nAChR. However, in *Xenopus oocytes* expressing human α7 homomeric nAChR, GTS-21 (1 mM) elicited a response 12% as large as the maximal response to (−)-nicotine (8). This differential may be due to greater sensitivity of the electrophysiologic approach used with oocyte nAChR expression, or it may result from a functional selectivity for α7 compared to α4β2 and α3-containing nAChR.

In behavioral studies, GTS-21 did not appear have anxiolytic-like activity in normal mice or rats. However, GTS-21 did produce a significant enhancement of DMTS performance in normal monkeys and had a selective effect on long-delay trials, which would require the greatest reliance on retention processes. This is consistent with previous reports demonstrating enhanced performance on several learning and memory tasks by intact, aged rats (3), rats with nucleus basalis lesions

TABLE 4

PHARMACOKINETIC PARAMETERS OF GTS-21 AFTER ORAL ADMINISTRATION IN DOG AND MONKEY

Species	C_{max} (ng/ml)	T_{max} (h)	F (%)
Monkey	1.4 ± 1.4	0.3	1.0 ± 1.0
Dog	12 ± 3	0.6 ± 0.5	27 ± 8

The oral dosage was 0.154 mg/kg (500 nmol/kg). Values represent the peak plasma concentration (C_{max}), the time after dosing at which the peak occurred (T_{max}), and the apparent bioavailabilty (F). T_{max} could be estimated in only one monkey because GTS-21 was not detectable in the other two. Other values are shown as mean ± SEM ($n = 3$).

(37), and older rabbits (50). Additionally, GTS-21 has been found to augment the formation of long-term potentiation in rat hippocampal slices from normal rats (31). These data, as a whole, suggest that acute and chronic GTS-21 treatment can enhance both acquisition and retention of cognitive tasks, consistent with other studies demonstrating the ability of (−)-nicotine to improve cognitive performance in monkeys (13,48), rats (23), and patients with AD (42). GTS-21, possibly due to selective activity at brain nAChRs, appears to have a reduced liability for the production of side effects which greatly limit the clinical potential of (−)-nicotine (6).

The profile of enhanced DMTS performance following GTS-21 administration differs from that of (−)-nicotine. Previous studies have demonstrated that (−)-nicotine itself enhances DMTS performance both immediately and 24 h after administration (13,48). GTS-21, however, produced significant enhancement of DMTS performance primarily 24 h after administration of the 64.8 nmol/kg dose. This delayed enhancement of performance has not been characterized and was unexpected in view of the 30-min plasma half-life of GTS-21, but potentially could be explained a longer half-life in brain or by formation of an active metabolite not measured in the pharmacokinetic studies. Additionally, GTS-21 may enhance the formation of a long-lasting neuronal process, such as long-term potentiation, that decays more slowly than the plasma or brain content of GTS-21.

Individual monkeys appeared to exhibit enhanced performance at 10 min after administration of the 64.8 nmol/kg dose and at both 10 min and 24 h after administration of 32.4 nmol/kg GTS-21. These apparent effects were associated with considerable variance and were not statistically significant. However, the possibility exists that with an increased sample size, GTS-21 may elicit an immediate enhancement of performance.

The selectivity of GTS-21 in enhancing retention processes independently of a general stimulatory effect is suggested by its lack of influence on response latencies. Baseline (control) choice response latencies for incorrect (non-matching) responses were significantly longer than for correct responses, theoretically due to an increase the duration of attempted recall of the sample stimulus. GTS-21 had no effect on this pattern of responding. Sample response latencies were similar for correct and incorrect response trials and also were unaffected by drug administration. Decreased latency to respond to sample stimuli may be an indicator of a stimulant drug effect, but was not observed with GTS-21. The prolonged effect of GTS-21 on DMTS performance resembles that of nicotine and may provide information as to which nicotinic receptor subtypes mediate the short-lived and long-term effects of nicotinic stimulation on cognitive performance.

Cardiovascular studies in dogs showed little adverse response to GTS-21 up to 2.5 μmol/kg i.v. (−)-Nicotine, in contrast, was found to elicit significant effects on arterial pressure and heart rate at doses as low as 100 nmol/kg i.v. These findings are consistent with the concept that it is possible to develop nAChR agents that have reduced cardiovascular and other liabilities compared to (−)-nicotine, yet are able to improve performance in at least some preclinical models for learning or memory.

In summary, GTS-21 has been reported to enhance learning/memory performance in aged or lesioned rats and rabbits, and in the present study was found to enhance the performance of normal monkeys in the delayed matching-to-sample (DMTS) task. GTS-21 was relatively effective in stimulating [^{3}H]dopamine release from rat striatal slices compared to its ability to stimulate cation efflux in cells bearing human α4β2 or ganglionic nAChR. However, unlike (−)-nicotine, GTS-21 did not appear to be anxiolytic-like in the rodent elevated plus maze model and did not cross-discriminate significantly in rodents trained to differentiate (−)-nicotine from saline (cf. 12). GTS-21 was less toxic than (−)-nicotine and had little cardiovascular effect in healthy dogs. Memory performance in monkey DMTS was enhanced 24 h after intramuscular administration despite the shorter plasma half-life observed in dog and monkey. Further studies are needed to determine which nAChR subtype(s) are critical in enhancing learning/memory performance, an undertaking which may be facilitated through the use of selective nAChR ligands such as GTS-21.

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