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GTS-21, A Mixed Nicotinic Receptor Agonist/ Antagonist, Does Not Affect The Nicotine Cue

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VAN HAAREN, F., K. G. ANDERSON, S. C. HAWORTH AND W. R. KEM. *GTS-21, a mixed nicotinic receptor agonist/antagonist, does not affect the nicotine cue.* PHARMACOL BIOCHEM BEHAV **64**(2) 439–444, 1999.—Identification of nicotinic receptor subtypes involved in nicotine dependence is required for guiding the design of more selective antagonists capable of blocking the nicotine cue and nicotine self-administration. Due to the multiplicity of nicotinic receptors in the mammalian brain, selective agonists and antagonists are needed to assess the functional involvement of a particular subtype in vivo. Only recently have a few nicotinic receptor subtype-selective antagonists and agonists been identified. GTS-21 (also known as DMBX-anabaseine) is the only agent so far reported that selectively stimulates the α7 nicotinic receptor. Here GTS-21 was used to assess the possible mediation of the nicotine cue by this receptor subtype. Long–Evans rats were trained to discriminate between presession administration of 0.10 or 0.40 mg/kg (−)-nicotine bitartrate and its vehicle. GTS-21 did not substitute for nicotine, as all subjects consistently chose the vehicle lever after GTS-21 substitution. In another experiment, different doses of GTS-21 were administered prior to nicotine administration to investigate whether GTS-21 would antagonize the nicotine cue. Such was not the case. The lack of effect of GTS-21 upon the nicotine cue is consistent with the notion that the cue is mediated by nicotinic receptors other than the α7 receptor. © 1999 Elsevier Science Inc.

Drug discrimination Nicotine bitartrate Nicotine cue GTS-21 Lever press Rats

NICOTINE is a drug of abuse that produces dependence (30). The search for effective drugs to counteract the addictive properties of nicotine has intensified in recent years as the magnitude of the public health problem caused by chronic tobacco self-administration has become vividly apparent. The drug-discrimination paradigm has proven to be a powerful analytic tool in this enterprise [cf. (24,27,28)]. The discriminative stimulus effects of nicotine are likely mediated by neuronal nicotinic acetylcholine receptors (nAChR), as the nicotine stimulus can be blocked by mecamylamine and chlorisondamine which penetrate the blood–brain barrier, but not by the antagonist hexamethonium, which does not readily enter the brain (9,22,24). The largest concentration of nicotinic receptors is observed in the cortex, thalamus, and interpeduncular nucleus, with binding also observed in the amygdala, septum, brain stem motor nuclei, and the locus coeruleus [cf. (2,6)]. Neuronal nicotinic acetylcholine receptors are pentameric complexes, usually composed of β (β2 through β5) as well as α (α2 through α9) subunits. In the brain, nicotinic receptors can be classified pharmacologically as high or low affinity with respect to nicotine binding. Receptors containing the β2 subunit and either α3 or α4 subunits are the most abundant high affinity receptors, and have been implicated in mediation of the nicotine cue (8,21). Unfortunately, selective inhibitors for these receptors, which can readily enter the brain after peripheral administration, are not yet available (20). The major low affinity brain nicotinic receptor is composed of α7 subunits. Several relatively selective antagonists are available for blocking this receptor, including α-bungarotoxin, α-conotoxin, and methyllycaconitine (5,11,32,34,35). Only the latter compound penetrates into the brain when administered peripherally (32). Assessment of the nicotinic receptor involvement in the nicotine cue should ideally involve experiments with subtype-selective nicotinic agonists as well as antagonists.

GTS-21, a 3-benzylidene adduct of anabaseine (14), selectively stimulates α7 nicotinic receptors but also inhibits some

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other nicotinic receptors, including neuronal receptors containing β2 subunits (7,12,13,18). In this article we report our initial analysis of the action of GTS-21 upon the nicotine cue. These studies addressed two questions: 1) to what extent does GTS-21 act as a nicotine cue, and 2) to what extent are the discriminative stimulus properties of nicotine antagonized by prior administration of GTS-21? We have found that even high doses of GTS-21 fail to either stimulate or block the nicotinic cue in rats. Our results indicate that α7 nicotinic receptors play little or no role in mediating the initial recognition of nicotine.

EXPERIMENT 1

This experiment was designed to investigate whether or not the administration of GTS-21 substitutes for the subjective effects of different training doses of nicotine (0.10 and 0.40 mg/kg). Different cues were established because the discriminative stimulus effects of nicotine, as those of other drugs, are directly related to the training dose [cf. (29)]. In this context it is noteworthy that the nicotine training dose has been shown to determine whether or not generalization to the nicotine analogs anabasine and cytisine occurs (23,29).

Method

Subjects. Six male, experimentally naive, Long–Evans rats served as subjects. They were obtained from a commercial supplier (Harlan–Sprague–Dawley, Indianapolis, IN) when they were approximately 90 days old. Upon arrival in the laboratory, they were housed in groups of three under a reversed light–dark cycle (lights on 1800 h) and constant temperature and humidity conditions. The subjects were allowed limited access to food for 22 h prior to each experimental session, but water was continuously available in the home cages.

Apparatus. Experiments were conducted in three standard, two-lever, rodent operant conditioning chambers (Coulbourn Instruments, Allentown, PA), each equipped with a house light, two levers, stimulus lights above the levers, a Sonalert, and a pellet dispenser. Each experimental chamber was enclosed in a sound-attenuating, ventilated cabinet. The chambers were connected to a PDP 11-23 microcomputer (Digital equipment Corporation, Maynard, MA) located in the experimental room itself. Experimental contingencies and data acquisition procedures were programmed in SKED-11 (25) obtained from State Systems, Inc. (Kalamazoo, MI).

Procedure. Subjects were first trained to respond on both levers using a procedure previously described in more detail (33). A 10-min blackout period preceded each experimental session, and sessions were terminated after 30 min had elapsed or after 40 reinforcer presentations, whichever occurred first. Once subjects reliably pressed the lever, the schedule of reinforcement was changed to a tandem Random Interval 60-s, fixed ratio 1 (TAND RI 60-s, FR 1), during which the first response that occurred after an average of 60 s completed the RI requirement, and the next response (completing the FR requirement) resulted in food presentation. As responding stabilized, the FR was incremented across sessions until the terminal schedule (TAND RI 60-s, FR 10) was reached. This terminal schedule has proven to be very useful in assessing the discriminative stimulus properties of numerous compounds, including nicotine, as it encourages stable response rates across both response alternatives (28).

Nicotine discrimination training. The houselight and the stimulus lights above the lever were illuminated at the start of the session, 15 min after the subcutaneous administration of 0.20 mg/kg (−)-nicotine bitartrate (obtained from Sigma, St. Louis, MO) or its vehicle. The nicotine concentration was calculated in terms of the free base form of nicotine. Nicotine was prepared fresh every week. Responding on the left lever was reinforced when nicotine was administered presession (D), and responding on the right lever was reinforced on days when the vehicle (V) had been administered. Nicotine and vehicle were administered according to different 5-day sequences (DVDDV or VDVVD) to minimize discrimination based on variables other than the subjective effects of the drug itself. Reinforcement of responses was arranged according to a TAND RI 60-s, FR 10 schedule. Pressing the wrong lever reset the FR requirement. Sessions were terminated after 30 min had elapsed since the end of the pretreatment period, or after 30 reinforcements, whichever occurred first. Accurate discrimination between nicotine and saline was assumed to be present when a minimum of 80% of all responses prior to the first reinforcer occurred on the injection-appropriate lever for five consecutive sessions. Once an accurate discrimination had been established, the subjects were assigned to two groups. For one group, the training dose of nicotine was decreased from 0.20 to 0.10 mg/kg; for the other group, it was increased from 0.20 to 0.40 mg/kg. Otherwise, sessions continued as before.

GTS-21 substitution test. Once subjects reliably discriminated between 0.10 mg/kg nicotine and its vehicle, or between 0.40 mg/kg nicotine and its vehicle, GTS-21 substitution tests were conducted. During these sessions, lever presses were recorded but had no scheduled consequences. Each dose of GTS-21 (1.0, 2.0, 4.0, and 8.0 mg/kg and vehicle) was administered via intraperitoneal (IP) injections after at least 3 consecutive days of stable baseline responding (nicotine or vehicle) without GTS-21 administration. Sessions were identical to the nicotine discrimination procedure [i.e., 15-min pretreatment period, TAND (RI 60-s, FR 10-resetting schedule)] except that they were terminated either (a) in place of the first reinforcement, or (b) after 5 min had elapsed since the end of the pretreatment period, whichever occurred first. Doses of GTS-21 were administered first in descending order, then in ascending order. For those rats whose accuracy and/or response rates were not stable after the second determination of a dose, subsequent administrations of that dose were conducted as necessary to determine a consistent range of responding.

Results

Figure 1 shows the percentage of total responses (prior to the delivery of the first reinforcer) on the nicotine lever during discrimination training and during test sessions when different doses of GTS-21 (vehicle, 1.0, 2.0, 4.0, and 8.0 mg/kg) were administered in lieu of the nicotine training dose. The data for subjects trained to discriminate 0.10 mg/kg nicotine from vehicle are shown in the top panel of the figure, while the data for those subjects trained to discriminate the 0.40 mg/kg dose of nicotine from vehicle are shown in the bottom panel. Figure 1 shows that subjects consistently discriminated between presession nicotine administration (NIC) and presession vehicle administration (VEH) prior to substitution testing. It can readily be seen that the different doses of GTS-21 did not substitute at all for either one of the two nicotine training doses, as all subjects consistently chose to respond on the lever previously associated with vehicle administration.

Table 1 presents an overview of overall session response rates maintained in the presence of the different nicotine training doses, following vehicle administration and when different doses of GTS-21 were substituted for the nicotine train-

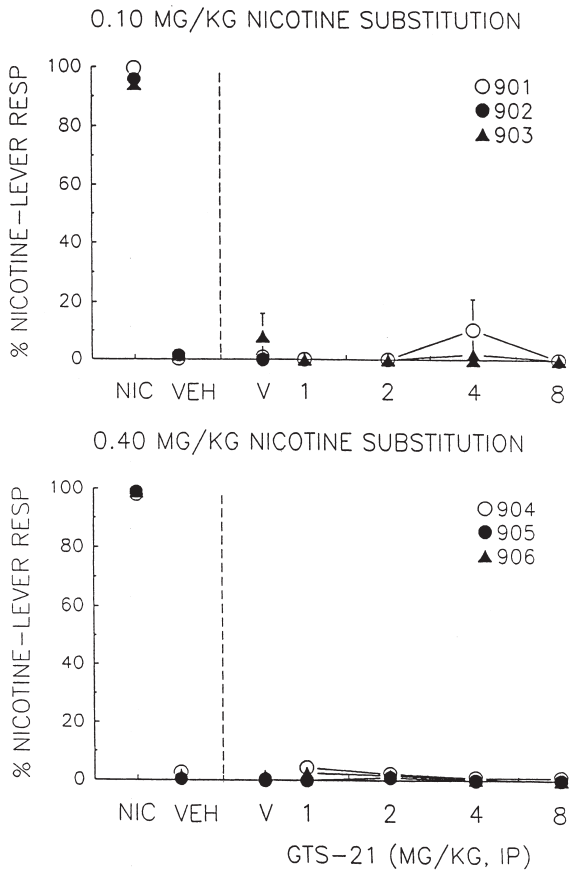

FIG. 1. The percentage of nicotine-lever responses prior to the delivery of the first reinforcer during discrimination training is shown on the left in each panel of the figure (NIC = nicotine, VEH = vehicle). The percentage of nicotine-lever responses following substitution of different doses of GTS-21 for the nicotine training dose is shown on the right side of each figure.

ing dose. Overall session response rates were lower following nicotine administration than following vehicle administration. There did not appear to be a systematic relationship between overall session response rates and GTS-21 substitution for the different training doses of nicotine. Some rates increased dose dependently following substitution up to the highest dose of GTS-21 (e.g., #901), others decreased (e.g., #902 and 905), while others yet were hardly affected (#904 and #906).

EXPERIMENT 2

This experiment was designed to investigate whether or not the administration of GTS-21 would antagonize the subjective effects of different nicotine training doses (nicotine at 0.10 and 0.40 mg/kg).

Method

Subjects. Six experimentally naive male Long–Evans rats served as subjects. They were obtained from a commercial supplier (Harlan–Sprague–Dawley, Indianapolis, IN) when they were approximately 90 days old. Upon arrival in the laboratory they were housed individually in wire mesh cages under a reversed light–dark cycle (lights on 1800 h) and constant temperature and humidity conditions.

TABLE 1

OVERALL SESSION RESPONSE RATES (RESPONSES/MINUTE) AS A FUNCTION OF THE DOSE OF NICOTINE, ITS VEHICLE AND FOLLOWING SUBSTITUTION WITH DIFFERENT DOSES OF GTS-21

	NIC (mg/kg)		GTS-21 (mg/kg)				
Subject	0.10	VEH	0.0	1.0	2.0	4.0	8.0
901	52	66	46	74	77	100	67
902	95	130	45	66	31		111
903	90	126	57	87	84	100	61
	NIC (mg/kg)		GTS-21 (mg/kg)				
Subject	0.40	VEH	0.0	1.0	2.0	4.0	8.0
904	60	75	58	85	59	75	46
905	70	101	63	55	59	39	45
906	38	80	72	71	71	88	36

Apparatus. Identical to the apparatus used in Experiment 1.

Procedure. Training procedures were similar to those described for Experiment 1, except that all subjects were first trained to discriminate 0.40 mg/kg nicotine from its vehicle. Following the assessment of the effects of GTS-21 on the discriminative stimulus engendered by this dose of nicotine, all subjects were retrained to respond to a 0.10 mg/kg nicotine discriminative stimulus and the subjective effects of GTS-21 were then assessed in the presence of this cue.

GTS-21 test. All subjects responded reliably under the TAND (RI 60-s, FR 10) resetting schedule of reinforcement in the presence of both nicotine and vehicle when antagonism tests were initiated. During these tests, subjects received an IP injection of different doses of GTS-21, 30 min prior to the start of the test session and 15 min prior to the administration of the nicotine training dose (0.40 mg/kg nicotine initially, then 0.10 mg/kg nicotine). Lever presses were recorded, but had no scheduled consequences during test sessions. The test session was terminated when subjects completed the requirements of the TAND (RI 60-s, FR 10) schedule on one of the levers (but reinforcement was not presented), or after 5 min, whichever came first.

Results

Figure 2 shows the percentage of nicotine-lever responses prior to the delivery of the first reinforcer in the presence of the 0.40 mg/kg nicotine cue (top panel) and in the presence of the 0.10 mg/kg nicotine cue (bottom panel). It can be seen in Figure 2 that all subjects reliably discriminated between the presession administration of 0.40 mg/kg nicotine or 0.10 mg/kg nicotine and its vehicle during discrimination training. Figure 2 also shows that all subjects continued to respond on the nicotine-appropriate lever following the administration of different doses of GTS-21 prior to nicotine administration independent of the valence of the nicotine cue (0.40 mg/kg or 0.10 mg/kg).

Table 2 shows overall session response rates (responses per minute as a function of nicotine and vehicle administration alone, or in the presence of different doses of GTS-21.

In this experiment subjects responded at higher rates following the administration of 0.40 mg/kg nicotine administration than following vehicle administration during discrimina-

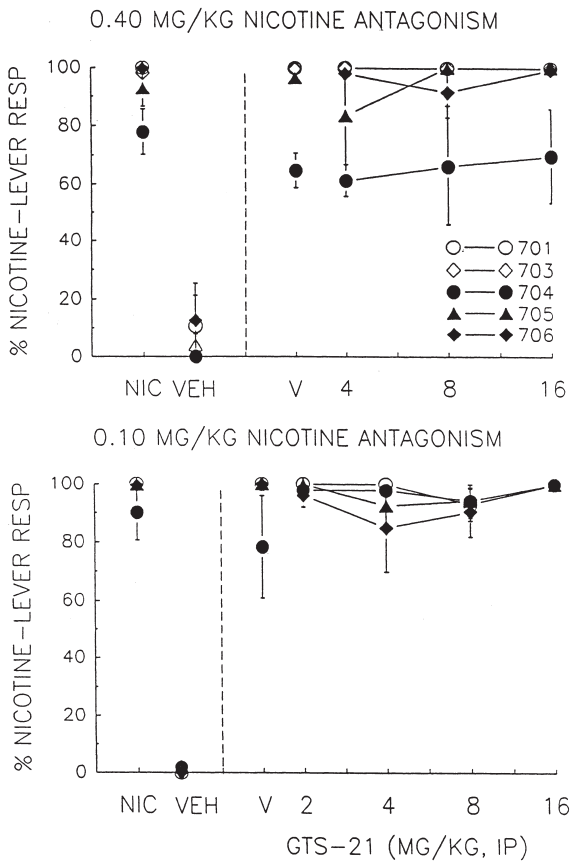

FIG. 2. The percentage of nicotine-lever responses prior to the delivery of the first reinforcer during discrimination training (NIC and VEH on the left in each panel of the figure). The percentage of nicotine-lever responses following prior administration of different doses of GTS-21 is shown on the right in each panel of the figure.

tion training. This difference in response rates was generally maintained when subjects were retrained in the presence of the 0.10 mg/kg nicotine stimulus. Pretreatment with GTS-21 in many cases (e.g., #701, 703 and 704) decreased response rates in the presence of the 0.40 mg/kg nicotine cue. Pretreatment with GTS-21 had less of an effect on response rates previously observed in the presence of 0.10 mg/kg nicotine.

GENERAL DISCUSSION

GTS-21 is a relatively lipophilic compound that rapidly enters the central nervous system when administered orally or by injection (15). It has numerous actions in the central nervous system, as reflected in the enhancement of cognitive behavior (1,3,17,18,36), auditory gating (26), and elevation of dopamine and norepinephrine levels in the frontal cortex (31). These actions are thought to be due to the rather unique ability of this drug candidate to activate only α7 type nicotinic receptors (7,18). GTS-21 also has been shown to act as an antagonist upon other central nicotinic receptors, but usually at much higher doses.

Our experiments were designed 1) to determine whether GTS-21 would substitute for different training doses of nicotine, and 2) whether or not GTS-21 would antagonize the discriminative stimulus properties of different training doses of

TABLE 2

OVERALL SESSION RESPONSE RATES (RESPONSES/MINUTE) AS A FUNCTION OF THE DOSE OF NICOTINE (mg/kg), ITS VEHICLE AND FOLLOWING PRETREATMENT WITH DIFFERENT DOSES OF GTS-21 (mg/kg)

	NIC (mg/kg)		GTS-21 (mg/kg)			
Subject	0.40	VEH	0.0	4.0	8.0	16.0
701	46	39	18	19	23	15
703	141	129	96	96	19	82
704	162	127	41	36	71	88
705	145	92	129	153	122	107
706	69	38	33	57	50	65

	NIC (mg/kg)		GTS-21 (mg/kg)				
Subject	0.10	VEH	0.0	2.0	4.0	8.0	16.0
701	52	67	28	38	47	44	54
703							
704	99	89	129	120	158	144	86
705	107	88	82	86	47	77	129
706	64	37	28	64	86	72	

nicotine. The results clearly show that GTS-21 does not substitute for, nor antagonize, nicotine cues at 0.10 or 0.40 mg/kg. Drugs which act as nicotine cues generally stimulate high affinity nicotinic receptors containing β subunits (21). Since GTS-21 is a moderate affinity antagonist of these nicotinic receptor subtypes, it is possible that a mild stimulating effect of the GTS-21 doses administered in our experiments may not have been expressed because of a simultaneous inhibition of the other, high affinity receptors. Brioni et al. (5) have shown that MLA, a rather selective α7 antagonist, does not affect the nicotine cue. Rats were trained to discriminate between 1.9 μmol/kg nicotine and its vehicle, and were then treated with methyllycaconitine administered either by the intraperitoneal or by the intracerebroventricular route. Methyllycaconitine failed to antagonize the nicotine cue, indicating (as do the results of the present experiment) that α7 receptors do not appear to play an important role in mediating the discriminative stimulus properties of nicotine.

Our experiments also sought to establish whether GTS-21 action in the context of a nicotine discrimination, might be a function of nicotine training dose. In Experiment 1 the effects of training dose were assessed in different groups of subjects who had first been trained to discriminate an intermediate dose of nicotine (0.20 mg/kg), and were subsequently retrained to discriminate a lower (0.10 mg/kg) or a higher (0.40 mg/kg) dose of nicotine. In Experiment 2, all subjects were first trained to discriminate a 0.40 mg/kg training dose prior to an experimental condition in which the training dose was reduced to 0.10 mg/kg nicotine. Although retraining was effective in both experiments, it is possible that the experimental conditions could have caused the development of some nicotine tolerance, which could have influenced the behavioral assessment of the effectiveness of GTS-21. Previous experiments have shown that prolonged exposure to nicotine may result in upregulation of nicotinic acetylcholine receptors (16). James et al. (10) have shown that desensitization of these receptors may occur after only one injection, and that there exists considerable variation among individual rats. If development of tolerance to nicotine had occurred, this would have

reduced nicotine choice following nicotine administration and also enhanced the ability of GTS-21 to block the nicotine cue. However, these changes were not observed.

In conclusion, our results indicate that GTS-21 does not substitute for or antagonize different nicotine cues, despite the fact that it affects some behaviors in a manner similar to that of nicotine. In particular, GTS-21, much like nicotine, enhances learning and memory performance in aged or lesioned rats, rabbits, and monkeys (1,3,4,17,36). The failure of GTS-21 to act as a nicotine cue should be advantageous in considering its possible chronic therapeutic use in enhancing memory (19).

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