A Comparative Study on the Effects of Nicotine and GTS-21, a New Nicotinic Agonist, on the Locomotor Activity and Brain Monoamine Level

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ABSTRACT—Effects of GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride], a selective nicotinic agonist, on locomotor activity and dopamine turnover were examined and compared to those of nicotine to test if GTS-21 exhibits side effects similar to those of nicotine. GTS-21 had no effect on locomotor activity in mice or dopamine turnover in rats. In contrast, nicotine produced a biphasic effect on locomotor activity. It also enhanced dopamine turnover rates in the striatum and cerebral cortex, suggesting the involvement of dopaminergic systems in the nicotine-induced changes in locomotor activity. GTS-21 exhibits fewer adverse effects, suggesting that it has therapeutic potential for cognitive disorders related to central cholinergic dysfunction.

Keywords: GTS-21, Nicotine, Nicotinic agonist

It has been proposed that the drugs capable of stimulating neuronal nicotinic acetylcholine receptors (nAChRs) may have therapeutic effects in Alzheimer's disease (AD) (1), since the decrease of nicotinic receptors in the frontal cortex was demonstrated in AD patients (2) and the administration of nicotine improved cognitive and attentional performance and reduced anxiety in AD patients (3). However, the clinical use of nicotine for AD patients is limited because of its adverse effects (cardiovascular, nausea and alternation of locomotor activity).

Recently, GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride], a selective nicotinic agonist, was reported to have 4 times higher affinity than nicotine for nicotinic receptors consisting of α7-subunits (4). In previous studies, we demonstrated that GTS-21 exerted protective effects against transient ischemia- and nucleus basalis magnocellularis (nBM) lesion-induced neuronal damage in experimental animals (5, 6) and attenuated cognitive impairment caused by cerebral ischemia in a passive avoidance task. These findings suggested that this compound may be useful for the treatment of cognitive disorders due to central cholinergic dysfunction. However, it remains unclear whether this compound produces adverse effects similar to those of nicotine. Thus, in the present study we investigated the effects of GTS-21 on locomotor activity in mice, dopamine turnover in the rat brain and cardiovascular systems in cats and compared these effects with those of nicotine.

Male ddY mice (3-week-old; Japan SLC, Inc., Shizuoka), male Wistar rats (6-week-old; Clea Japan, Inc., Tokyo) and mongrel cats (2.5–5.0 kg; Clea Japan, Inc.) were used. The animals were housed in a thermostatically regulated environment at 23±3℃ with constant humidity (30–70%) and a 12-hr light-dark cycle (lights on 06:00–18:00), and they were given free access to food and water.

The following compounds were used: GTS-21 (synthesized at University of Florida or Taiho Pharmaceutical Co., Ltd., as described previously (7)), (−)-nicotine ditartrate salt (Wako Pure Chemical Industries, Ltd., Osaka), and pentobarbital-Na (Nembutal®; Abbot Laboratories, North Chicago, IL, USA). Drugs were dissolved in distilled water or physiological saline. For i.v. injection, drugs were dissolved in physiological saline and neutralized with NaOH (pH 6.5). The doses are expressed in terms of the salts.

In the motor activity test, 5 mice were placed in a cage (35×40×35 cm), and the motor activity was measured

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using Animex (Muromachi Kikai Co., Ltd., Tokyo). After a 1-hr of habituation period, mice were injected with GTS-21 (0.5 and 2 mg/kg, i.p. or 10 mg/kg, p.o.) or nicotine (0.3 and 1.5 mg/kg, i.p. or 10 mg/kg, p.o.). Measurement of motor activity was started immediately after drug administration, and data was recorded at 15-min intervals over 45 min. Each animal group consisted of 15 mice.

To determine the contents of monoamine and monoamine metabolites in the rat brain, a whole brain was removed immediately after decapitation and divided into the cerebral cortex and striatum according to the method of Glowinski and Iversen (8). Each tissue was homogenized in 4 ml of 0.1 M perchloric acid solution containing 0.1 mM sodium pyrosulfite and 0.02 mM Na_2-EDTA. After centrifugation at $13,000 \times g$ for 20 min, the supernatant was filtrated through a 0.45-mm filter (Millipore Co., Bedford, MA, USA) and used for the determinations. The concentrations of dopamine (DA) and its metabolites were determined by high performance liquid chromatography (HPLC) (Shimadzu Co., Kyoto) with an electrochemical detector (Bioanalytical System, West Lafayette, IN, USA). The mobile phase consisted of 0.1 M citric acid, 0.1 M sodium acetate, 17% methanol, 3 mg/l Na_2EDTA and 350 mg/l Na octanesulfonate. The following standard chemicals were used: DA-HCl, 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) (Sigma Chem., St. Louis, MO, USA). One hour after GTS-21 (1, 3 and 10 mg/kg), nicotine (1, 3 and 10 mg/kg) or distilled water (D.W.) was injected p.o. to rats. After oral administration of 10 mg/kg of GTS-21 in rats, the T_{max} value in plasma was 1.00 ± 0.71 hr, and the brain concentration was parallel to it in the plasma (9). In the present study, the levels of DA and its metabolites are expressed as a percent of the control values. Control levels at 1 hr after D.W. treatment in rats were as follows (mean$\pm$S.E.M.; n=5): in the cerebral cortex: DA, 661.7 ± 25.1; DOPAC, 83.5 ± 6.0; HVA, 92.5 ± 4.4 and in the striatum: DA, 4517.0 ± 271.4; DOPAC, 823.2 ± 42.5; HVA, 654.3 ± 38.2 ng/g wet tissue.

When testing the effects of GTS-21 on the cardiovascular system, the right common carotid artery of a cat was cannulated under pentobarbital anesthesia and systemic blood pressure was continuously measured via a transducer (Model P-23ID; San-ei, Tokyo) and recorded on a polygraph (Model 361, San-ei). Drugs were injected i.v. through a catheter inserted into the right femoral vein.

Behavioral and neurochemical data were analyzed by Dunnett's test. A difference with $P < 0.05$ was considered significant.

As shown in Fig. 1, the i.p. injection (0.5 and 2 mg/kg) or the p.o. administration (10 mg/kg) of GTS-21 did not change the motor activity during the same observation periods. The i.p. injection of nicotine (0.3 mg/kg) had no effect on the motor activity recorded during the observation period. In contrast, the animals treated with 1.5 mg/kg nicotine showed a significantly decreased motor activity during the first 15-min, and a significantly increased motor activity during the second 15-min observation period compared to the activity of the vehicle-treated group. The p.o. administration of 10 mg/kg nicotine also exhibited a tendency to increase the motor activity during

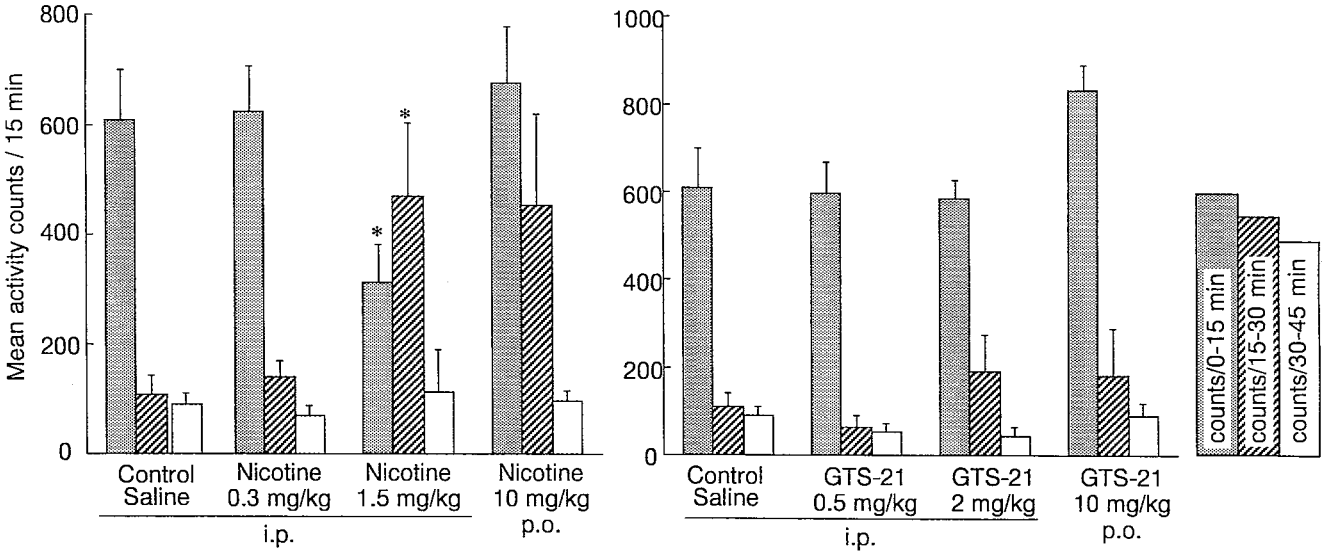

Fig. 1. The effects of nicotine and GTS-21 on locomotor activity in mice. Animals were tested for 45 min, beginning immediately after subcutaneous injection of saline or drugs. Each column with a vertical bar represents the mean$\pm$S.E.M. of 15 animals/group. *$P < 0.05$, significantly different from the saline group by Dunnett's test.

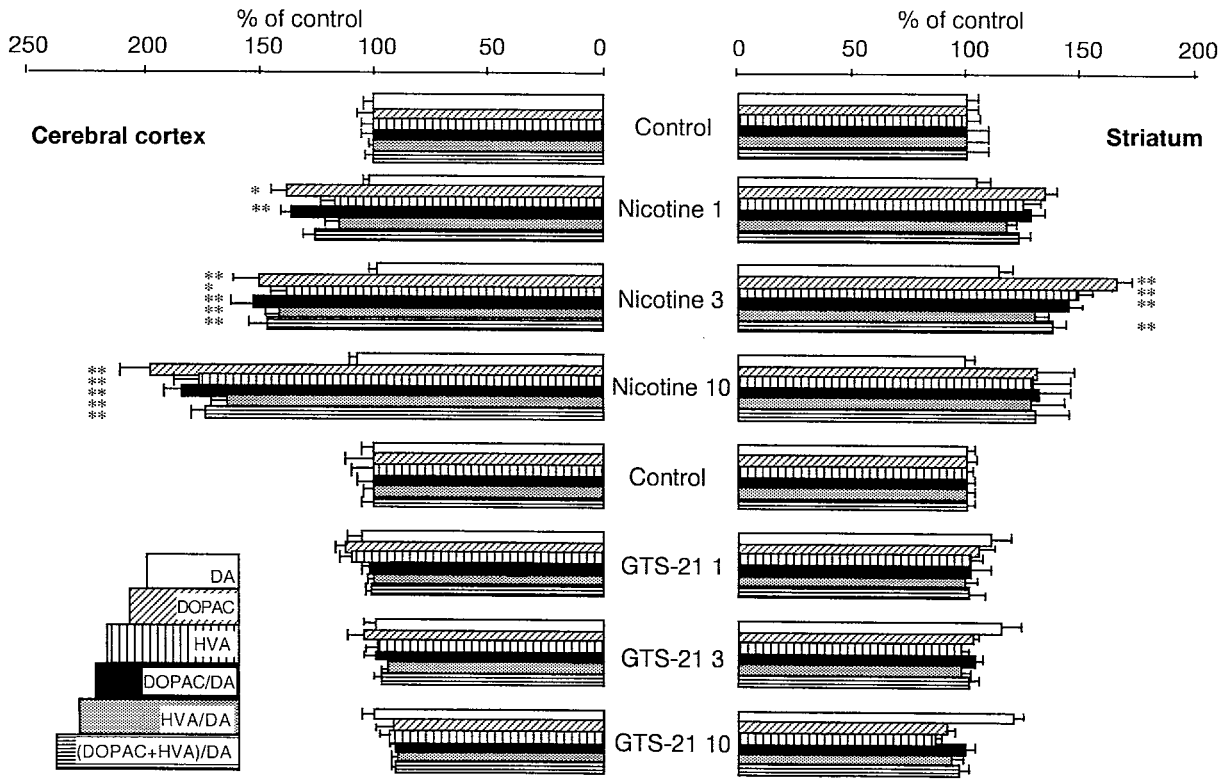

Fig. 2. Effects of nicotine and GTS-21 on dopamine and its metabolites in the cerebral cortex and striatum. A drug or distilled water was orally administered, and then the animals killed 1 hr later. Results are the mean±S.E.M. of 5 animals/group and express as a percent of the control values. *$P<0.05$ and **$P<0.01$, significantly different from the vehicle by Dunnett's test.

the second 15-min period, but the effect was non-significant.

Figure 2 summarizes the effects of p.o. administered GTS-21 (1, 3 and 10 mg/kg) and nicotine (1, 3 and 10 mg/kg) on the contents of DA and its metabolites in the cerebral cortex and striatum. The administration of GTS-21 did not cause any change in the levels of DA or its metabolites, while nicotine increased DOPAC and HVA levels in the cerebral cortex and striatum.

GTS-21 had no effect on blood pressure, heart rate or respiration (approximately 100 μg/kg), although GTS-21 at high doses exhibited a very weak vasodepressor response with an ED_{50} value of 9.8 mg/kg (i.v.). In contrast, the i.v. administration of nicotine (10−40 μg/kg) produced an initial transient vasodepressor response followed by a sustained vasopressor response, and the increase of blood pressure was accompanied by increases of the heart rate and respiration rate (Fig. 3). The ED_{50} value of nicotine to produce a sustained vasopressor response was 32.1 μg/kg.

The present study demonstrated that although GTS-21 was a highly selective nAChR agonist, it failed to exhibit side effects similar to those of nicotine. Systemically administered nicotine but not GTS-21 initially depressed and then enhanced locomotor activity in mice. In the early phase of the behavioral test, fear and anxiety were evoked by the novel environmental stimuli. Anxiolic-like effects of systemic administration of nicotine have been reported in experimental animals (10). Nicotine seems to reduce the animal's feeling of fear and anxiety in a novel environment, so nicotine decreased locomotor activity in the early phase. Furthermore, it has been reported that nicotine enhances the activity of dopaminergic systems in the brain via stimulation of nAChRs and that this enhancement is involved in the hyperactivity caused by systemic nicotine (11). Consistent with these findings, we found that nicotine administration significantly increased the DA turnover in the striatum and cortex of the rat brain. In contrast, GTS-21, at the same dose range as nicotine, produced no effect on the DA turnover, suggesting that the failure of GTS-21 to affect the motor activity of mice may be due to its inability to stimulate central dopaminergic systems.

When the effects on the cardiovascular and respiration responses were tested in cats, nicotine produced a change in blood pressure and increases in heart rate and excitation of respiration at the dose of μg order. However, GTS-21 showed very few effects on these responses. Con-

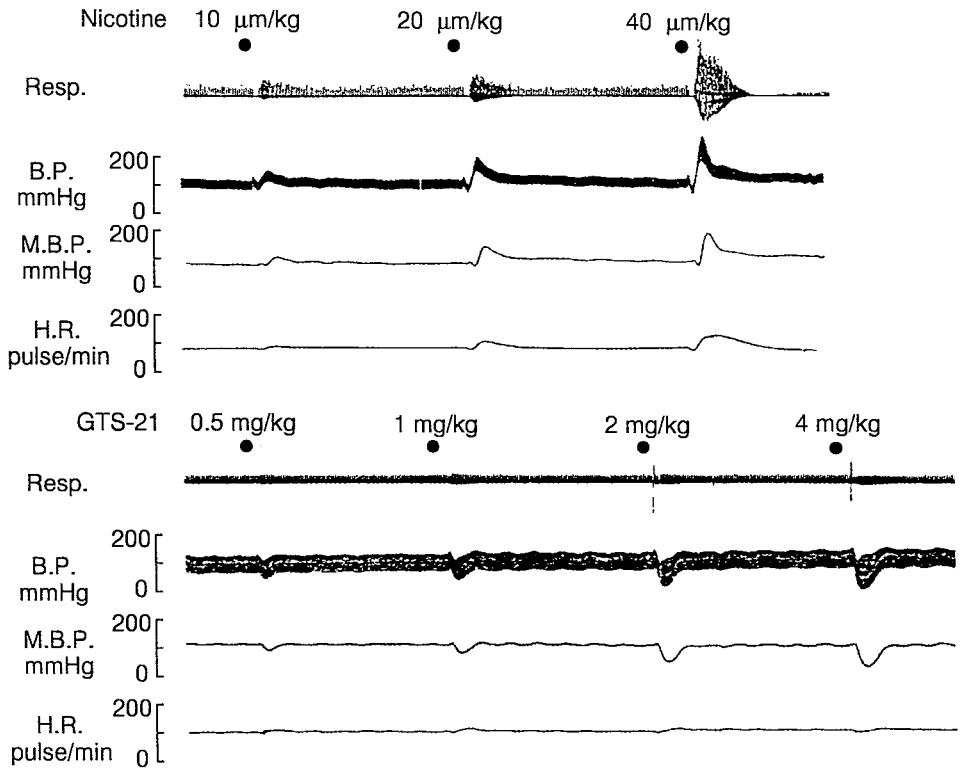

Fig. 3. Effects of intravenous administration of nicotine and GTS-21 on respiration (Resp.), blood pressure (B.P.), mean blood pressure (M.B.P.) and heart rate (H.R.) in anesthetized cats.

sidering evidence that the effects of nicotine on the cardiovascular system are known to be mediated by its agonistic properties at nicotinic receptors in the autonomic nervous system and neuromuscular junction of skeletal muscle (12), the present findings indicate that GTS-21 has higher selectivity for the CNS nAChR subtype consisting of the $\alpha 7$ subunit rather than for peripheral ganglionic and muscle-type nAChRs. For GTS-21, stimulation of the $\alpha 7$-subunits of neuronal nAChRs did not contribute to the cardiovascular response at the peripheral system.

Our previous studies indicated that GTS-21 has cytoprotective effects against neuronal damage in experimental animals (5, 6) at the dose range of 0.1–10 mg/kg (p.o.). In addition, in vitro studies demonstrated that GTS-21, at 1–10 μM, has a protective action against glutamate-induced neurotoxicity in cultured cortical neurons and that this effect is antagonized by α-bungarotoxin, an $\alpha 7$-selective antagonist (13). Taking into account the effective dose of GTS-21 to exert a neuroprotective activity, the lower potential of GTS-21 to produce undesirable pharmacological effects similar to those of nicotine is beneficial when GTS-21 is used for the treatment of AD patients or the cognitive impairment caused by neuropathological changes in central cholinergic systems.

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