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Modulatory effect of ginseng total saponin on dopamine release and tyrosine hydroxylase gene expression induced by nicotine in the rat

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Abstract

Several studies have demonstrated that behavioral activation induced by psychostimulants is prevented by ginseng total saponin (GTS), which has been known to act on the central dopaminergic system. In an attempt to investigate whether the effect of GTS is through its inhibitory action on the elevated dopaminergic transmission, we examined the effect of GTS on nicotine-induced dopamine (DA) release in the nucleus accumbens (NA) of freely moving rats using in vivo microdialysis. Systemic injection of nicotine (3 mg/kg; i.p.) produced a mild increase in extracellular DA of dialysates samples in the NA ($132 \pm 13\%$ over basal levels at the peak). GTS (100 mg/kg; i.p.) had no effect on resting levels of extracelluar DA. However, an increase in accumbens DA release produced by systemic nicotine was completely blocked by systemic pre-treatment with GTS (100 mg/kg; i.p.). In addition, the effect of GTS on nicotine-induced tyrosine hydroxylase (TH) and immediate early gene expression in ventral tegmental area (VTA) or NA regions was examined. A single injection of nicotine increased TH mRNA level at VTA region. GTS, which did not affect the basal TH mRNA expression, attenuated nicotine-induced TH mRNA expression. Nicotine slightly increased both c-fos and c-jun mRNA level and GTS, which did not affect the basal c-fos and c-jun mRNA expression, further enhanced nicotine-induced c-fos and c-jun mRNA level at both VTA and NA regions. Our results suggest that GTS may have an inhibitory action against nicotine-induced DA release in NA region and TH mRNA expression in VTA region. GTS may exert an potentiative effect on both c-fos and c-jun mRNA expression at NA region through inhibiting the release of DA in NA. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Nucleus accumbens; Dopamine release; Ginseng total saponin; Nicotine; Tyrosine hydroxylase; c-fos; c-jun

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1. Introduction

The mesolimbic dopaminergic system projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NA) is known to play an important role in the reinforcing effects of drugs of abuse such as nicotine, an alkaloid agonist of nicotinic acetylcholine receptors. For example, several studies have shown that nicotine can produce a marked increase in dopamine (DA) release in the NA (Imperato et al., 1986; Damsma et al., 1989; Mirza et al., 1996; Pontieri et al., 1996; Marshall et al., 1997). Lesions of this system abolished both locomotor activating and the reinforcing effects of nicotine (Clarks et al., 1998). The rewarding and behavioral effects of nicotine seem to be related with an increase in DA release in the NA (Benwell and Balfour, 1992; Corrigall et al., 1992; Di Chiara and Imperato, 1998).

On the other hand, it has been demonstrated that behavioral activation produced by drugs of abuse may be prevented by ginseng total saponin (GTS), a fraction with active components of ginseng extract. For example, methamphetamine-, morphine- or cocaine-induced behavioral activities were blocked by pre-treatment with GTS (Tokuyama et al., 1992; Kim et al., 1995a,b). Conditioned place preferences induced by methamphetamine were also antagonized by GTS (Kim et al., 1996). Since the reinforcing effects of DA release in the nucleus accumbens produced by drugs of abuse may be associated with a behavioral hyperactivity, it seems possible that an inhibitory effect of GTS on behavioral activity might reflect blockade of dopaminergic transmission in NA.

Several lines of evidence have demonstrated that systemic injection of nicotine increases tyrosine hydroxylase (TH) activity in VTA and immediate early gene expression in VTA and NA regions (Pang et al., 1993; Pagliusi et al., 1996; Panagis et al., 1996; Pich et al., 1997; Mathieu-Kia et al., 1998; Pich et al., 1998). The treatment with ginsenoside Rg1 itself tends to increase c-fos induction in the hippocampus (Liu and Zhang, 1996). Recently, Kim et al. (1998) have reported that GTS inhibits TH expression in the bovine adrenal gland. However, the role of GTS in the regulation of TH and immediate early genes induced by nicotine in VTA or NA region has not been well characterized.

In order to investigate whether the effect of GTS is through its inhibitory action on the elevated central dopaminergic transmission produced by nicotine, we examined the effect of GTS on the nicotine-induced extracellular DA release in the NA of freely moving rats using in vivo microdialysis technique. Furthermore, effects of GTS on nicotine-induced TH, c-fos and c-jun mRNA levels in VTA or NA region were investigated in the present study.

2. Material and methods

2.1. Subjects

Subjects were adult male Sprague–Dawley rats, weighing between 280 and 320 g at the time of surgery. Rats were kept on a 12:12 h light:dark cycle in individual home cages with food and water available ad libitum.

2.2. Surgery and dialysis procedure

Rats were anesthetized with sodium pentobarbital (50 mg/kg). Using aseptic techniques, guide cannulae (Carnegie Medicine, Stockholm, Sweden) aimed to terminate within the NA (AP, 2.0; L, 1.2 from bregma; H, 8.0 from dura) (Paxinos and Watson, 1986) were stereotaxically implanted and attached to the skull using skull screws and dental cement as previously described. Briefly, the cannula was closed with a tight-fitting stainless steel obturator. Following 1 day recovery, a 2 mm vertical dialysis probe (CMA12, Carnegie Medicine) connected via a dual liquid swivel to a syringe pump containing an artificial cerebrospinal fluid (ACSF; 145 mM NaCl, 2.7 mM KCl and 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, pH 7.4) was perfused at a constant rate of 1.5 μl/min, and was inserted into the guide cannula in the rats. Rats were then placed in the polycarbonate container and the outlet tubing was connected to a microfraction collector (Carnegie Medicine). The dialysate was collected

during 30 min sampling intervals in plastic microvials on the fraction collector. Animals were pre-treated with GTS (100 mg/kg; i.p.) or saline 90 min before any drug injections. Three baseline samples were collected and then injected with systemic nicotine (3 mg/kg; i.p.) or saline. Samples were then collected for 210 min following drug treatments. In order to examine the effect of GTS on the resting DA release in the NA, another group of rats was injected with GTS (100 mg/kg; i.p.) alone.

2.3. Analytical procedure

Sample (injection volume, 30 μl) was assayed for DA using HPLC equipped with electrochemical detector (ESA, coulochem 5200B). The detector was equipped with the high performance analytical cell (ESA model 5014) which is tailored for use in microdialysis applications. The first potential was set at −100 mV, and the second was at +320 mV. A guard cell was set at +400 mV followed by Nova-Pak C18 column (150 × 3.9 mm, Waters, Milford, MA). The mobile phase contained 75 mM sodium phosphate, 0.1 mM EDTA, 1.4 mM octan sulfonic acid, 10% acetonitrile, pH 3.2 with H_3PO_4, and pumped at a flow rate of 1.0 ml/min. DA in dialysates was expressed as percentage of baseline release measured as the mean of the last three samples collected immediately before any experimental treatment.

2.4. Histological procedure

At the end of the experiments, rats were perfused transcardially, under deep pentobarbital anesthesia, with normal saline followed by a 10% formalin solution. The brains were removed from the skulls and stored in 10% formalin for at least 2 weeks after which 50 μm cryostat sections were cut through the sites of the microdialysis probes and the injection cannulae and subsequently stained with cresyl violet.

2.5. Isolation of total RNA

Total cellular RNA were extracted from VTA and NA regions using a rapid guanidine thiocyanate-water saturated phenol/chloroform extraction and subsequent precipitation with acidic sodium acetate (Chomczynski and Sacchi, 1987). Total cellular RNA in the aqueous phase were precipitated with cold isopropyl alcohol. Isolated RNA samples were subjected to spectrophotometric analysis at 260 and 280 nm.

2.6. Non-isotope northern blot hybridization analysis

Ten micrograms of total RNA were denatured and electrophoresised on 1% agarose-formaldehyde gels (Kopchik et al., 1981) and transferred to nylon hybond-N hybridization membrane sheets (Amersham, Buckinghamshire, England). After baking for 1–2 h at 80°C, the membranes were prehybridized at 68°C for at least 1 h in prehybridization buffer (5× SSC, 50% formamide, 0.02% SDS, 0.1% sodium *N*-lauroyl sarcosine, 2% blocking reagent). The Dig-labeled TH, *c-fos* and *c-jun* probes were added to prehybridization buffer containing 50% formamide. The membranes were incubated overnight at 68°C in a shaking water bath, and washed twice for 10 min per wash in 2× wash solution (2× SSC, 0.1% SDS) at room temperature. Then, the membranes were washed twice for 15 min per wash in 0.1× wash solution (0.1× SSC, 0.1% SDS). After equilibrating the membranes in buffer I (100 mM maleic acid, 150 mM NaCl, pH 7.5) for 1 min, the membranes were gently agitated in buffer II (1% blocking reagent in buffer I) for 30–60 min. The membranes were hybridized with the diluted anti-DIG-alkaline phosphatase [1: 10 000 (75 mU/ml)] in buffer II for 30 min. After washing the membrane twice for 15 min per wash in 0.3% Tween 20 (in buffer I), the membranes were equilibrated in buffer III (100 mM Tris–HCl, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$) for 2 min. Approximately 0.5 ml (per 100 cm^2) of Lumi-Phos TM 530 was spread over the surface of membrane. After incubation of membrane at 37°C for 15–20 min, the membranes were exposed to Hyperfilm-MP (Amersham) for detection of the chemi-luminescent signal. For rehybridization, blots were washed for 20 min at room temperature in sterile millipore water, then the membranes were washed

for overnight at 65°C in 50 mM Tris–HCl, pH 8.0, 50% dimethylformamide and 1% SDS to remove the hybridized probe and rehybridized to Dig-labeled rat cyclophilin cRNA probe, a gene encoding peptidyl-prolyl *cis-trans* isomerase which is constitutively expressed in most mammalian tissues with the exception of skeletal muscle (Danielson et al., 1988; Takahashi et al., 1989). The cRNA probes for TH (35), *c-fos* (Curran et al., 1987), *c-jun* (Hattori et al., 1988), and cyclophilin (Danielson et al., 1988; Takahashi et al., 1989) were synthesized in vitro from linearized expression vector with using DIG-UTP, as suggested by the manufacturer (Boehringer Mannheim, Germany).

2.7. Statistical analysis

Data were statistically analyzed by two-way analysis of variance (ANOVA) (drug × time) with repeated measures on the time factor. Contrasts between individual means were carried out to identify the origin of differences between the groups.

2.8. Drugs

Nicotine was purchased from Sigma (St Louis, MO). GTS fraction was obtained from Korea Ginseng and Tobacco Research Institute (Taejon, Korea). Total saponin fraction is composed of R_{b1} (18%), R_{b2} (9%), R_c (10%), R_d (8%), R_e (9%), R_f (3%), R_{g1} (6%), R_{g2} (4%), R_{g3} (5%), R_0 (4%), R_a (3%), other ginsenosides and components (21%). All drugs used for injection were dissolved in sterile saline (0.9% NaCl solution).

3. Results

3.1. Histological analysis

Examination of stained sections revealed that all dialysis probes terminated within the NA.

3.2. Dopamine analysis

Analysis of raw data obtained from the three baseline samples by two-way ANOVA with repeated measures (6 × 3, drug treatment × time, 30 min blocks) indicated that there were no significant differences among the groups in DA levels ($F_{5,23} = 0.93$, $P > 0.047$). Changes in DA release after injections of drugs are shown in Fig. 1A, where it can be seen that nicotine (3 mg/kg; i.p.) resulted in a peak increase in $132 \pm 13\%$ over baseline at 120 min and tended to return towards baseline values at the end of the 3 h testing period. An ANOVA (6 × 7, drug × time) performed on the DA values after drug injections, expressed as percentage of baseline release, indicated a significant effect of drug treatments ($F_{5,23} = 10.5$, $P < 0.0001$). Post hoc contrasts indicated that nicotine injection ($F_{7,17} = 3.5$, $P < 0.01$) significantly increased DA output compared to the group injected with saline. As shown in Fig. 1B, no significant DA changes were observed following injections of saline or nicotine with GTS.

GTS (100mg/kg; i.p.) had no effects on resting levels of extracellular DA ($F_{7,17} = 0.6$, $P > 0.7$), but completely blocked the increase in DA release in NA induced by a systemic injection of nicotine ($F_{7,17} = 2.8$, $P < 0.03$).

3.3. Effect of GTS on nicotine-induced TH mRNA expression in VTA region.

The TH mRNA level in rat VTA was examined 30 min after nicotine (3 mg/kg) administration. As shown in Fig. 2, nicotine markedly increased the TH mRNA level. The treatment with GTS alone did not affect the basal TH mRNA level. However, the treatment with GTS abolished nicotine-induced TH mRNA increase (Fig. 2). The elevations of TH mRNA level induced by nicotine was completely inhibited by pre-administration with GTS (100 mg/kg) (Fig. 2).

3.4. Effect of GTS on nicotine-induced c-fos and c-jun mRNA expression in VTA and NA regions.

A single injection of nicotine also caused inductions of c-fos and c-jun mRNA expression in

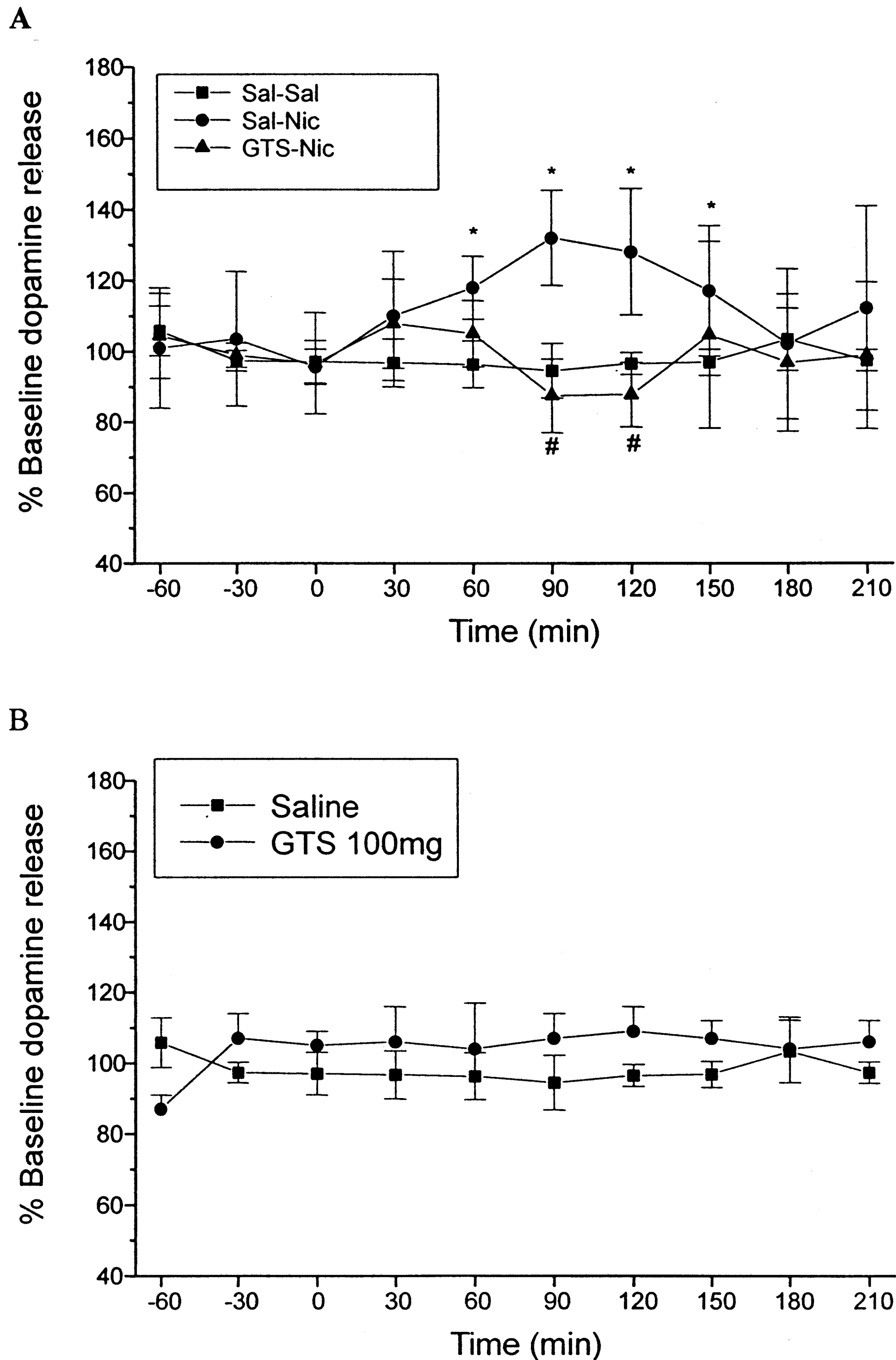

Fig. 1. Effect of GTS on changes in the levels of dopamine in the NA after systemic injections of saline-saline ($n = 6$), saline-nicotine (3 mg/kg; i.p., $n = 4$) and GTS (100 mg/kg; i.p., $n = 4$)-nicotine ($n = 4$) (A) and the time course of extracellular DA changes in the NA after injections of GTS (100 mg/kg; i.p., $n = 3$) (B). Results were expressed as a mean $\pm$ SD expressed as a percentage of the three baseline samples. Significant difference from saline-saline group: $*P < 0.05$; significantly different from saline-nicotine group: $\# P < 0.05$.

both VTA and NA regions (Figs. 3 and 4). GTS alone did not affect the basal levels of c-fos and c-jun mRNA in both regions. Both c-fos and c-jun mRNA increases induced by nicotine were further enhanced by the pre-treatment with GTS (Figs. 3 and 4).

4. Discussion and conclusion

The results of the present study demonstrate that systemic injection of nicotine produces an increase of DA release in the NA. The current results are consistent with previous studies (Imperato et al., 1986; Damsma et al., 1989; Benwell and Balfour, 1992; Pontieri et al., 1996; Marshall et al., 1997), indicating that the mesolimbic DA system plays an important role in mediating addictive effects of nicotine.

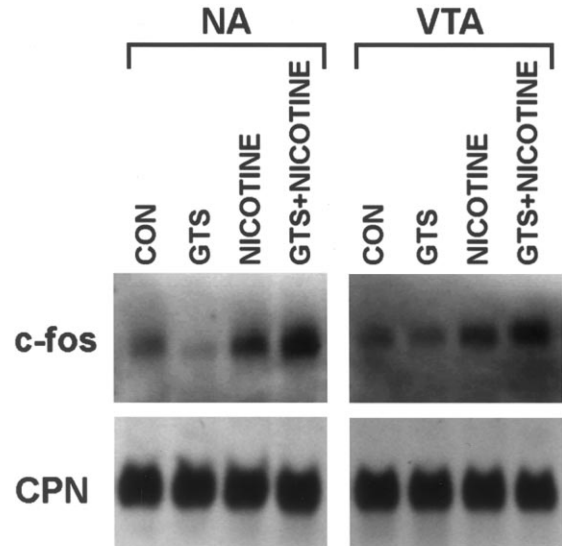

Fig. 3. Effect of GTS on nicotine-induced c-fos mRNA level. After the pre-treatment with saline or 100 mg/kg of GTS for 30 min, either saline or nicotine (3 mg/kg) was administered intraperitoneally. The animals were sacrificed at 30 min after the treatment. Ten micrograms of total RNA, which were extracted from pooled rat VTA and NA ($n = 3$/group) using a guanidium thiocyanate/phenol/chloroform gradient method, were used for determination of c-fos mRNA level. The unregulated mRNA encoding cyclophilin (CPN) was used as an internal loading control.

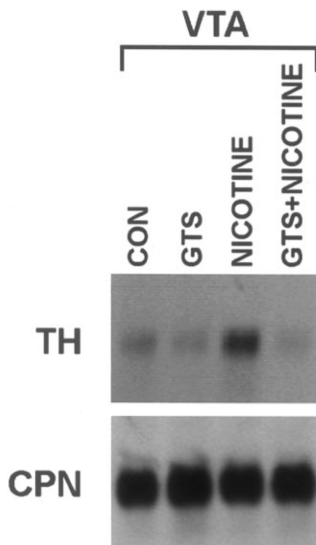

Fig. 2. Effect of GTS on nicotine-induced TH mRNA level in VTA region. After the pre-treatment with saline or 100 mg/kg of GTS for 30 min, either saline or nicotine (3 mg/kg) was administered intraperitoneally. The animals were sacrificed at 30 min after the treatment. Ten micrograms of total RNA, which were extracted from pooled rat VTA ($n = 3$/group) using a guanidium thiocyanate/phenol/chloroform gradient method, were used for determination of TH mRNA level. The unregulated mRNA encoding cyclophilin (CPN) was used as an internal loading control.

The present findings demonstrated that the pre-treatment with GTS clearly attenuates nicotine-induced DA release in the NA. These data strongly suggest that GTS may act on dopaminergic transmission produced by nicotine. In this context, the current results are in agreement with other studies which have shown that GTS or ginseng extract may act on the central dopaminergic system. For example, GTS is able to inhibit DA receptor supersensitivity produced by morphine or cocaine (Kim et al., 1995a,b). Furthermore, GTS has been demonstrated to prevent methamphetamine-induced depletion of DA (Oh et al., 1997).

Numerous studies have reported that GTS is able to block psychostimulant-induced behavioral sensitization which is persistent, enhanced stereotypy and locomotor activity produced by repeated injections of psychostimulants such as cocaine or amphetamine (Robinson and Becker, 1982, 1986). For example, it has been shown that GTS or

ginseng extract may inhibit the development of behavioral sensitization produced by methamphetamine, cocaine or morphine (Bhargava and Ramarao, 1991; Tokuyama et al., 1992; Kim et al., 1995a,b, 1996). The current results may explain blocking effect of GTS on behavioral hyperactivity induced by nicotine as well as other psychostimulants.

Nicotinic cholinergic receptors are known to be located on the cell bodies of dopaminergic neurons in the NA (Clarks and Pert, 1985) and it has been suggested that nicotine may stimulate presynaptic nicotinic cholinergic receptors on DA terminals to increase extracellular DA release in the NA (Wonnacott et al., 1990). In simple explanation of our current results, GTS may act on nicotinic receptors of DA terminals and block actions of nicotine on DA transmission. This suggestion is supported by a finding that ginseng saponins reduce acetylcholine-induced catecholamine secretion in bovine adrenal cells (Tachikawa et al., 1995).

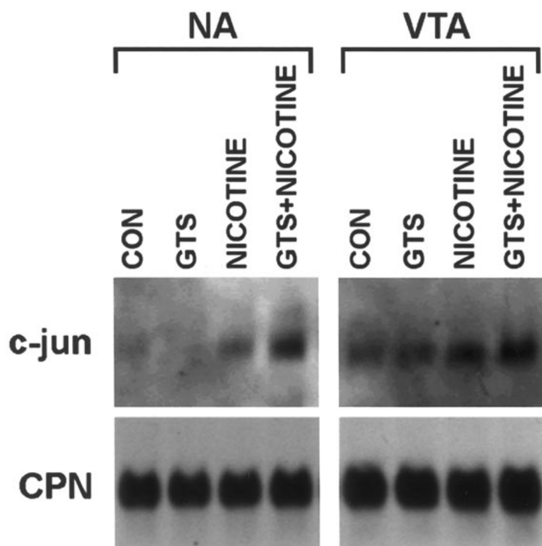

Fig. 4. Effect of GTS on nicotine-induced c-jun mRNA level. After the pre-treatment with saline or 100 mg/kg of GTS for 30 min, either saline or nicotine (3 mg/kg) was administered intraperitoneally. The animals were sacrificed at 30 min after the treatment. Ten micrograms of total RNA, which were extracted from pooled rat VTA and NA ($n = 3$/group) using a guanidium thiocyanate/phenol/chloroform gradient method, were used for determination of c-jun mRNA level. The unregulated mRNA encoding cyclophilin (CPN) was used as an internal loading control.

Little has been known about action of GTS on the central dopaminergic system at the cellular or molecular levels. However, in the present study, we found that the treatment with GTS also abolished TH mRNA upregulation induced by nicotine in VTA region. Thus, it is speculated that there are two possible mechanisms of GTS in the regulation of TH mRNA expression in VTA region. First, the inhibitory action of GTS on DA release from the neurotermials located at NA. This inhibition may turn on the negative feedback mechanism, leading to the reduction of transcriptional activity in the cell bodies located at VTA region. Second, GTS may directly exert the inhibitory action against TH mRNA increase induced by nicotine in VTA region.

In addition to TH mRNA level, it has been well known that nicotine induces immediate early genes in various regions of the brain (Pang et al., 1993; Pich et al., 1997, 1998; Mathieu-Kia et al., 1998). In the present study, we found that nicotine also increases both c-fos and c-jun mRNA levels in VTA and NA regions. This result observed in the northern blot analysis is in line with that observed in in situ hybridization studies (Pang et al., 1993; Pich et al., 1997, 1998; Mathieu-Kia et al., 1998). Watanabe et al. (1998) have reported that c-fos expression induced by a nicotinic receptor agonist (epibatidine) was attenuated by a DA receptor antagonist, suggesting that DA released from the DA neuroterminal located in NA may induce c-fos mRNA expression. However, we found in the present study that GTS exerts a potentiative interaction for the upregulation of both c-fos and c-jun mRNA expression in NA region. The finding of inhibitory action of GTS against nicotine-induced DA release suggests that at least the potentative effect of GTS in the regulation of c-fos and c-jun mRNA level in NA may not be mediated by inhibiting DA release. Although the exact mechanisms of the potentiative interaction for c-fos and c-jun mRNA expression between nicotine and GTS are unclear at present, it is speculated that GTS may exert an inhibitory action against the action of enzymes that are involved in degradation of c-fos

and c-jun mRNA levels. However, it cannot be excluded that GTS may have a direct action on the transcriptional activity induced by nicotine. Thus, further studies are required for the detailed delineation in relation to the action of GTS in the regulation of nicotine-induced immediate early gene.

In summary, the current data suggest that GTS may act on presynaptic DA terminals to inhibit nicotine-induced enhancement of DA release. Furthermore, GTS may have an inhibitory action against nicotine-induced TH mRNA expression in VTA region, whereas GTS may have a potentiative effect on the upregulation of c-fos and c-jun mRNA expression in both VTA and NA regions. This may reflect blocking effect of GTS on some of behavioral activities induced by nicotine.

Acknowledgements

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