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Blockade of nicotine-induced locomotor sensitization by a novel neurotensin analog in rats

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Abstract

Neurotensin is a tridecapeptide with anatomic and functional relationships to dopaminergic neurons. Previously we showed that one of our brain-penetrating neurotensin analogs, NT69L (*N*-met-L-Arg, L-Lys, L-Pro, L-neo-Trp, L-*tert*-Leu, L-Leu), blocks cocaine- and D-amphetamine-induced hyperactivity in rats. We have now performed a similar study in rats sensitized to nicotine over 15 days of administration. Male Sprague–Dawley rats were randomly assigned to receive daily injections for 15 days with one of the following combinations: saline/nicotine (0.35 mg/kg), NT69L (1 mg/kg)/nicotine, saline/saline, or NT69L/saline with a 30-min period between injections. On day 15 each group was given saline/nicotine or NT69L/nicotine and tested in an activity chamber. One-time administration of NT69L attenuated nicotine-induced activity with an ED_{50} of 1.6 μg/kg. Rats injected with nicotine over the 15 days had a significant increase in locomotor activity, consistent with nicotine-induced locomotor sensitization. A single injection of NT69L on day 15 prior to nicotine markedly decreased nicotine-induced hyperactivity. Although daily injections of NT69L lessened its effect, statistically significant reductions in hyperactivity to nicotine persisted throughout the study. There was no significant difference in activity between rats injected with NT69L/saline and saline/saline. Thus, the activity reduction was not due to sedation. Acute and chronic nicotine injection caused an increase in cytisine binding in prefrontal cortex. NT69L significantly reduced the increase caused by acute but not chronic injection of nicotine. Nicotine injection resulted in an increase in dopamine levels in the striatum and dopamine and norepinephrine levels in the prefrontal cortex. NT69L lowered the norepinephrine and dopamine levels in the prefrontal cortex but did not affect striatal dopamine. The present study is the first report, to our knowledge, of a possible role for neurotensin in the development of nicotine dependence, and suggests that neurotensin analogs such as NT69L may be explored as treatment for nicotine and other psychostimulant abuse.

Keywords: Nicotine; Addiction; Neurotensin; Sensitization; Psychostimulant

1. Introduction

Cigarette smoking is the single most preventable cause of morbidity and mortality in the United States, accounting for approximately 427,000 deaths each year (MMWR, 2000). Prevalence of smoking has declined from 42% of adults in 1965 to a plateau of around 25% in recent years (MMWR, 1998). Individuals with psychiatric illness are more likely to be smokers and to have a poorer prognosis (Covey et al., 1994; Hall et al., 1993). Smoking rates approach 90% in schizophrenia, and high rates are also associated with affective illness (Glassman et al., 1990; Hughes et al., 1986). Current pharmacotherapies, principally nicotine replacement and bupropion, approximately double the cessation rate vs. placebo (Hughes et al., 1999). However, even with concomitant behavioral therapy, long-term success rates range from 25% to 45% (Fiore, 2000). New therapies are needed, particularly for those with psychiatric co-morbidity and for anyone failing existing therapies (Hughes et al., 1999; Rennard and Daughton, 2000). Our experience over the past 15 years with the development of novel neurotensin analogs and their possible role in treating psychostimulant abuse led us to suspect neurotensin receptor agonists may be explored as potential therapeutic agents for nicotine dependence.

Nicotine is widely accepted as the tobacco substance that causes addiction (Benowitz, 1991; Henningfield et al., 1983; Stolerman and Jarvis, 1995). The preponderance of

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evidence is that nicotine's reinforcing properties are due to stimulation of mesocorticolimbic dopamine systems (Di Chiara, 2000; George et al., 2000; Stein et al., 1998). Chronic nicotine administration produces behavioral sensitization in animals and addiction in humans (Miller et al., 2001). Behaviorally, animals naive to nicotine exhibit an initial depression in motor activity (Morrison and Stephenson, 1972). Repeated administration results in tolerance to the depressant effect and sensitization to nicotine's stimulant effects (Domino, 2001; Ksir et al., 1985). Thus, constant doses of nicotine produce increasing degrees of locomotor activity after repeated administration (Clarke and Kumar, 1983). This animal model of locomotor sensitization depends on activation of dopaminergic neurons (Clarke et al., 1988), and has been proposed as a model for the acquisition of addiction and for relapse in humans (De Vries et al., 2002; Miller et al., 2001).

Neurotensin is an endogenous tridecapeptide discovered nearly three decades ago (Carraway and Leeman, 1973). The amino acid sequence is pyroGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH. Most, if not all, of the activity mediated by neurotensin-(1–13) is seen with the shorter fragment, neurotensin-(8–13). The accumulated evidence has shown that neurotensin behaves as a neurotransmitter or neuromodulator in the CNS and that there are striking interactions between neurotensin via its receptors and central dopaminergic systems (Lambert et al., 1995; Tyler-McMahon et al., 2000a). Our group has developed brain-penetrating analogs of neurotensin-(8–13) that incorporate our novel amino acid, L-neo-tryptophan (Fauq et al., 1998). Given extracranially, one of these compounds, called NT69L, induces a significant reduction in core body temperature, antinociception, blockade of apomorphine-induced climbing behavior, and blockade of haloperidol-induced catalepsy (Cusack et al., 2000; Tyler-McMahon et al., 2000b). We have previously reported on blockade by NT69L of cocaine- and D-amphetamine-induced hyperactivity in rats (Boules et al., 2001). The present study was undertaken to extend these results to nicotine-induced hyperactivity, and to survey neurochemical correlates of changes in activity level.

2. Materials and methods

2.1. Animals and treatment protocol

Male Sprague–Dawley rats weighing 200–250 g were housed in a temperature-controlled room with free access to food and water under artificial 12 h light/dark cycle. All tests were conducted during the light cycle. All procedures were approved by the Mayo Foundation Institutional Animal Use and Care Committee. The animals received an intraperitoneal injection of either NT69L (1 mg/kg) or an equivalent volume (100 μl) of saline. They were next administered nicotine 0.35 mg/kg or saline subcutaneously 30 min after the first injection. These injections were repeated once a day for 15 days. On days 1 and 15, rats were placed in a Plexiglass Opto-Varimax Minor motility chamber (Columbus Instruments, Columbus, OH) for 1 h for acclimation. Baseline activity was recorded for 30 min for each rat. The rats were then injected with either saline or NT69L and placed in the chamber for 30 min, after which each rat was removed and injected with either nicotine or saline and returned to the chamber. Activity, in counts per 10-min intervals was recorded for 60 min. On day 15, animals were sacrificed after being tested for locomotor activity. Different sections of the brain were dissected on ice and kept on dry ice until they were frozen at −80 °C at which temperature they were kept until assayed.

To determine the ED_{50} for the ability of NT69L to attenuate the nicotine-induced activity in rats, groups of four rats each were injected with varying doses of NT69L and 30 min later injected with nicotine and activity was recorded as explained above.

2.2. Analytical procedures

2.2.1. NT69L synthesis

NT69L (*N*-met-L-Arg, L-Lys, L-Pro, L-neo-trp, L-*tert*-leu, L-leu) was synthesized as described previously (Cusack et al., 2000). The synthesis of L-neo-Trp was completed by the Mayo Clinic Jacksonville, Organic Synthesis Core Facility (Fauq et al., 1998). NT69L was dissolved in saline and administered intraperitoneally.

2.2.2. [^{3}H]cytisine binding

Nicotinic receptor binding sites in membrane homogenates of frozen tissue were measured using [^{3}H]cytisine according to the method of Pabreza et al. (1991). The frozen tissue was resuspended in 50 mM Tris–HCl buffer (pH 7.4), homogenized with a Brinkmann Polytron (setting 6), and washed twice in fresh buffer by centrifugation at 35 000 × *g*. The resulting pellets were resuspended in buffer. Aliquots of homogenates were added to tubes containing [^{3}H]cytisine in the presence or absence of 100 μM nicotine and incubated in a final volume of 250 μl for 75 min at 4 °C. The incubations were terminated by filtration through GF/B filter paper that was mounted on a Brandel Cell Harvester. Filters had been soaked in 0.5% polyethylenimine to reduce binding of radioligand to the filter. The filters were washed 3 × with cold 0.9% NaCl and counted in a scintillation counter. The data was analyzed using the PC version of the ligand software program. Specific binding was defined as the difference between the binding in the presence and absence of 100 μM nicotine.

2.2.3. Dopamine D2 receptor binding assay

Homogenized brain tissue was assayed for dopamine D2 receptor binding by the method of Bowden et al. (1997). Briefly, the brain tissue was homogenized with buffer (50 mM Tris–HCl, 5 mM KCl and 120 mM NaCl, pH 7.5) and

centrifuged at 26000 × *g* (10 min, 4 °C). The pellet was resuspended in buffer to 1 mg/10 μl and immediately used for binding. Aliquots of 20 μl tissue homogenates were added to 25 μl [^{3}H] raclopride and 25 μl buffer (for total binding) or 25 μl sulpiride 30 nM (for nonspecific binding) in a total volume of 250 μl and incubated at room temperature for 90 min. Membrane bound radioactivity was recovered by filtration under vacuum through Whatman GF/B filters using Brandel cell harvester. Filters were washed with ice-cold phosphate buffered saline and radioactivity determined by liquid scintillation counting. Protein was quantified using the Micro BCA assay (Pierce, Rockford, IL).

2.2.4. Striatal and prefrontal cortex dopamine

Analysis of brain dopamine was done using high performance liquid chromatography with electrochemical detection (ESA, Chelmsford, MA), according to previously described procedures (Kilts et al., 1981; Krstulovic, 1982). The rats were killed by decapitation and the striatal tissue was weighed and homogenized in 10 volumes of ice-cold 0.1 N perchloric acid containing 10 mM EDTA. The tissue homogenates were centrifuged for 10 min (26000 × *g*, 4 °C). A 20 μl of the diluted supernatant was injected onto the high performance liquid chromatography system with MD-150/RP-C18 column (ESA). The mobile phase was 10% acetonitrile containing 75 mM sodium dihydrogen phosphate, 1.7 mM 1-octane sulfonic acid sodium salt, 0.01% triethylamine and 10 μM EDTA, pH 3.0. The flow rate was maintained at 0.5 ml/min. The detector potentials were set at: 350 mV; E1: −175 mV and E2: 250 mV. The concentration of dopamine in each sample was quantified by comparison of area under curve between samples and external standards run on the same day.

2.3. Statistical analysis

Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, using Sigma Stat software, with $P < 0.05$ being considered significant. Graphs were generated with the use of GraphPad Software (San Diego, CA).

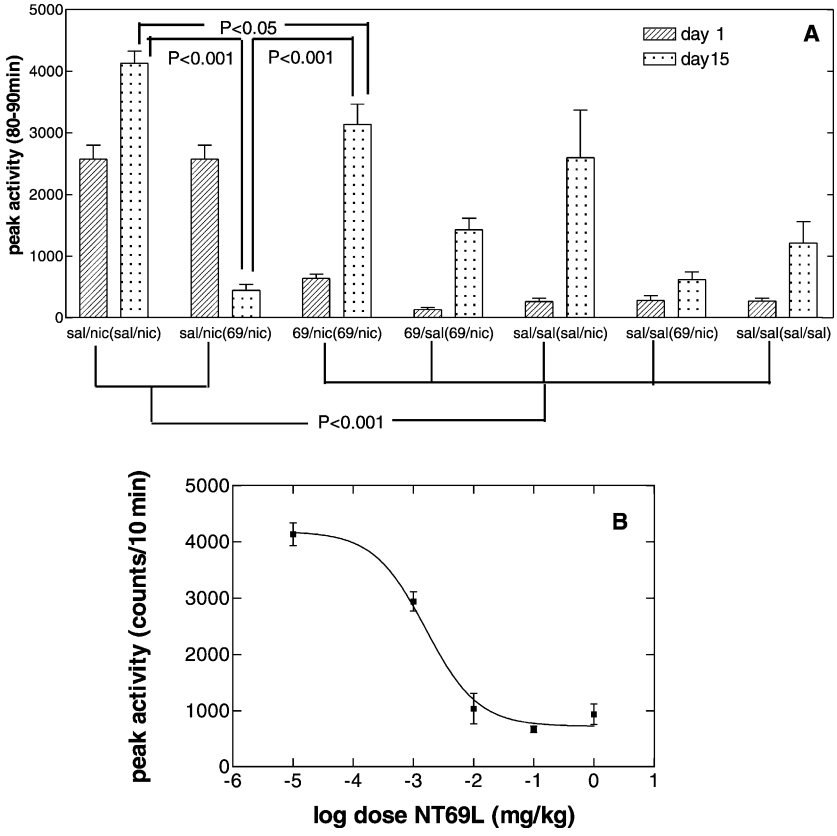

Fig. 1. (A) Effect of NT69L on nicotine-induced activity in rats. Sprague–Dawley rats (200 g) were injected daily for 14 days with the treatment outside the parentheses. On day 15 the rats were injected as shown with treatment inside the parentheses. On days 1 and 15 activity was recorded. Rats were placed in a Plexiglass Opto-Varimax Minor motility chamber (Columbus Instruments, Columbus, OH) for 1 h for acclimation. A 30-min baseline was recorded for each rat. The rats were then injected with either saline or NT69L and placed in the chamber for 30 min after which each rat was removed and injected with either nicotine or saline and returned to the chamber. Activity, in counts per 10 min intervals was recorded for 60 min. Abbreviations: sal—saline; nic—nicotine; 69—NT69L. (B) Dose response for NT69L for the attenuation of nicotine-induced activity in rats. The experimental design was the same as that described in (A), except that the dose of NT69L varied as indicated.

3. Results

3.1. Nicotine-induced hyperactivity

Fig. 1A shows peak activity level for each treatment group at days 1 and 15. A single injection of nicotine (0.35 mg/kg, s.c.) significantly ($P<0.001$) increased locomotor activity (955%) as compared to control group [sal/nic(sal/nic) vs. sal/sal(sal/sal)]. Acute injection of NT69L (1 mg/kg, i.p.) on day 1, 30 min prior to nicotine injection, significantly reduced nicotine-induced hyperactivity by 75% ($P<0.001$) [sal/nic(69/nic) vs. 69/nic(69/nic)]. With one-time administration of NT69L, its ED_{50} for attenuating the nicotine-induced activity was 1.6 μg/kg as determined by non-linear regression analysis, 95% confidence interval: 0.0008926–0.002877 mg/kg; $R=0.97$ (Fig. 1B). The reduction in activity was not due to sedation since there was no significant difference between the 69/sal and the sal/sal groups on day 1. Rats receiving daily nicotine injections responded with more than a 60% increase in peak activity, indicating sensitization to the stimulant effects of nicotine [sal/nic(sal/nic) day 1 vs. day 15]. This effect was markedly reduced (500%) by a single injection of NT69L given 30 min prior to nicotine on the fifteenth day of nicotine administration, $P<0.001$ [sal/nic(sal/nic) vs. sal/nic(69/nic), day 15]. Daily injection of NT69L reduced the magnitude of locomotor sensitization by 24%, but did not completely block it. Nonetheless, statistically significant reductions ($P<0.05$) in activity, compared with saline, were seen after 15 days [sal/nic(sal/nic) vs. 69/nic(69/nic) day 15].

3.2. Cytisine binding

Fig. 2 shows the effect of nicotine and NT69L on cytisine binding in the prefrontal cortex and the ventral tegmental area. The effect of acute nicotine injection was assessed by comparing the sal/sal(sal/nic) group to the sal/sal(sal/sal) group. A single nicotine injection tended to increase cytisine binding in the prefrontal cortex, but not to a significant level. However, 15 daily injections of nicotine significantly increased (62%), $P<0.05$, cytisine binding in prefrontal cortex when compared to that for the saline control group [sal/nic(sal/nic) vs. sal/sal(sal/sal)], as well as to that for rats given NT69L [sal/nic(69/nic) vs. sal/sal(69/nic)] (Fig. 2A). Injection of NT69L daily for 15 days significantly reduced (62%) cytisine binding caused by acute, but not by chronic, nicotine injections [69/sal(69/nic) vs. 69/nic(69/nic)] (Fig. 2A).

Both nicotine and NT69L resulted in a 35% increase in cytisine binding in the ventral tegmental area as compared

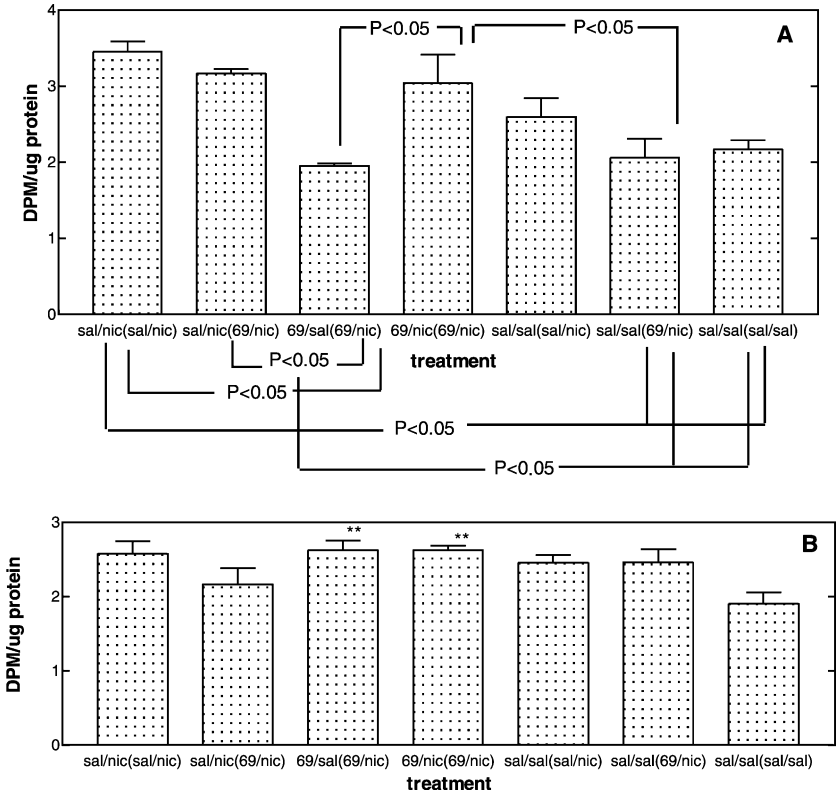

Fig. 2. (A) Effect of NT69L on cytisine binding in prefrontal cortex. Sprague Dawley rats were maintained and treated as mentioned for Fig. 1. On day 15, animals were sacrificed after being tested for locomotor activity. Prefrontal cortex was harvested on ice, kept on dry ice until frozen at −80 °C until assayed as detailed in Materials and methods. (B) Effect of NT69L on cytisine binding in ventral tegmental area Sprague–Dawley rats were maintained and treated as mentioned for Fig. 1. On day 15, animals were sacrificed after being tested for locomotor activity. Ventral tegmental area was harvested on ice, kept on dry ice until frozen at −80 °C until assayed as detailed in Materials and methods. **Significantly different from sal/sal(sal/sal), $P<0.05$.

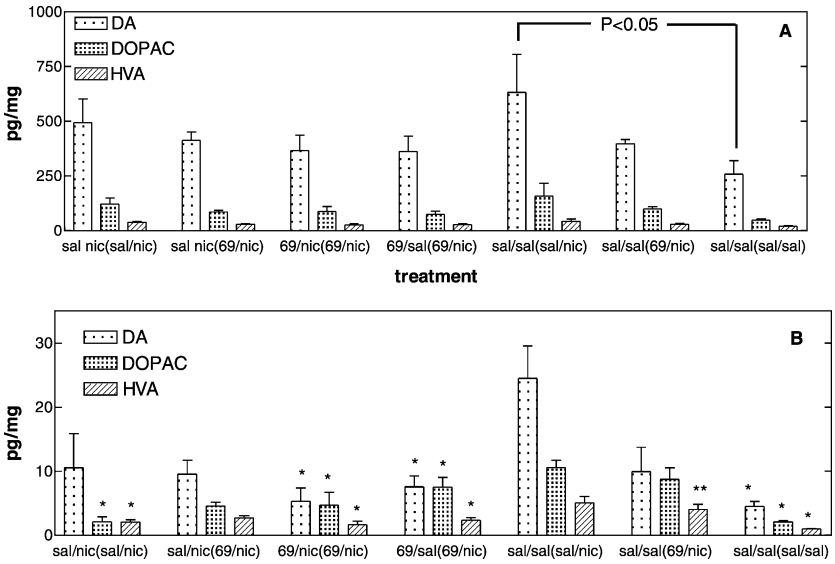

Fig. 3. (A) Effect of nicotine and NT69L on dopamine and its metabolites in striatum. Sprague–Dawley rats were treated as mentioned for Fig. 1. On day 15, animals were sacrificed after being tested for locomotor activity. Striatum was harvested on ice, kept on dry ice until frozen at − 80 °C until assayed using high performance liquid chromatography with electrochemical detection as detailed in Materials and methods. (B) Effect of nicotine and NT69L on dopamine and its metabolites in prefrontal cortex Sprague-Dawley rats were treated as mentioned for Fig. 1. On day 15, animals were sacrificed after being tested for locomotor activity. Prefrontal cortex was harvested on ice, kept on dry ice until frozen at − 80 °C until assayed using high performance liquid chromatography with electrochemical detection as detailed in Materials and methods. *Significantly different from sal/sal(sal/nic). **Significantly different from sal/sal(sal/sal), $P<0.05$.

to saline control, but only chronic injection of NT69L was significant ($P<0.05$) (Fig. 2B).

3.3. Dopamine and metabolites

A single injection of nicotine significantly ($P<0.05$) increased dopamine levels in striatum by 192% [sal/sal (sal/nic) vs. sal/sal(sal/sal) (Fig. 3A). Pretreatment with NT69L did not affect dopamine levels in the striatum.

In the prefrontal cortex (Fig. 3B), a single injection of nicotine significantly ($P<0.05$) increased (400%) dopamine and its metabolites when measured by high performance liquid chromatography with electrochemical detection [sal/sal(sal/nic) vs. sal/sal(sal/sal)]. Fifteen daily injections of nicotine reduced this effect to 232% as compared to saline control [sal/sal(sal/sal) vs. sal/nic(sal/nic)]. Injection of NT69L for 15 days lowered the nicotine-induced increase in dopamine to control levels.

3.4. Dopamine receptor binding

Dopamine (D2) receptor binding in striatum was higher in saline-treated animals than in any other group. No difference, however, was seen across any of the nicotine and NT69L treatment groups.

3.5. Norepinephrine

Acute (1 injection) and chronic (15 daily injections) of nicotine significantly ($P<0.05$) increased norepinephrine in prefrontal cortex to 213–300% as compared to saline control [sal/nic(sal/nic), sal/nic(69/nic) and sal/sal(sal/nic) vs. sal/

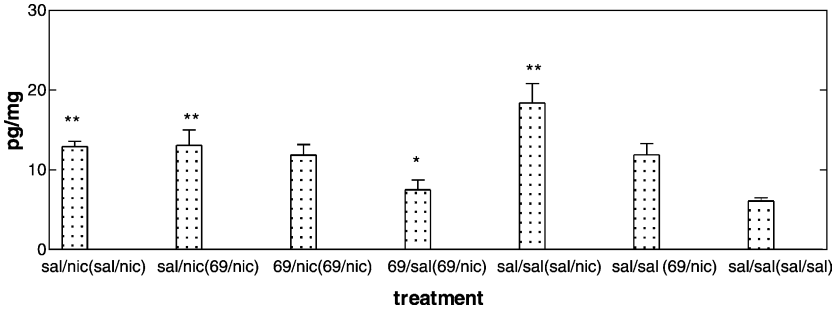

Fig. 4. Effect of nicotine and NT69L on norepinephrine in prefrontal cortex. Sprague–Dawley rats were treated as mentioned for Fig. 1. On day 15, animals were sacrificed after being tested for locomotor activity. Prefrontal cortex was harvested on ice, kept on dry ice until frozen at − 80 °C until assayed using high performance liquid chromatography with electrochemical detection as detailed in Materials and methods. *Significantly different from sal/sal(sal/nic). **Significantly different from sal/sal(sal/sal), $P<0.05$.

sal(sal/sal)]. Daily injections of NT69L blocked the increase caused by a single injection of nicotine resulting in a 40% reduction in norepinephrine [69/sal(69/nic) vs. sal/sal(sal/nic)] (Fig. 4). No significant differences were observed among treatment groups in the striatum, data not shown.

4. Discussion

The primary aim of this study was to determine if a blood–brain barrier penetrating neurotensin analog could block nicotine-induced hyperactivity in rats. This work is an extension of our previous research, in which we demonstrated that one of our novel neurotensin analogs blocked the effect of cocaine- and D-amphetamine-induced hyperactivity (Boules et al., 2001). The experimental paradigm we chose, behavioral sensitization, is considered to be an animal model for the acquisition of addiction, and to the process of relapse, in humans (De Vries et al., 2002; Miller et al., 2001). For this study we employed an analog of neurotensin-(8–13) called NT69L, which incorporates our novel amino acid L-neo-tryptophan. NT69L, given extracranially, produces antinociception, reduction in core body temperature, blockade of apomorphine-induced climbing behavior and haloperidol-induced catalepsy, and blocks psychostimulant-induced hyperactivity (Boules et al., 2001; Cusack et al., 2000; Tyler-McMahon et al., 2000b).

As expected from the literature (Domino, 2001; Miller et al., 2001), rats administered daily doses of nicotine developed increased locomotor activity, consistent with behavioral sensitization. Administration of a single dose of NT69L significantly reduced the nicotine-induced activity with an ED_{50} of 1.6 μg/kg. This ED_{50} is much lower than those for NT69L's for hypothermic, antinociceptive and anticataleptic (against haloperidol) effects (260–400 μg/kg) (Cusack et al, 2000; Tyler-McMahon et al., 2000b) demonstrating a highly potent effect of NT69L on the blockade of nicotine-induced hyperactivity. NT69L produced a marked reduction in activity when administered on day 15 to rats treated daily with nicotine. When NT69L was administered daily, the effect of activity blockade lessened over time, but remained statistically significant through day 15. In prior studies on the effect of NT69L on cocaine- and D-amphetamine-induced hyperactivity, no tolerance to NT69L was seen through 3 days of administration (Boules et al., 2001); however, with 7–10 days of twice daily dosing of D-amphetamine (Hertel et al., 2001) or 10 daily injections of cocaine (unpublished data from our laboratory), there was an eventual loss of activity against D-amphetamine and cocaine-induced hyperactivity, respectively. In the present study, the delay in development of tolerance against nicotine was even more striking. This finding is significant in light of the development of tolerance to antinociception, hypothermia, reduction of food intake, and blockade of haloperidol-induced catalepsy (but not apomorphine-induced climbing behavior) after just a single dose of NT69L (Boules et al., 2001; unpublished data from our laboratory).

Neurotensin exerts its effects through its receptors (Le et al., 1996; Tyler-McMahon et al., 2000a; Vincent et al., 1999). A high-affinity receptor, called NTS-1 and a lower affinity receptor, NTS-2 have been cloned from rat (Chalon et al., 1996; Tanaka et al., 1990) and human (Vita et al., 1997; Watson et al., 1993). A third binding site, called NTS-3, is presently without known functional response (Mazella et al., 1998). Neurotensin cell bodies are found in the ventral tegmental area, and the vast majority of the neurons also contain tyrosine hydroxylase, the enzyme that is the rate-limiting step for dopamine and norepinephrine synthesis. These neurons project to the nucleus accumbens, prefrontal cortex, and amygdala (Kinkead et al., 1999; McMahon et al., 2002). Neurotensin antagonizes dopamine and stimulant-induced transmission in mesolimbic pathways (Ervin et al., 1981; Kalivas et al., 1981), although its effect may depend on the activation state of the dopaminergic system (Brun et al., 2001). Thus, neurotensin participates in regulation of dopaminergic pathways implicated in nicotine addiction. In addition, neurotensin interacts with gamma-aminobutyric acid neurons in the nucleus accumbens, glutamatergic neurons in prefrontal cortex, and cholinergic afferents from the laterodorsal tegmental nucleus to the ventral tegmental area (Kinkead et al., 1999; Tyler-McMahon et al., 2000a).

Behavioral sensitization to psychostimulants is a complex process thought to involve mesocorticolimbic dopamine neurons, with influences from glutamate, gamma-aminobutyric acid, K-opioid and other neurotransmitter systems that produce long-term changes in neurotransmission (Hahn et al., 2000; Pierce and Kalivas, 1997). Also, the initiation and expression of sensitization have been reported to be behaviorally, neurochemically and temporally distinct (Pierce and Kalivas, 1997). Since systemic nicotine exerts its effects on mesocorticolimbic dopamine neurons by stimulating nicotinic receptors located on the somatodendritic membranes of the cells and sensitization is mediated by receptors located in the ventral tegmental area, we were interested to test the effect of NT69L on the nicotinic receptors. Daily injections of nicotine increased cytisine binding in prefrontal cortex similar to results published by Shoaib et al. (1997). Our results are also similar to results shown previously where behavioral sensitization to nicotine was associated with an increase in brain nicotinic binding (Ksir et al., 1985; Lapchak et al., 1989). NT69L blocked the effect of a single dose of nicotine, but it did not block the effect of chronic nicotine administration. Thus NT69L might block the initiation of sensitization and it attenuated but did not completely inhibit sensitization expression.

Since the action of nicotine on dopamine neurons may result in changes in dopamine receptor sensitivity (Nisell et al., 1996), we tested the effect of NT69L on the dopamine binding due to the close relationship between dopamine and neurotensin. Dopamine receptor binding in the striatum was

not influenced by any of the treatments, although all were lower than the saline control group. These results may be due to somatodendritic dopamine autoreceptor down-regulation that has been reported with the expression of sensitized responses to cocaine (Henry et al., 1989). However, studies examining binding to sub-cortical dopamine receptors after chronic nicotine administration, yielded no conclusive results (Nisell et al., 1996).

Several lines of experimental evidence indicate that stimulation of brain dopamine systems is of major significance for the reinforcing and dependence producing properties of nicotine. Development of sensitization has been reported to affect noradrenaline projections to the hippocampus and to be associated with an increased formation of noradrenaline in the nerve terminals (Mitchell et al., 1989). In this study we found an increase of noradrenaline in the prefrontal cortex after acute and chronic nicotine injection. This agrees with previous nicotine studies (Di Chiara, 2000) on the release of adrenaline and serotonin in the brain. It is also in agreement with others (Fu et al., 1998; Summers and Giacobini, 1995), who reported a significant increase in the release of noradrenaline, dopamine, and serotonin in the rat cortex with repeated nicotine injection.

Acute nicotine injection increased dopamine levels in the striatum and prefrontal cortex. Injection of NT69L for 15 days blocked that increase in the prefrontal cortex. Previous in vivo studies showed an increase of dopamine release after 12 days of nicotine injection in the prefrontal cortex and extracellular dopamine concentration in nucleus accumbens in drug naive rats (Nisell et al., 1996), while others (Benwell and Balfour, 1992) found that pretreatment with nicotine produced an increase in accumbal dopamine release, but failed to show an increase in dopamine release in the nucleus accumbens in response to acute injection. For practical reasons, dopamine and norepinephrine have been determined in whole striatal tissue preparations. Future studies, with in vivo microdialysis will be performed in the nucleus accumbens shell verses core, prefrontal cortex, and striatum to study the effect of NT69L on the release of dopamine and norepinephrine after acute and sub-chronic injection of nicotine.

Meng et al. (1998) hypothesized that a reduction in neurotensinergic tone in the nucleus accumbens shell may contribute to behavioral sensitization to amphetamine. A core vs. shell heterogeneity in the responsiveness of dopamine transmission to chronic nicotine exposure (Di Chiara, 2000) could therefore, also reflect changes in neurotensin regulation. Repeating the present study, but with less frequent exposure to nicotine (Miller et al., 2001), while monitoring dopamine release in the nucleus accumbens core vs. shell in response to NT69L may shed more light on its mechanism of action. Nevertheless, our results provide additional evidence for the involvement of neurotensin in the behavioral and/or addictive properties of psychostimulants. Our data suggest that NT69L warrants further study for a possible role in treating nicotine dependence.

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