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Induction of tolerance to the suppressant effect of the neurotensin analogue NT69L on amphetamine-induced hyperactivity

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Abstract

Although several studies have indicated that neurotensin administered acutely has several pharmacological properties common with those of antipsychotic drugs, the effects of repeated exposure to neurotensin receptor agonism have been less well characterised. Here, we investigated the effect of the novel neurotensin-(8–13) analogue NT69L [(*N*-methyl-Arg), Lys, Pro, L-*neo*-Trp, tert-Leu, Leu] in animal models sensitive to central neurotensin receptor stimulation as well as in predictive models for antipsychotic activity and motor side-effect liability. Acute injection of NT69L (0.19–6.1 μmol/kg, s.c./i.p.) caused hypothermia (> 2.5 °C) and reduction in spontaneous locomotor activity but failed to induce catalepsy. Furthermore, NT69L (0.10 μmol/kg, s.c.) counteracted the hyperlocomotion elicited by amphetamine (0.5 mg/kg, s.c.). However, repeated injections of NT69L (0.19 μmol/kg, s.c. for 6 days, twice daily) significantly reduced its effect on spontaneous locomotor activity and completely abolished its effect on amphetamine-elicited hyperactivity. Our data obtained after single injections of NT69L indicate that this drug stimulates central neurotensin receptors after peripheral administration and collectively support the notion that neurotensin receptor agonism is associated with an attractive pre-clinical profile as regards both antipsychotic activity and motor side-effect liability. However, the present results also indicate that repeated neurotensin receptor stimulation may cause a desensitisation of neurotensin receptor mediated effects.

Keywords: Neurotensin; Hyperactivity; Amphetamine; Antipsychotic; NT69L

1. Introduction

The endogenous tridecapeptide neurotensin is heterogeneously distributed throughout the brain and exerts a wide range of biological effects in laboratory animals including hypothermia and antinociception (Bissette et al., 1976; Clineschmidt et al., 1979). Neurotensin appears to be particularly closely associated with the mesolimbic dopamine system. For example, neurotensin has been found co-localised with dopamine in neurones of the mesolimbic dopamine system (Hökfelt et al., 1984) and seems to act as a modulator of dopaminergic neurotransmission in such brain regions (Kasckow and Nemeroff, 1991). Interestingly, several pre-clinical studies have shown that acute neurotensin receptor stimulation induces antipsychotic-like effects without causing severe motor disturbances (Kinkead et al., 1999). Such findings, together with studies showing that administration of antipsychotic drugs increase neurotensin content in discrete brain nuclei (Govoni et al., 1980; Kinkead et al., 2000) have encouraged researchers to suggest that at least some of the beneficial clinical effects of antipsychotic treatment are mediated via enhanced neurotensin neurotransmission (Nemeroff, 1980; Kinkead et al., 1999). However, given the fact that clinical onset of antipsychotic action typically requires long-term treatment, surprisingly few studies have investigated the effects of repeated exposure to neurotensin or neurotensin receptor agonists in the context of antipsychotic-like activity.

Consequently, the main objective of the present study was to compare the effects of acute versus repeated neurotensin receptor stimulation on amphetamine-induced hyperactivity, a pre-clinical assay frequently used for predicting clinical antipsychotic activity (Arnt, 2000). To this end, we used the novel neurotensin receptor agonist NT69L [(*N*-methyl-Arg), Lys, Pro, L-*neo*-Trp, tert-Leu, Leu], a neurotensin-(8–13) analogue which recently has been reported to induce centrally mediated effects after peripheral administration (Cusack et al., 2000; Tyler-McMahon et al., 2000). The ability of NT69L to affect spontaneous locomotor activity and induce catalepsy in rats was also assessed. In addition, the effect of peripherally administrated NT69L

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on core body temperature was examined to verify that the drug penetrates into the brain as neurotensin-induced hypothermia is generally attributed to central neurotensin receptor stimulation (Bissette et al., 1976).

2. Materials and methods

2.1. Animals

Male Wistar rats (170–240 g; Møllegaard, Denmark) were used in all experiments. Animals were housed under controlled laboratory conditions (temperature: 21 ± 2 °C; humidity: 55 ± 5% relative) on a 12:12 h light–dark cycle (lights on at 06:00) and allowed free access to standard rodent food and water. All experiments were performed in accordance with the ethical guidelines of H. Lundbeck.

2.2. Drugs

NT69L ($M_w = 825$ g/mol) was synthesised by Mayo Protein Core Facility (Rochester, MN) whereas amphetamine was purchased from Nomeco (Denmark). Both drugs were dissolved in saline (0.9% NaCl).

2.3. Measurement of core body temperature

Core body temperature was measured by means of a thermistor probe (Ellab DM852 thermometer) inserted 3 cm into the rectum of the animal. Rectal temperature was monitored immediately before and at various time points during a 240-min time period after i.p. drug injection. Experiments were performed between 11:00 and 16:00 in a temperature-controlled environment. Control animals received saline injections.

2.4. Measurement of catalepsy

Catalepsy was evaluated using a standard wire mesh method (Arnt, 1982). Briefly, rats were placed on a vertical wire netting (50 cm × 50 cm) and classified as either cataleptic or not. Rats were considered cataleptic if they remained immobile during a 15-s time period. Animals that showed muscle relaxation (i.e. passively sliding down) or failed to move their paws but exhibited active body or head movements were not considered as cataleptic. The rats were tested every hour during the first 6 h and once 24 h after s.c. drug administration. Control animals received saline injections.

2.5. Measurement of spontaneous motility and amphetamine-induced hyperactivity

Locomotor activity of rats was assessed using standard test cages (42 cm × 26 cm) equipped with four infrared light sources and photocells placed 4 cm above the bottom of the cage. Activity counts were registered each time two adjacent light beams were consecutively interrupted in order to avoid registration of stationary movements of the animals.

To assess the spontaneous motility, rats were placed in the test cages immediately after s.c. administration of drug or vehicle and the activity was monitored during a 15-min time period, starting 15 min after the injection. In order to assess a potential effect on amphetamine-induced hyperlocomotion, rats were pretreated with NT69L (30 min, s.c.) and subsequently injected with amphetamine (0.5 mg/kg, s.c.). Immediately after the second injection, rats were placed in the test cages and the locomotor activity was recorded for a time period of 120 min. Control rats received saline injections.

Seventy-two rats were repeatedly treated with NT69L (0.19 μmol/kg, s.c., twice daily for 6 days). On the seventh day, the effect of several doses of NT69L on spontaneous motility and amphetamine-induced hyperactivity were tested using identical methodology as described above. Control rats received repeated saline injections.

2.6. Statistical analysis

Group comparisons were performed using one- or two-way analysis of variance (ANOVA). The ANOVAs were followed by the Tukey test for multiple comparisons. A P-value less than 0.05 was considered significant. All statistical analysis were performed using the SigmaStat (5.0) software.

The ED_{50}-values were calculated by log-probit analysis on the basis of percent inhibition responses obtained at each dose.

3. Results

3.1. Induction of hypothermia

Systemic administration of NT69L (0.19–1.52 μmol/kg, i.p.) induced a dose-dependent hypothermia in rats (Fig. 1A,B). Statistical analysis of the temporal effects of NT69L on body temperature (Fig. 1A) indicated a significant treatment effect ($F_{3,21} = 18.46$; $P < 0.001$), time effect ($F_{8,168} = 9.687$; $P < 0.01$) and overall interaction ($F_{24,168} = 6.988$; $P < 0.001$). Similarly, statistical evaluation of the overall effect of NT69L on body temperature (Fig. 1B) revealed a significant dose effect ($F_{3,21} = 20.84$; $P < 0.01$).

3.2. Cataleptic potential

A high dose of NT69L (6.1 μmol/kg, s.c.) failed to induce catalepsy in rats during the 24-h observation period ($n = 4$; data not shown).

3.3. Spontaneous motility: effect of acute versus repeated administration

Acute challenge with NT69L (0.38–6.1 μmol/kg, s.c.) dose-dependently reduced the spontaneous motility in rats pretreated with either vehicle or NT69L (0.19 μmol/kg, s.c., twice daily for 6 days). However, the NT69L pretreatment significantly reduced the inhibitory effect of the drug (Fig. 2A). A two-way ANOVA indicated a significant pretreatment effect (vehicle versus NT69L pretreatment; $F_{1,56} = 12.31$; $P < 0.001$), treatment effect (acute challenge; $F_{3,56} = 88.61$; $P < 0.001$) and overall interaction ($F_{3.56} = 2.779$; $P < 0.05$). The ED_{50}-values of NT69L for inhibiting spontaneous motility in the vehicle or NT69L pretreatment groups were calculated to < 0.38 or 1.10 μmol/kg, respectively.

3.4. Amphetamine-induced hyperactivity: effect of acute versus repeated administration

Acute injection of amphetamine (0.5 mg/kg, s.c.) profoundly increased the locomotor activity to approximately the same extent in rats pretreated with either vehicle or NT69L (0.19 μmol/kg, s.c., twice daily for 6 days). In the vehicle pretreated group, acute challenge with NT69L (0.10 μmol/kg, s.c.) significantly counteracted the hyperactivity induced by amphetamine. However, the NT69L pretreatment completely abolished such inhibitory effect of the drug on amphetamine-induced hyperactivity (Fig. 2B). A two-way ANOVA indicated a significant pretreatment effect (vehicle versus NT69L pretreatment; $F_{1,70} = 5.330$; $P < 0.05$), treatment effect (acute challenge; $F_{4,70} = 13.18$; $P < 0.001$) and overall interaction ($F_{4,70} = 5.651$; $P < 0.001$).

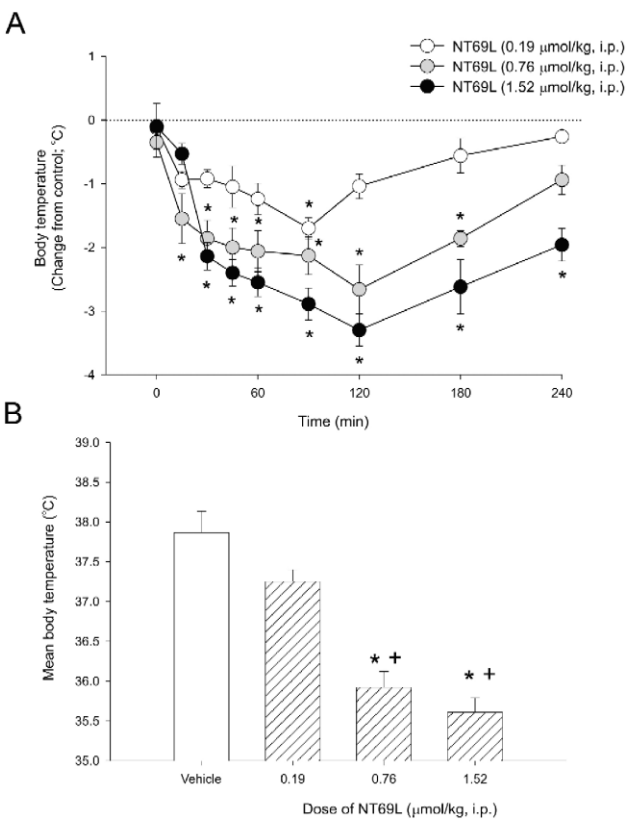

Fig. 1. Effect of acute administration of NT69L on body temperature in rats. $^{*}P < 0.05$ compared to the vehicle control group. $^{+}P < 0.05$ compared to the NT69L (0.19 μmol/kg, i.p.) treatment group. Each point represents the mean (±S.E.M.) temperature (°C) change from control whereas each bar represents the mean (±S.E.M.) body temperature during the entire postinjection sampling period. Two (A)- or one (B)-way ANOVA followed by Tukey test was used to determine significance ($n = 10$ in control group and $n = 5$ in all NT69L treatment groups).

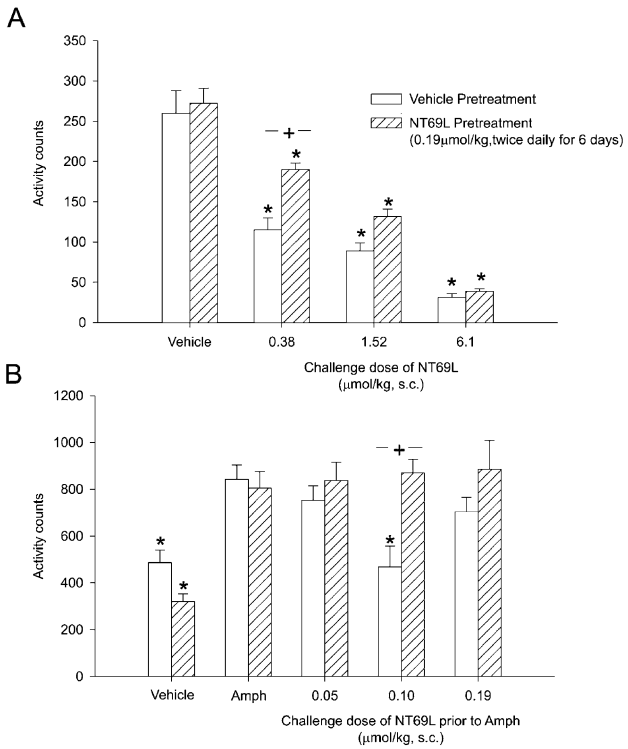

Fig. 2. Effect of pretreatment with NT69L (0.19 μmol/kg, s.c. for 6 days, twice daily) on its inhibitory effect on (A) spontaneous motility and (B) on amphetamine (Amph)-induced hyperactivity. Each bar represents the mean (±S.E.M.) number of activity counts during the (A) 15-min or (B) 120-min sampling period. $^{*}P < 0.05$ compared to (A) the respective vehicle control group or to (B) the respective Amph treatment group. $^{+}P < 0.05$ for comparisons between pretreatment groups. Two-way ANOVA followed by Tukey test was used to determine significance ($n = 8$ in all groups).

4. Discussion

The hexapeptide NT69L is an analogue of neurotensin-(8–13), the biologically active fragment of the native neurotensin peptide. NT69L contains the novel aminoacid L-*neo*-tryptophan at position 11, which has been suggested to be the key feature underlying its potent binding to neurotensin receptors and low susceptibility to peptidase degradation (Tyler et al., 1999). Indeed, NT69L exhibit

high affinity for both human- and rat-cloned neurotensin NTS1 receptors with equilibrium dissociation constants of 0.83 and 1.55 nM, respectively, and appears in contrast to the native neurotensin peptide rather stabile in both human and rat plasma (Cusack et al., 2000). In agreement with previous literature, we found that NT69L induced a significant reduction in core body temperature in rats (Cusack et al., 2000; Tyler-McMahon et al., 2000). Although the responsible neurotensin receptor subtype(s) remains to be conclusively identified (Gully et al., 1997; Dubuc et al., 1999), the hypothermic effect of neurotensin is thought to be mediated via centrally located neurotensin receptors (Bissette et al., 1976). Therefore, our data support the notion that NT69L has the ability to cross the blood–brain barrier and subsequently activate central neurotensin receptors after extracranial administration (Cusack et al., 2000; Tyler et al., 1999; Tyler-McMahon et al., 2000).

By now it is well established that acute, central administration of neurotensin produces behavioural effects similar to those of antipsychotic drugs (Kinkead et al., 1999). For example, several preclinical studies have shown that neurotensin given intracranially, in similarity to antipsychotic drugs, reduces spontaneous locomotor activity and counteracts behavioural responses induced by dopamine receptor stimulation (Ervin et al., 1981; Skoog et al., 1986; Dubuc et al., 1994; Feifel et al., 1997; Sarhan et al., 1997). Interestingly, it has been shown that neurotensin as well as the classical, dopamine D_2 receptor blocking antipsychotic drug haloperidol reduce spontaneous locomotor activity and antagonise the behavioural effects induced by amphetamine when injected locally in the nucleus accumbens. In contrast, intracaudate injections of neurotensin failed to affect amphetamine-induced stereotypic behaviours whereas haloperidol is quite effective in this regard (Ervin et al., 1981). Thus, it appears that neurotensin receptor stimulation preferably compromises dopamine transmission in the mesolimbic as compared to the nigrostriatal system. This is in line with the present finding showing that NT69L failed to induce catalepsy, a behavioural response associated with blockade of dopaminergic transmission within the nigrostriatal pathway, within a dose interval that markedly reduced spontaneous locomotor activity. It should be pointed out that the cataleptic potential of NT69L was determined using a single dose, which is indeed inappropriate if a non-linear dose–response relationship exists. However, previous studies using a variety of doses of neurotensin, NT69L or other neurotensin analogues have concluded that neurotensin receptor stimulation is not associated with induction of catalepsy (Jolicoeur et al., 1981; Sarhan et al., 1997; Cusack et al., 2000). In fact, NT69L has been shown to counteract the catalepsy induced by dopamine D_2 receptor blockade (Cusack et al., 2000).

Previous studies have indicated that the hyperactivity induced by low doses of amphetamine is largely mediated via increased output of dopamine in mesolimbic areas, whereas the behavioural signs seen after higher doses of amphetamine, such as stereotypies, are attributed to stimulation of dopamine receptors located in nigrostriatal projection areas (Kelly et al., 1975; Costall et al., 1977; Arnt, 1995). Thus, the present finding that NT69L reversed the hyperactivity elicited by a low dose of amphetamine further supports our conclusion that NT69L antagonises dopamine transmission in mesolimbic areas. Recent experiments showing that NT69L potently counteracted the climbing behaviour but failed to affect the oro-facial stereotypies induced by the non-selective dopamine D_2 receptor agonist apomorphine are in agreement with this interpretation (Cusack et al., 2000). Moreover, the present data indicate that acute administration of NT69L may specifically counteract the hyperactivity induced by amphetamine without affecting the spontaneous motor activity as a relative large difference between effective doses in these two paradigms was observed. It should be noted that the dose–response curve for antagonising amphetamine-induced hyperactivity was biphasic with only the intermediate dose being effective. Such complex dose–response relationship has not been observed in previous studies investigating the effect of neurotensin on amphetamine-induced hyperactivity (Ervin et al., 1981; Sarhan et al., 1997). Although the precise reason for this inverted U-shaped relationship is currently not known, it is interesting to note that similar dose–response curve has been obtained when investigating the ability of neurotensin to counteract the amphetamine-induced disruption of sensorimotor gating (Feifel et al., 1997). Nevertheless, given that reversal of amphetamine-induced hyperactivity and induction of catalepsy are two preclinical tests frequently used for predicting antipsychotic potential and motor side-effect profile, respectively (Arnt, 2000), our behavioural data obtained using single administrations of NT69L collectively predict an attractive profile of this drug as regards both antipsychotic activity and extrapyramidal side-effect liability.

Interestingly, the present experiments indicated that repeated administration of NT69L attenuates its ability to reduce spontaneous motility and totally abolishes its inhibitory effect on amphetamine-induced hyperactivity. Thus, it is possible that repeated neurotensin receptor stimulation may cause a functional down regulation of these receptors. Clearly, further investigations to reveal the underlying mechanism for this phenomenon is warranted. Attenuation of the inhibitory effect of neurotensin receptor agonists on spontaneous motor activity after repeated i.c.v. treatment has previously been described (Meisenberg and Simmons, 1985), whereas the present data on repeated neurotensin receptor stimulation and amphetamine-induced locomotor activity appear more controversial. Thus, it has previously been reported that repeated intracerebroventricular injections of neurotensin modestly, but significantly, sensitised rats to the stimulatory effect of amphetamine (Rompre, 1997). Such effect was not observed in the

present study where no significant difference in response to amphetamine could be detected between animals chronically treated with NT69L or vehicle. One reason for this discrepancy may well be the fact that the previous study allowed much longer time period, i.e. one week versus 16–18 h, between the last injection of neurotensin or NT69L and the amphetamine challenge. It cannot be ruled out that the mechanisms underlying the purported neurotensin-induced sensitisation to amphetamine requires a relatively long wash-out period to develop and was therefore not detected in the present study.

Our data collectively suggest that, although acute neurotensin receptor stimulation induces behavioural effects predictive of antipsychotic efficacy, repeated neurotensin receptor activation appears to cause a down regulation of neurotensin receptors mediated effects. Such phenomenon should be considered during development of new therapy strategies against schizophrenia based on neurotensin receptor agonism.

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