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Repeated administration of the neurotensin analogue NT69L induces tolerance to its suppressant effect on conditioned avoidance behaviour

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Abstract

Although acute neurotensin receptor stimulation exerts diverse behavioural effects that resemble those seen after administration of antipsychotic drugs, data on effects after repeated exposure to neurotensin receptor agonism is relatively sparse. Here, we demonstrate that repeated administration of the novel neurotensin-(8–13) analogue NT69L [(*N*-methyl-Arg), Lys, Pro, L-*neo*-Trp, *tert*-Leu, Leu] induce tolerance to its suppressant effect on conditioned avoidance behaviour in rats, a predictive assay for antipsychotic activity. In contrast, the inhibitory effect of haloperidol on this behaviour was sustained despite repeated administration of this classical antipsychotic drug. These findings indicate that repeated exposure to neurotensin receptor stimulation induces tolerance to the antipsychotic-like effects of neurotensin receptor agonists.

Keywords: Neurotensin; Antipsychotic; Conditioned avoidance behaviour; Haloperidol; NT69L

1. Introduction

It is well accepted that the endogenous tridecapeptide neurotensin exerts a wide range of central nervous system (CNS)-mediated effects in rodents including hypothermia and antinociception (Bissette et al., 1976; Clineschmidt et al., 1979). In addition, studies have shown that acute neurotensin receptor stimulation induce antipsychotic-like effects without concomitantly causing major motor disturbances (Kinkead et al., 1999; Binder et al., 2001). For example, several investigators have reported that stimulation of neurotensin receptors in brain counteract the hyperlocomotion elicited by psychostimulants like amphetamine and phencyclidine (Ervin et al., 1981; Skoog et al., 1986; Sarhan et al., 1997; Boules et al., 2001a; Hertel et al., 2001). Such findings, together with the fact that administration of antipsychotic drugs increase neurotensin content in discrete brain nuclei (Govoni et al., 1980; Kinkead et al., 2000), indicate that at least some of the beneficial clinical effects of antipsychotic drugs are associated with enhanced neurotensin neurotransmission (Nemeroff, 1980; Kinkead et al., 1999).

Although there is little doubt that acute neurotensin receptor stimulation induces antipsychotic-like behaviours, literature on chronic administration of neurotensin receptor agonists is surprisingly rare. We have recently reported that repeated administration of the novel neurotensin-(8–13) analogue NT69L (Cusack et al., 2000; Tyler-McMahon et al., 2000) induces desensitisation to its suppressant effect on spontaneous locomotion and its ability to counteract amphetamine-induced hyperactivity in rats (Hertel et al., 2001). Such desensitisation may indeed have bearing on the feasibility to develop a novel therapy strategy against schizophrenia based on neurotensin receptor agonism, particularly as clinical onset of antipsychotic action typically requires long-term treatment.

To further elucidate possible tolerance phenomenon due to repeated neurotensin receptor stimulation in the context of antipsychotic-like behaviours, we investigated the effect of acute and repeated NT69L administration on conditioned avoidance behaviour, a preclinical assay with high predictive validity for clinical antipsychotic efficacy (see Arnt, 2000). The effects of NT69L were compared to those of the classical antipsychotic drug haloperidol.

2. Materials and methods

2.1. Animals

Male Wistar rats (Møllegaard, Denmark) with an initial weight of around 150 g were used in all experiments. Ani-

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mals 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). Animals were kept on a restricted diet in order to counteract excessive weight gain. Water was available ad libitum. All experiments were performed in accordance with the ethical guidelines of H. Lundbeck.

2.2. Drugs

NT69L [(*N*-methyl-Arg), Lys, Pro, L-*neo*-Trp, *tert*-Leu, Leu] (M_w = 825 g/mol), was synthesised by Mayo Protein Core Facility and haloperidol (M_w = 376 g/mol) was purchased at Sigma. NT69L was dissolved in saline (0.9% NaCl) whereas haloperidol was dissolved in distilled water with a minimal addition of diluted tataric acid. Injections were given subcutaneously (s.c.) in a volume of 5 ml/kg.

2.3. Conditioned avoidance behaviour

Conditioned avoidance behaviour was assessed using four automated shuttle-boxes (ENV-010M, MED-Associates) each placed in a sound-attenuated chamber. The boxes were divided into two compartments by a partition with one opening. The boxes were equipped with photocells sensitive to infrared light on each side of the dividing wall in order to automatically register movement from one compartment to the other. Rats were trained to move into the adjacent compartment (response) within 10 s upon presentation of the conditioned stimuli (tone and light) in order to avoid the unconditioned stimulus, a 0.5-mA scrambled electric shock in the grid floor. The following variables were recorded: avoidance (response to conditioned stimuli within 10 s); escape (no response to conditioned stimuli but response to the unconditioned stimulus within 10 s) and escape failure (no response to either conditioned stimuli or the unconditioned stimulus within 10 s).

Rats were habituated to the shuttle-box 3 min before each test session. During training, each test session consisted of 30 trials with intertrial intervals varying randomly between 20 and 30 s. Training was carried out 5 days/week until the animals performed avoidance in >80% of the trials on three consecutive days. All experimental sessions consisted of 10 trials with intertrial intervals varying randomly between 20 and 30 s.

2.4. Drug treatment

Four groups of animals (n = 8) receive twice daily injections of either vehicle, 0.08, 0.16 or 0.31 mg/kg NT69L. This dose–regimen was selected based on our previous experiments with NT69L (Hertel et al., 2001). The rats were tested the day before treatment (pre-test), day 1 of the treatment period (acute), day 7 as well as 1 day after the treatment period (withdrawal).

In the second experiment, four groups of animals (n = 7) receive daily injections of either vehicle, 0.04, 0.08 or 0.16 mg/kg haloperidol. Single daily injections were preferred due to the relatively long half-life and the long-lasting dopamine D2 receptor antagonistic effects of haloperidol (see e.g. Matsubara et al., 1993). The rats were tested the day before treatment (pre-test), day 1 of the treatment period (acute), days 7, 14 and 21 as well as 1 day after the treatment period (withdrawal).

All injections were given 30 min before the start of testing.

2.5. Statistical analysis

Group comparisons were performed using two-way analysis of variance (ANOVA; treatment × treatment duration) with repeated measures followed by Student–Newman–Keuls test for multiple comparisons. A P-value less than 0.05 was considered significant. All statistical analyses were performed using the SigmaStat (2.03) software.

3. Results

3.1. Effect of NT69l on conditioned avoidance behaviour

Acute administration of NT69L (0.08, 0.16 and 0.31 mg/kg, s.c.) suppressed the conditioned avoidance behaviour

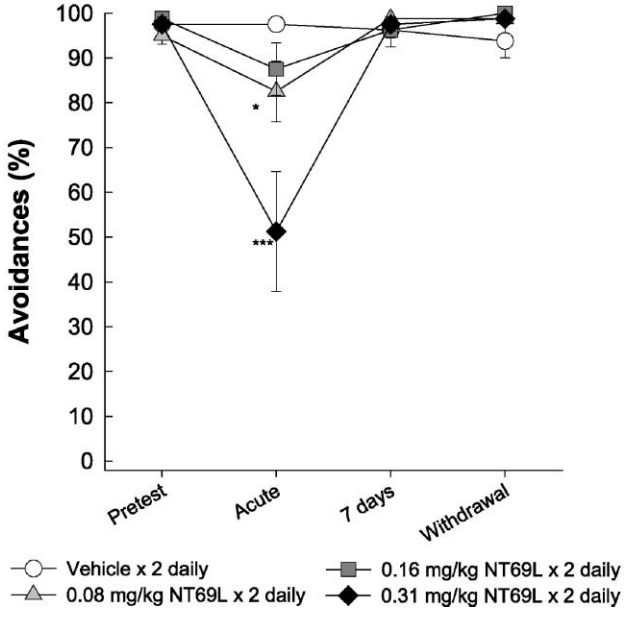

Fig. 1. Effect of acute and repeated administration of NT69L (0.08, 0.16 and 0.32 mg/kg, s.c., twice daily) on conditioned avoidance behaviour. Conditioned avoidance behaviour was also assessed 1 day before the start of NT69L administration (pre-test) as well as 1 day after the discontinuation of the drug treatment (withdrawal). Each point represents the mean (± S.E.M.) percent avoidance. Two-way ANOVA (treatment × treatment duration) with repeated measures followed by Student–Newman–Keuls test for multiple comparisons was used to determine significance (n = 8 in all groups). *P < 0.05, ***P < 0.001 compared to vehicle control group.

(day 1 of the treatment period; Fig. 1). However, the suppressant effect was completely abolished after 7 days of repeated NT69L treatment (0.08, 0.16 and 0.31 mg/kg, s.c., twice daily). The percent avoidance was still at pre-test levels 1 day after the withdrawal of NT69L. Statistical analysis of the conditioned avoidance data indicated a significant overall interaction (treatment × treatment duration; $F(9,84) = 5.3$; $P < 0.001$). NT69L did not induce escape failures at any dose or at any measured time point throughout the entire treatment period (data not shown).

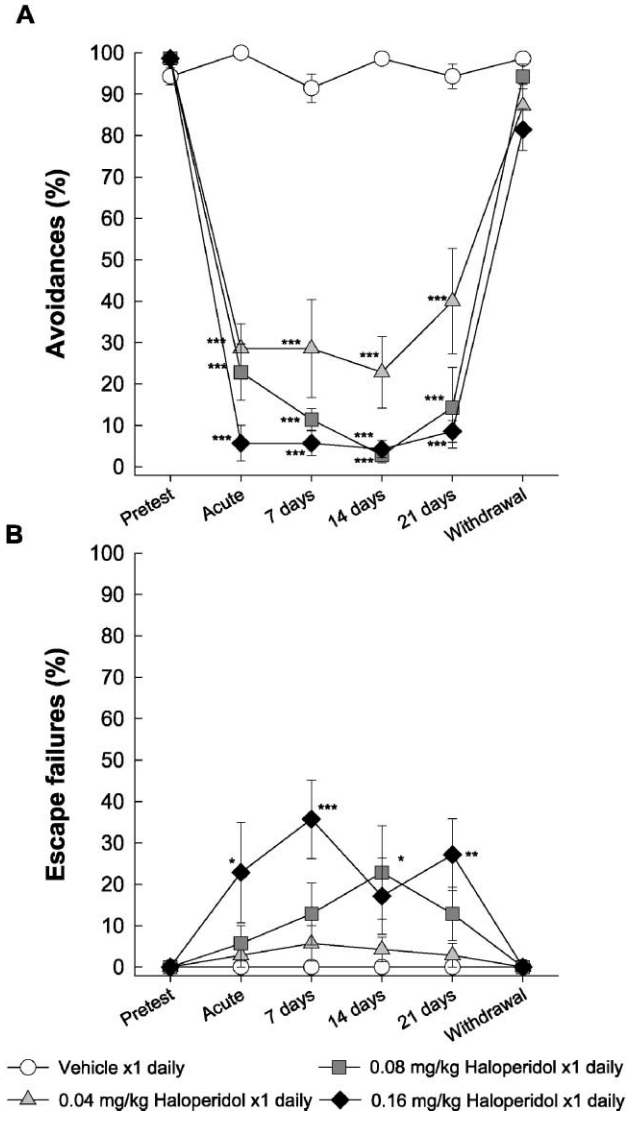

Fig. 2. Effect of acute and repeated administration of haloperidol (0.04, 0.08 and 0.16 mg/kg, s.c., once daily) on (A) conditioned avoidance behaviour and (B) escape failures. These parameters were also assessed 1 day before the start of haloperidol administration (pre-test) as well as 1 day after the discontinuation of the drug treatment (withdrawal). Each point represents the mean (± S.E.M.) percent avoidance or escape failures. Two-way ANOVA (treatment × treatment duration) with repeated measures followed by Student–Newman–Keuls test for multiple comparisons was used to determine significance ($n = 7$ in all groups). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to vehicle control group.

3.2. Effect of haloperidol on conditioned avoidance behaviour

Acute administration of haloperidol (0.04, 0.08 and 0.16 mg/kg, s.c.) markedly suppressed the conditioned avoidance behaviour (day 1 of the treatment period; Fig. 2A). Its suppressant effect was still evident after 7, 14 and 21 days of repeated haloperidol treatment (0.04, 0.08 and 0.16 mg/kg, s.c., once daily), i.e. throughout the entire treatment period. The conditioned avoidance behaviour returned to pre-test levels 1 day after the withdrawal of haloperidol. Statistical analysis of the conditioned avoidance data indicated a significant overall interaction (treatment × treatment duration; $F(15,120) = 16$; $P < 0.001$). Treatment with haloperidol increased the incidence of escape failures, an effect that was observed throughout the entire treatment period (Fig. 2B). The number of escape failures returned to pre-test levels 1 day after the withdrawal of haloperidol. Statistical analysis of the escape data indicated a significant overall interaction (treatment × treatment duration; $F(15,120) = 2.4$; $P < 0.01$).

4. Discussion

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 apparently underlie its potent binding to neurotensin receptors and low susceptibility to peptidase degradation (Tyler et al., 1999; Cusack et al., 2000). We and others have shown that extracranial administration of NT69L induces a significant reduction in core body temperature in rats (Cusack et al., 2000; Tyler-McMahon et al., 2000; Hertel et al., 2001), an effect in accordance with a blood–brain barrier penetrating property of this peptide (Bissette et al., 1976).

Numerous studies have shown that stimulation of central neurotensin receptors produces behavioural effects similar to those of antipsychotic drugs (Kinkead et al., 1999). For example, we have previously reported that acute administration of NT69L antagonises amphetamine-induced hyperactivity (Hertel et al., 2001), a finding recently replicated by others (Boules et al., 2001a). In line with these findings, we found in the present study that acute administration of NT69L selectively suppressed conditioned avoidance behaviour without disrupting escape behaviour, a behavioural profile predicting antipsychotic efficacy (see Ellenbroek, 1993; Arnt, 2000). This effect of NT69L is in all probability mediated via stimulation of central neurotensin receptors as previous experiments have shown that intra cranial injections of neurotensin suppress such behaviour (Luttinger et al., 1982). The precise mechanism responsible for suppressant effect of NT69L is currently not known. It is clear that dopaminergic transmission plays a major role in the expression of this behaviour as exemplified by the attenuating effect of the dopamine D2 receptor antagonist haloperidol (this study).

Furthermore, previous studies have underlined the importance of dopaminergic transmission within the nucleus accumbens in this context (Wadenberg et al., 1990). Given that local accumbal administration of neurotensin receptor agonists are able to counteract dopamine-mediated behaviours such as hyperlocomotion (Ervin et al., 1981; Skoog et al., 1986), it seems reasonable that stimulation of neurotensin receptors located in this area are responsible for the observed effects of NT69L. This is in accordance with the extensive expression of neurotensin receptors within the nucleus accumbens (Lambert et al., 1995). Regardless of the underlying mechanism, the present as well as previous findings (Hertel et al., 2001; Boules et al., 2001a) clearly indicate that acute administration of NT69L induces behavioural effects predictive of antipsychotic efficacy.

In our previous study, we observed that tolerance developed to the inhibitory effects of NT69L on spontaneous locomotion and amphetamine-induced hyperactivity after repeated treatment with the peptide (Hertel et al., 2001). In parallel with these data, we found in present study that repeated administration of NT69L completely abolishes its inhibitory effect on conditioned avoidance behaviour. This tolerance to the antipsychotic-like effect is in all probability not due to an unspecific compensatory mechanism as the suppressant effect of haloperidol on conditioned avoidance behaviour was still evident after repeated treatment, a result in line with previous literature (Ensler et al., 1993). Our finding contrasts somewhat with those of Boules et al. (2001b) who recently reported that the attenuating effect of NT69L on dopamine receptor mediated behaviours in 6-hydroxydopamine lesioned rats was unchanged after repeated treatment with the drug (Boules et al., 2001b). Apart from the fact that the effect of repeated treatment of NT69L was investigated in different models, this discrepancy may well be due to the differences in the dosing regimes used. Thus, Boules et al. (2001b) investigated the effects of NT69L after 3 days of daily treatment whereas in the present study the drug was administered twice daily for 7 days. It cannot be ruled out that development of tolerance requires a sustained neurotensin receptor stimulation and was therefore not detected by Boules et al. (2001b) who used a relatively short and infrequent administration regime of NT69L. It should also be mentioned that earlier studies from other laboratories have suggested that tolerance develop to acute behavioural effects of neurotensin agonists after their repeated administration. For example, the inhibitory effects of neurotensin receptor agonists on spontaneous motor activity have been found to be significantly attenuated after repeated i.c.v. treatment (Meisenberg and Simmons, 1985).

Although the precise mechanism underlying the observed tolerance phenomenon of NT69L is currently unknown, it has previously been shown that neurotensin receptors undergoes desensitisation and internalisation after stimulation with agonists in a variety of cellular and tissue based systems (see Hermans and Maloteaux, 1998). Therefore, it is possible that the observed development of tolerance to the antipsychotic-like effects of NT69L on conditioned avoidance behaviour (this study) as well as on spontaneous locomotor activity and amphetamine-induced hyperactivity (Hertel et al., 2001) is caused by neurotensin receptor desensitisation and/or internalisation. Further investigations to reveal the mechanism responsible for this tolerance phenomenon are clearly warranted.

The present findings collectively support the notion that acute neurotensin receptor stimulation induces behavioural effects predictive of antipsychotic efficacy. However, they also suggest that this beneficial antipsychotic-like effect of neurotensin receptor stimulation may be abolished, or at least profoundly reduced, after repeated exposure to neurotensin receptor agonists. Such phenomenon should be considered during development of new therapy strategies against schizophrenia based on neurotensin receptor agonism.

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