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Research report

Chronic NT69L potently prevents drug-induced disruption of prepulse inhibition without causing tolerance

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ABSTRACT

NT69L is a neurotensin receptor agonist with antipsychotic-like activity. NT69L blocks apomorphine-induced climbing in rats with no effect on stereotypic behavior, attenuates D-amphetamine-induced hyperactivity, and blocks pharmacologically induced disruption of prepulse inhibition (PPI) of the startle response. Repeated administration of NT69L results in tolerance to some, but not to all of its effects. Because schizophrenic patients require long-term treatment, chronic (21-day) administration of NT69L was tested in PPI with comparisons to chronic haloperidol and clozapine treatment.

Sprague–Dawley rats received acute or 21 daily, subcutaneous injections of NT69L (1.0 mg/kg). On days 1 and 21 the NT69L injection was followed 30 min later by treatment with either saline; the dopamine agonist, D-amphetamine (5.0 mg/kg); or the serotonin 5-HT_{2A} psychotomimetic receptor agonist [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] DOI (0.5 mg/kg). Experiments were repeated with either haloperidol (1 mg/kg) or clozapine (20 mg/kg) in place of NT69L. Acute injection of NT69L significantly blocked D-amphetamine and DOI disruption of PPI. As with the acute injection, 21 daily administrations of NT69L also blocked D-amphetamine- and DOI-induced disruption of PPI. The data show that animals do not develop tolerance to the antipsychotic-like effects of NT69L when tested in the PPI of the startle response. The persistent efficacy of NT69L with chronic treatment provides further support for the therapeutic use of neurotensin (NT) agonists to treat schizophrenia and possibly other disorders that are characterized by PPI deficits. The modulatory role of NT69L on the dopaminergic and serotonergic neurotransmission systems both of which are implicated in the pathophysiology of schizophrenia is discussed.

1. Introduction

Schizophrenia is a life-long debilitating disease, which usually begins in early adulthood. The currently available, typical antipsychotic drugs (APD) can control the positive manifestations of the disease, but are associated with extrapyramidal side effects (EPS). The newer, atypical APD such as clozapine provide major advances, but result in metabolic and other side effects [17]. Additionally, the National Institute of Mental Health (NIMH) Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study found that three-quarters of the participants stopped taking their first antipsychotic medication before the end of 18 months. Reasons such as medication not controlling the symptoms or intolerable side effects were cited for lack of compliance [34,35,53]. Thus there is a great need for the development of new APD.

Neurotensin (NT) is an endogenous tridecapeptide neurotransmitter with potent central nervous system effects including hypothermia [6], antinociception [40], modulation of dopamine (DA) neurotransmission [20,33,41,42], and stimulation of anterior pituitary hormone secretion [37,43,45]. Additionally, NT and NT receptor agonists demonstrate antipsychotic-like activities [9,11]. NT69L, a NT agonist that can be administered peripherally [55] shows antipsychotic-like activity in various animal models predictive of efficacy in treatment of psychosis. Specifically, NT69L blocks apomorphine-induced climbing without causing catalepsy [19], attenuates amphetamine-induced hyperactivity [12], and prevents the disruption of prepulse inhibition (PPI) induced by D-amphetamine (indirect DA agonist), [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride] (DOI, a 5-HT_{2A} agonist), and dizocilpine (a non-competitive NMDA antagonist)[46,48]. Repeated administration of NT69L results in tolerance to some, but not to all of the effects of NT69L [10]. Since effective pharmacological treatment of schizophrenia typically requires that patients take an antipsychotic drug (APD) indefinitely, an ideal APD should demonstrate persistent efficacy as the result of continuous administration. Thus, the chronic

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administration of NT69L was tested in an animal model with high predictive validity for antipsychotic drugs, namely, blockade of drug-induced disruption of PPI of the startle reflex, an operational measure of sensorimotor gating that is deficient in schizophrenic patients [13,14]. However, in addition to schizophrenia, there are other disorders that are characterized by PPI deficits. These include Huntington's disease [52], Tourette's syndrome [15], and obsessive–compulsive disorder (OCD) [50] and might benefit in some way from NT therapy.

Our previous findings [10] showed no tolerance developing to NT69L at blocking effects of the direct DA receptor agonist apomorphine, which stimulates pre- and post-synaptic DA receptors, or blocking the indirect DA receptor agonist D-amphetamine, which releases DA and other amines and blocks DA uptake [18]. Therefore, we predicted that chronic administration of NT69L will block drug-induced disruption of PPI without the development of tolerance.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats, $n = 4$-52, (initial weight 220–250 g) obtained from Harlan Laboratories, San Diego, were used in all experiments. The animals were housed in groups of two or three in a temperature controlled room with free access to water and food (Irridated rat diet (Teklad LH-485) from Harlan, Madison WI, USA) on a 12:12 h light/dark cycle. Animals were allowed a minimum of 7 days habituation to home cages during which time they were handled for 2 min daily as previously described [28] before experimental testing. On test days (day 1 and day 21), animals were habituated to the experimental room for 60 min prior to behavioral testing, performed between 9 a.m. and 4 p.m. Primarily an acute study was conducted where animals in each group were used once. For the chronic study, in each separate group the same animals were tested on days 1 and 21. The data from the acute study and day 1 in the chronic study were averaged and presented as day 1. All animal protocols are approved by the Mayo Clinic Institutional Animal Care and Use Committee in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

NT69L (1 mg/kg) was synthesized in the Mayo Proteomics Research Center (Rochester, MN) as previously described [23]. DOI (0.5 mg/kg), clozapine (20 mg/kg), and haloperidol (1 mg/kg) were purchased from Sigma–Aldrich (St. Louis, MO). NT69L, DOI, and D-amphetamine (Sigma–Aldrich (St. Louis, MO) were dissolved in saline (0.9% sodium chloride injection, Baxter Healthcare Cooperation, Deerfield, IL, USA); haloperidol and clozapine were dissolved in saline with ethanol and/or saline with 0.04% (v/v) acetic acid (vehicle). Doses of D-amphetamine and DOI used in this study have been previously shown consistently to disrupt PPI [48]. Additionally, similar doses of NT69L, haloperidol, and clozapine were used in previous experiments to block disruption of PPI [4,26,48]. All drugs were injected subcutaneously (s.c.). Control groups were injected with 100 μl of sterile saline or vehicle.

2.3. Behavioral testing

2.3.1. Equipment

PPI was measured using four identical startle chambers (San Diego Instruments, San Diego, CA). Each chamber consisted of a clear Plexiglas cylinder that rested on a Plexiglas platform inside a ventilated and illuminated enclosure housed in a sound attenuated room. A continuous background noise of 65 dB and the various acoustic stimuli were produced within each chamber by a high-frequency loudspeaker. The whole-body startle response of each animal resulted in vibrations that were transduced into analog signals by a piezoelectric unit mounted beneath the platform.

2.3.2. Test sessions

Tests were performed as previously described [47]. The test session consisted of five trial types within a 15 min time period, including a 5 min acclimation to a 65 dB background noise that continued throughout the trial. Trials were: no stimulus, presented to measure baseline movements within the cylinder; pulse-alone (a 120 dB startle pulse for 40 ms) and; prepulse plus pulse (prepulses 4, 8, or 12 dB above background lasting 20 ms). Prepulses were presented 100 ms (onset to onset) before the pulse. All trial types were presented in pseudo-random order with an average inter-trial interval of 15 s (range 8–23 s). In addition, four pulse-alone trials were presented at the beginning and end of the session. These were not used in the calculation of prepulse inhibition. %PPI was calculated according to the formula: [1 − (startle magnitude after prepulse-pulse pair/startle magnitude after pulse only] × 100. Rats were administered 21 daily s.c. injections of NT69L (1 mg/kg), haloperidol (1 mg/kg), clozapine (20 mg/kg), saline, or vehicle. On test days, the rats were injected with NT69L, haloperidol, or clozapine and 30 min later they were injected s.c. with either saline or the PPI disrupting drug (D-amphetamine at 5 mg/kg or DOI at 0.5 mg/kg). Twenty minutes later the animals were placed in the startle chambers and PPI was measured.

2.4. Determination of the dose response curves for NT69L in reversing D-amphetamine- and DOI-induced disruption of PPI

Various doses (0.01–2 mg/kg) of NT69L were injected into different groups of rats ($n \geq 8$) followed by D-amphetamine (5 mg/kg) or DOI (0.5 mg/kg). PPI was conducted as described previously. The ED_{50} for NT69L in blocking D-amphetamine- or DOI- induced disruption of PPI was determined using GraphPad Prism software (La Jolla, CA).

2.5. Statistical analysis

The data was analyzed with one-way ANOVA followed by Tukey's test for multiple comparisons. Two-way ANOVA was used to determine the interaction between the treatment and the pulse intensities. Data analysis was done using Sigma Stat software (SPSS, Inc., Chicago, IL) with $P < 0.05$ being considered significant.

3. Results

The %PPI for vehicle/saline group was not significantly different from the saline/saline group at day 1 (29 ± 4 and 32 ± 3, 49 ± 3 and 51 ± 3, 63 ± 2 and 64 ± 2 for vehicle and saline groups at 4, 8, and 12 dB pulse intensities, respectively), thus both group averages were used as controls. Twenty-one daily injections of saline did not alter %PPI as compared to acute injections (32 ± 3 and 40 ± 10, 51 ± 3 and 51 ± 7, 64 ± 2 and 63 ± 7 for acute and chronic injections at 4, 8 and 12 pulse intensities, respectively). Chronic injection of haloperidol, clozapine, or NT69L did not cause any significant change in body weight gain as compared to the saline control group. All groups showed an average of 17–21% increase in body weight at the end of the 21 days.

3.1. The effect of chronic injections of haloperidol and NT69L on D-amphetamine-induced disruption of PPI

Fig. 1 shows the significant effect of drug treatment (haloperidol and NT69L) in preventing the D-amphetamine-induced disruption of PPI ($F_{10,145} = 7.61$; $F_{10,144} = 10.79$; $F_{10,147} = 15.12$, $P < 0.001$) at 4, 8, and 12 dB pulse intensities, respectively. Comparisons among the groups show that haloperidol significantly ($P = 0.001$) blocked D-amphetamine-induced disruption of PPI after acute and 21 daily injections (4, 8, and 12 dB). Repeated injections of haloperidol attenuated the reversal potential, when compared to acute injections. However, the difference between the acute and chronic haloperidol injections did not reach a significant level ($P = 0.348$). Similarly, NT69L significantly blocked D-amphetamine-induced disruption of PPI after acute (4, 8, and 12 dB) and 21 daily injections (12 dB) ($P < 0.05$) without causing tolerance. Two-way ANOVA shows no significant interaction between the treatment and the pulse intensity ($P = 0.628$). Acute NT69L prevented PPI disruption caused by D-amphetamine with an ED_{50} of 0.08 mg/kg (Fig. 2). When compared to the effect of the typical APD haloperidol, NT69L had a significantly diminished effect after acute injection, but reached a similar level of significance after 21 daily injections. Thus, repeated administration of NT69L enhanced PPI without tolerance, a result similar to that of haloperidol. The effect of NT69L on blocking D-amphetamine-induced disruption of PPI was not significantly different between day 1 and day 21, although the inability to develop tolerance was shown to be significant only at 12 dB. Fig.3 shows the average %PPI and the controls. One-way ANOVA shows a significant effect of treatment ($F_{5,111} = 24.65$, $P < 0.001$) on the average %PPI. Comparisons among groups show that D-amphetamine significantly disrupted PPI as compared to all other treatments ($P < 0.001$). Acute pretreatment with NT69L had a significantly ($P < 0.05$) diminished effect in blocking the D-amphetamine-induced disruption of PPI as compared to acute