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Effect of N^1Dansylspermine and Ro25,6981 on locomotor activity in naïve mice and in the reserpinised mouse model of Parkinson's Disease

Polyamine antagonists and motor activity

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Abstract

The effect of N^1Dansylspermine, a polyamine analogue and competitive polyamine antagonist and Ro25,6981, a non-competitive polyamine antagonist with good affinity and selectivity for the GluN2B subunit, on locomotor activity in naïve mice was investigated. Furthermore the ability of the polyamine antagonists to reverse reserpine-induced hypokinesia was assessed, 24 hours after injection of a catecholamine depleting dose of reserpine (5 mg/kg, s.c.), to investigate the therapeutic potential of polyamine antagonists in Parkinson's Disease.

N^1-dansylspermine, significantly decreased locomotor activity in naïve animals ($p<0.001$), but caused a mild, but significant increase in locomotor activity in reserpinised mice at the highest dose tested ($p<0.05$). Ro25,6981 significantly stimulated locomotor activity in naïve animals ($p<0.001$) and had a slight significant stimulatory effect on reserpine-induced hypokinesia ($p=0.05$).

N^1Dansylspermine and Ro25,6981 had opposite effects on locomotor activity in naïve mice, but both had a mild antiparkinsonian effect in the reserpine model. These findings suggest that antagonism of the polyamine binding site on the GluN2B subunit can reduce hypokinesia, albeit only to a limited extent.

Keywords

Locomotor activity; Reserpine mouse model; polyamine antagonist; GluN2B antagonist; N^1Dansylspermine; Ro25,6981; Parkinson's Disease.

Introduction

Parkinson's Disease is a progressive movement disorder associated with neurodegeneration in the basal ganglia. It is characterised by difficulty in initiating motor activity, postural instability and a resting tremor. A classical hallmark of Parkinson's Disease is the loss of dopaminergic neurones in the substantia nigra pars compacta causing dopamine denervation of the striatum [1]. This degeneration is associated with a second hallmark of the disease, the deposition of misfolded and ubiquitinated α-synuclein containing Lewy bodies and Lewy neurites within neurones [2]. Another common feature accompanying the degeneration of dopamine neurones in Parkinson's Disease is the overactivity of the glutamatergic system in the subthalamic nucleus and corpus striatum of the basal ganglia [3]. Surgical inactivation of glutamatergic neurones in the subthalamic nucleus or their efferents in the internal segment of the globus pallidus have reversed Parkinson-like symptoms in animal models [4]. Amantadine, a drug used clinically to reduce the symptoms of Parkinsonism, increases dopamine release and also may act as a weak NMDA receptor antagonist [5, 6]. The non-competitive polyamine and NMDA receptor (GluN2B) antagonist ifenprodil displayed antiparkinsonian activity in the reserpine treated rat and MPTP lesioned marmoset models with a far better side effect profile than the competitive or channel blocking NMDA receptor antagonists [7, 8]. The polyamines are a group of naturally occurring amines including spermine and spermidine that positively modulate the activity of NMDA receptors [9], specifically through a binding sites on the GluN1, GluN2A and in particular, GluN2B subunits [10]. Interestingly, the physiological concentrations of the polyamines have been shown to accelerate the aggregation of α-synuclein in vitro [11]. Spermine and spermidine levels are raised in red blood cells of patients with Parkinson's Disease

[12]. Reserpine-induced monoamine depletion in rodents allows the rapid assessment of anti-akinetic potential of drugs, and was previously shown to detect the antiparkinsonian potential of ifenprodil [7] but the limited effect of its sister drug eliprodil [13]. The aim of this study was to assess the effect of N^1Dansylspermine, a polyamine analogue and competitive polyamine antagonist [14, 15] and Ro25,6981, a non-competitive polyamine antagonist with good affinity and selectivity for the GluN2B subunit [16] on locomotor activity in naïve mice and in the reserpinised mouse model of Parkinson's disease to investigate the therapeutic potential of polyamine antagonists in Parkinson's Disease.

Materials and Methods

Male albino TO mice (20 - 40 g) were obtained from Tuck & Sons, U.K., and were housed in groups of 6 under a twelve hour light / dark cycle (on: 07.00 - 19.00 hr) at an ambient temperature of 21 ± 1^{o} C, with food and water *ad libitum*. All experiments were conducted according to the requirements of Cruelty To Animals Act, 1876, European Community Directive, 86/609/EC.

Drugs and protocol

Very little is known of the pharmacodynamics and kinetics of N^1-dansylspermine and Ro25,6981. An initial observation window of 2 hours was chosen to give an indication of behavioural effects of these drugs. As it was observed that locomotor effects developed rapidly in naïve mice, the same duration of observation was chosen to assess the anti-dyskinetic effect of the drugs at a range of doses. Reserpine (5 mg/kg, s.c.) (Sigma, U.K.) was dissolved in the minimum quantity of glacial acetic acid in 2 / 3 drops of boiled distilled water, and subsequently made to volume with distilled water. Following reserpine or vehicle injection, mice were kept at an ambient temperature of 28^{o} C, to prevent hypothermia (which occurs in response to reserpine). The polyamine antagonists were administered 24 hours following reserpine or vehicle treatment. All drug treatments in the N^1-dansylspermine dose-response study and the Ro25,6981 dose-response study were administered the same batch of reserpine as the relevant reserpine control. Immediately following antagonist administration, animals were placed individually into a PanLab Actisystem chamber and locomotor count was recorded in 10 minute time-bins every 10 minutes for 2 hours.

N1-dansylspermine (2-20 μg, i.c.v ; gift of Prof. Graham Shaw, Trinity College, Dublin) was dissolved in distilled water. Mice were briefly anaesthetised with 5% isoflurane (Rhone Merieux, U.K.), prior to i.c.v. injection in a dose volume of 5 μl into the left cerebral ventricle using the method of Brittain [17]. Animals administered vehicle by the i.c.v. route began to recover within 15 seconds of injection, moving freely around the locomotor chamber, showing no overt signs of unusual behaviour. Ro 25-6981 (1-40 $mg.kg^{-1}$; La Roche Pharmaceuticals, Switzerland) was dissolved in distilled water and administered i.p. in a volume of 5 $ml.kg^{-1}$.

Data Analysis

Results are expressed as mean locomotor activity +/- s.e.m. over time for each treatment. 2-way ANOVA, with Bonferroni post hoc analysis was performed.

Results

Effects in naïve mice:

A significant main effect of N^1Dansylspermine on locomotor count was observed [$F(4,300) = 25.73$, $p<0.001$], with posthoc analysis demonstrating a significant reduction in locomotor count at all doses in comparison to control (Figure 1a and 1b). This was particularly apparent with the highest dose in the first 90 minutes of observation (Figure 1a and 1b). A significant effect of time was also observed, [$F(11,300) = 35.58$; $p<0.001$]. The i.c.v injection procedure briefly reduced locomotor activity in all groups. Following recovery, the high locomotor activity reduced over

time (Figure 1a). Treatment did not have the same effect at all times, as there was a significant time x treatment interaction observed [$F(44,300) = 2.86$; $p<0.001$].

A significant main effect of Ro25,6981 on locomotor activity in naïve mice was also found [$F(5,360) = 109.96$, $p<0.001$], reflecting the significant stimulatory effect on locomotor count evident at each concentration (Figure 2a and Figure 2b). No abnormalities in movement were observed, only increased exploratory behaviour that was maintained for the duration of the observation period. There was a significant effect of time [$F(11, 360) = 22.67$; $p<0.001$], and treatment did not have the same effect at all times, as there was a significant time x treatment interaction observed [$F(55,360) = 4.01$; $p<0.001$].

Effects in the reserpinised mouse model:

The reserpine treated animals all exhibited hypokinesia, tremor and hunched posture 24 hours after reserpine administration. A control group administered reserpine vehicle 24 hours prior to the study showed no abnormalities in locomotor activity. The reserpine used in the N1-dansylspermine and Ro25,6981 studies was from different batches of the drug, and produced some variability in extent of dyskinesia produced. Nonetheless, very clear dyskinesia was produced in both dose-response experiments, and all drug treatments were administered the same batch of reserpine as the relevant reserpine control to enable assessment of anti-dyskinesia effect.

A mild, but significant, stimulatory effect of N^1Dansylspermine on locomotor activity in reserpinised animals was observed [$F(4,300) = 2.96$, $p<0.05$] (Figure 1c). Posthoc analysis showed a significantly higher locomotor count with the 20μg dose in comparison to control, (Figure 1d; $p<0.05$). There was a significant effect of time [F

(11,300) = 11.8, p<0.001], and no time x treatment interaction was found [F (44,300)=1.04, NS].

Ro25,6981 had a mild, stimulatory effect on locomotor activity in reserpinised animals [F (5,360) = 2.28, p=0.05], but posthoc analysis did not identify a significant effect of any dose in comparison to control (Figure 2c and Figure 2d). There was a significant effect of time [F (11,360) = 9.32, p<0.001], and no time x treatment interaction was found [F (55,360) = 0.75, NS].

Discussion

Amantadine, a weak NMDA receptor antagonist is used clinically to treat the symptoms of Parkinson's Disease. Its sister compound, memantine has been studied in clinical trials for Parkinson's disease with some limited success [18], particularly in the treatment of dementia in Parkinsons disease [19]. The polyamines are positive modulators of NMDA receptor function in vivo [20], and as such, antagonists of polyamine binding sites may have therapeutic potential as motor stimulants, possibly without many of the disabling side effects of the more direct competitive and open channel antagonists of the NMDA receptor [21]. Ifenprodil has been shown to have anti-parkinsonian actions in reserpinised rat, 6-hydroxydopamine-lesioned primate and MPTP-lesioned primate models [7, 8, 22]. This anti-parkinsonian action is thought to occur through inhibition of overactive NR2B-containing NMDA receptors [7]. However, it is also known that ifenprodil and its sister compound eliprodil possess a degree of calcium and sodium channel antagonist potential [23, 24], inhibit inwardly rectifying potassium channels [25] and can also block the NMDA receptor evoked release of neuromodulators in the striatum [26]. There is also evidence that

eliprodil suppresses the uptake and enhances the efflux of dopamine in striatal tissues [27]. In addition, ifenprodil-like GluN2B antagonists have a high degree of interaction with α1-adrenergic receptors, which have been suggested to contribute to adverse side effects of these compounds [28]. The extent to which the interactions with GluN2B subunits or sites other than GluN2B subunits contribute to the overall effect of ifenprodil-like GluN2B antagonists *in vivo* is, as yet, unclear.

The ifenprodil-like analogue used in this study, Ro25,6981, has a very high affinity for GluN2B subunits [16]. Ro25,6981 (at a dose of 10 $mg.kg^{-1}$ i.p.) has recently been shown to be as effective at inhibiting conditioned fear as MK801, however without the attendant hyperlocomotion, ataxia and stereotypy associated with MK801 [29]. In the present study, we investigated significantly higher doses in mice (up to 40 $mg.kg^{-1}$) and observed hyperlocomotion that was maintained for the duration of the observation period, suggesting a duration of effect of Ro25,6981 of greater than 2 hours. It is also notable that no other motor abnormalities were observed with even the highest dose (40 $mg.kg^{-1}$). Ro25,6981 had a slight significant stimulatory effect on reserpine-induced hypokinesia, suggesting a mild anti-parkinsonian effect. This finding is in line with a recent study which showed that Ro25,6981 enhanced the anti-akinetic effect of L-DOPA in rats with 6-hydroxydopamine lesions [30].

N^1Dansylspermine is a potent polyamine competitive antagonist acting via the positive polyamine modulatory binding site on the NMDA receptor [31]. In naïve mice, N^1Dansylspermine caused reduced locomotor activity. Sedation has previously been reported following i.c.v. administration of 1,10-diaminodecane and diethylenetriamine, two polyamine analogues with some antagonist activity [32]. In

this study, N^1Dansylspermine had a mild, but significant stimulatory effect in reserpinised mice, indicating that an interaction with the polyamine binding site on the GluN2B subunit can reduce hypokinesia, albeit mildly.

Conclusions

The GluN2B antagonist, Ro25,6981 and the competitive polyamine antagonist, N^1Dansylspermine, both mildly reduced hypokinesia in the reserpinised mouse model of Parkinson's Disease. These findings suggest that antagonism of the polyamine binding site on the GluN2B subunit can reduce hypokinesia, albeit only to a limited extent. It may be worthwhile to assess the effect of repeated administration of polyamine antagonists in a more dopamine-specific neurotoxic or genetic model of Parkinson's disease, but it may be that their therapeutic potential in Parkinson's disease is limited.

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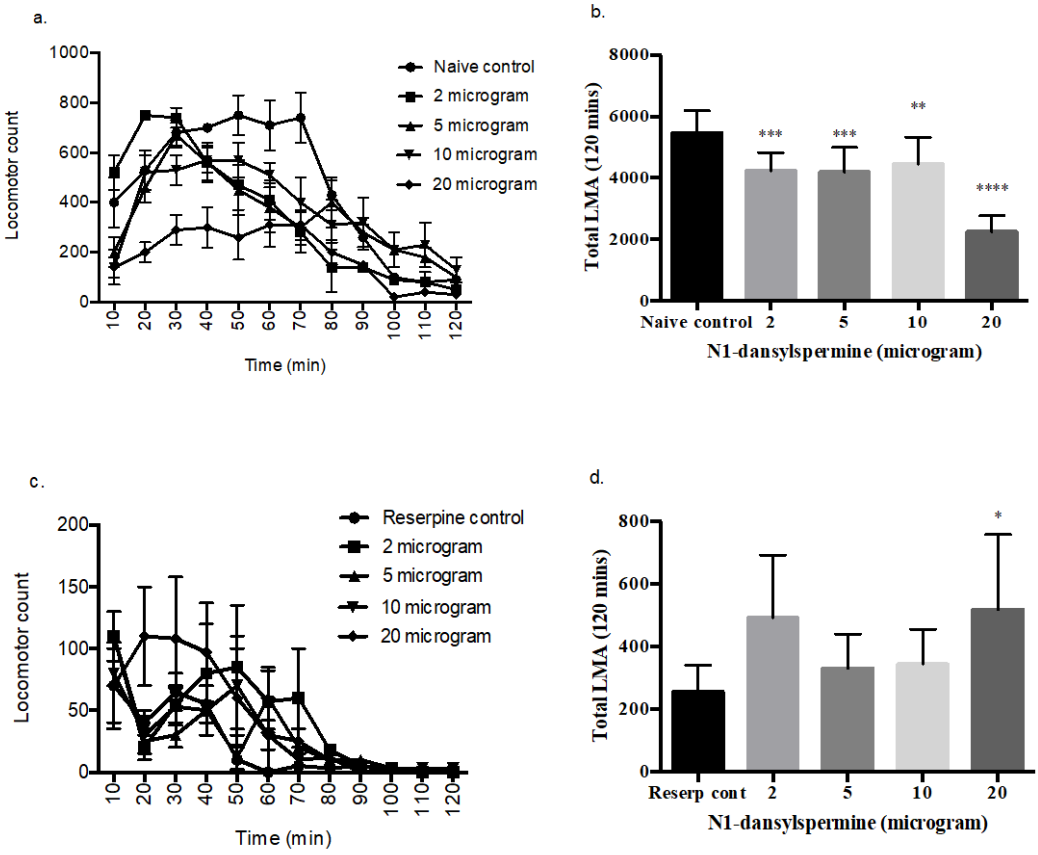
Figure 1: Effect of N1-dansylspermine on locomotor activity in naïve mice over time (Figure 1a; n=6 per treatment). Locomotor activity was recorded every 10 minutes for up to 2 hours. Figure 1b shows total locomotor activity (LMA) in the 2 hour observation window in naïve mice administered a range of doses of N1-dansylspermine. Figures 1c and 1d show the effect of N1-dansylspermine in reserpinised mice (n=6 per treatment).. Data is expressed as mean locomotor count ± sem; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, 2-way ANOVA with Bonferroni post-hoc analysis.

Figure 2: Effect of Ro 25,6981 on locomotor activity in naïve mice over time (Figure 2a; n=6 per treatment). Locomotor activity was recorded every 10 minutes for up to 2 hours. Figure 2b shows total locomotor activity (LMA) in the 2 hour observation window in naïve mice administered a range of doses of Ro 25,6981. Figures 2c and 2d show the effect of Ro 25,6981 in reserpinised mice (n=6 per treatment). Data is expressed as mean locomotor count ± sem; ****p<0.0001, 2-way ANOVA with Bonferroni post-hoc analysis.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Locomotor count
Time (min)
Naive control
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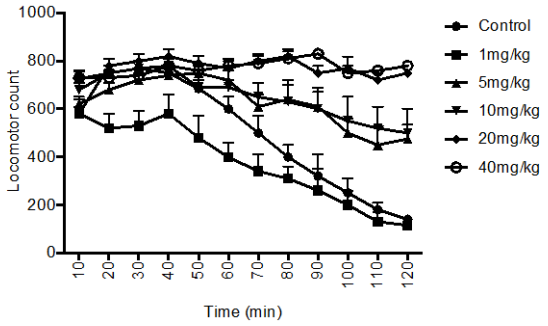
5 microgram
10 microgram
20 microgram
b.
Total LMA (120 mins)
Naive control
2
5
10
20
N1-dansylspermine (microgram)
c.
Locomotor count
Time (min)
Reserpine control
2 microgram
5 microgram
10 microgram
20 microgram
d.
Total LMA (120 mins)
Reserp cont
2
5
10
20
N1-dansylspermine (microgram)

a.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600
400
200
0
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)
Control
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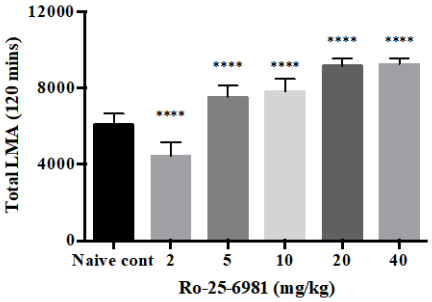
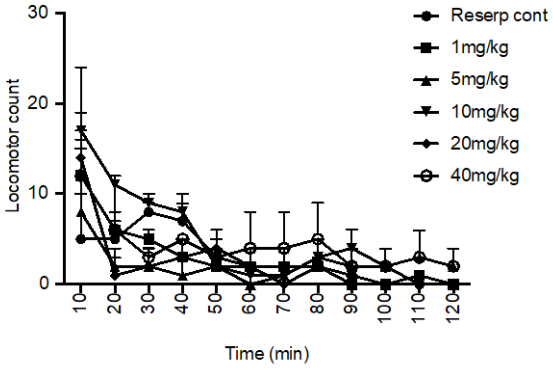
5mg/kg
10mg/kg
20mg/kg
40mg/kg

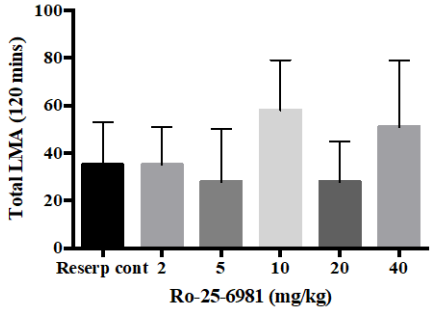
b.


Total LMA (120 mins)
12000
8000
4000
0
****
****
****
****
****
Naive cont
2
5
10
20
40
Ro-25-6981 (mg/kg)

c.


Locomotor count
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)
Reserp cont
1mg/kg
5mg/kg
10mg/kg
20mg/kg
40mg/kg

d.


Total LMA (120 mins)
100
80
60
40
20
0
Reserp cont
2
5
10
20
40
Ro-25-6981 (mg/kg)