



Effect of N1-Dansylspermine and Ro25,6981 on locomotor activity in naive mice and in the reserpinised mouse model of Parkinson's disease

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3 mouse model of Parkinson's Disease

4

5 Polyamine antagonists and motor activity

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23 Abstract

24 The effect of N¹Dansylspermine, a polyamine analogue and competitive polyamine
25 antagonist and Ro25,6981, a non-competitive polyamine antagonist with good
26 affinity and selectivity for the GluN2B subunit, on locomotor activity in naïve mice
27 was investigated. Furthermore the ability of the polyamine antagonists to reverse
28 reserpine-induced hypokinesia was assessed, 24 hours after injection of a
29 catecholamine depleting dose of reserpine (5 mg/kg, s.c.), to investigate the
30 therapeutic potential of polyamine antagonists in Parkinson's Disease.

31 N¹-dansylspermine, significantly decreased locomotor activity in naïve animals
32 ($p < 0.001$), but caused a mild, but significant increase in locomotor activity in
33 reserpinised mice at the highest dose tested ($p < 0.05$). Ro25,6981 significantly
34 stimulated locomotor activity in naïve animals ($p < 0.001$) and had a slight significant
35 stimulatory effect on reserpine-induced hypokinesia ($p = 0.05$).

36 N¹Dansylspermine and Ro25,6981 had opposite effects on locomotor activity in naïve
37 mice, but both had a mild antiparkinsonian effect in the reserpine model. These
38 findings suggest that antagonism of the polyamine binding site on the GluN2B
39 subunit can reduce hypokinesia, albeit only to a limited extent.

40

41 Keywords

42 Locomotor activity; Reserpine mouse model; polyamine antagonist; GluN2B
43 antagonist; N¹Dansylspermine; Ro25,6981; Parkinson's Disease.

44

45 **Introduction**

46 Parkinson's Disease is a progressive movement disorder associated with
47 neurodegeneration in the basal ganglia. It is characterised by difficulty in initiating
48 motor activity, postural instability and a resting tremor. A classical hallmark of
49 Parkinson's Disease is the loss of dopaminergic neurones in the substantia nigra pars
50 compacta causing dopamine denervation of the striatum [1]. This degeneration is
51 associated with a second hallmark of the disease, the deposition of misfolded and
52 ubiquitinated α -synuclein containing Lewy bodies and Lewy neurites within neurones
53 [2]. Another common feature accompanying the degeneration of dopamine neurones
54 in Parkinson's Disease is the overactivity of the glutamatergic system in the
55 subthalamic nucleus and corpus striatum of the basal ganglia [3]. Surgical
56 inactivation of glutamatergic neurones in the subthalamic nucleus or their efferents in
57 the internal segment of the globus pallidus have reversed Parkinson-like symptoms in
58 animal models [4]. Amantadine, a drug used clinically to reduce the symptoms of
59 Parkinsonism, increases dopamine release and also may act as a weak NMDA
60 receptor antagonist [5, 6]. The non-competitive polyamine and NMDA receptor
61 (GluN2B) antagonist ifenprodil displayed antiparkinsonian activity in the reserpine
62 treated rat and MPTP lesioned marmoset models with a far better side effect profile
63 than the competitive or channel blocking NMDA receptor antagonists [7, 8]. The
64 polyamines are a group of naturally occurring amines including spermine and
65 spermidine that positively modulate the activity of NMDA receptors [9], specifically
66 through a binding sites on the GluN1, GluN2A and in particular, GluN2B subunits
67 [10]. Interestingly, the physiological concentrations of the polyamines have been
68 shown to accelerate the aggregation of α -synuclein in vitro [11]. Spermine and
69 spermidine levels are raised in red blood cells of patients with Parkinson's Disease

70 [12]. Reserpine-induced monoamine depletion in rodents allows the rapid assessment
71 of anti-akinetic potential of drugs, and was previously shown to detect the
72 antiparkinsonian potential of ifenprodil [7] but the limited effect of its sister drug
73 eliprodil [13]. The aim of this study was to assess the effect of N¹Dansylspermine, a
74 polyamine analogue and competitive polyamine antagonist [14, 15] and Ro25,6981, a
75 non-competitive polyamine antagonist with good affinity and selectivity for the
76 GluN2B subunit [16] on locomotor activity in naïve mice and in the reserpinised
77 mouse model of Parkinson's disease to investigate the therapeutic potential of
78 polyamine antagonists in Parkinson's Disease.

79

80 **Materials and Methods**

81

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

87

88 *Drugs and protocol*

89 Very little is known of the pharmacodynamics and kinetics of N¹-dansylspermine and
90 Ro25,6981. An initial observation window of 2 hours was chosen to give an
91 indication of behavioural effects of these drugs. As it was observed that locomotor
92 effects developed rapidly in naïve mice, the same duration of observation was chosen
93 to assess the anti-dyskinetic effect of the drugs at a range of doses. Reserpine (5
94 mg/kg, s.c.) (Sigma, U.K.) was dissolved in the minimum quantity of glacial acetic
95 acid in 2 / 3 drops of boiled distilled water, and subsequently made to volume with
96 distilled water. Following reserpine or vehicle injection, mice were kept at an ambient
97 temperature of 28° C, to prevent hypothermia (which occurs in response to reserpine).
98 The polyamine antagonists were administered 24 hours following reserpine or vehicle
99 treatment. All drug treatments in the N¹-dansylspermine dose-response study and the
100 Ro25,6981 dose-response study were administered the same batch of reserpine as the
101 relevant reserpine control. Immediately following antagonist administration, animals
102 were placed individually into a PanLab Actisystem chamber and locomotor count was
103 recorded in 10 minute time-bins every 10 minutes for 2 hours.

104

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

114

115 *Data Analysis*

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

118

119 **Results**

120

121 *Effects in naïve mice:*

122 A significant main effect of N¹Dansylspermine on locomotor count was observed [F
123 (4,300) = 25.73, $p < 0.001$], with posthoc analysis demonstrating a significant
124 reduction in locomotor count at all doses in comparison to control (Figure 1a and 1b).
125 This was particularly apparent with the highest dose in the first 90 minutes of
126 observation (Figure 1a and 1b). A significant effect of time was also observed, [F
127 (11,300) = 35.58; $p < 0.001$]. The i.c.v injection procedure briefly reduced locomotor
128 activity in all groups. Following recovery, the high locomotor activity reduced over

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

131 A significant main effect of Ro25,6981 on locomotor activity in naïve mice was also
132 found [F (5,360) = 109.96, p<0.001], reflecting the significant stimulatory effect on
133 locomotor count evident at each concentration (Figure 2a and Figure 2b). No
134 abnormalities in movement were observed, only increased exploratory behaviour that
135 was maintained for the duration of the observation period. There was a significant
136 effect of time [F (11, 360) = 22.67; p<0.001], and treatment did not have the same
137 effect at all times, as there was a significant time x treatment interaction observed [F
138 (55,360) = 4.01; p<0.001].

139

140 *Effects in the reserpinised mouse model:*

141 The reserpine treated animals all exhibited hypokinesia, tremor and hunched posture
142 24 hours after reserpine administration. A control group administered reserpine
143 vehicle 24 hours prior to the study showed no abnormalities in locomotor activity.
144 The reserpine used in the N1-dansylspermine and Ro25,6981 studies was from
145 different batches of the drug, and produced some variability in extent of dyskinesia
146 produced. Nonetheless, very clear dyskinesia was produced in both dose-response
147 experiments, and all drug treatments were administered the same batch of reserpine as
148 the relevant reserpine control to enable assessment of anti-dyskinesia effect.

149 A mild, but significant, stimulatory effect of N¹Dansylspermine on locomotor activity
150 in reserpinised animals was observed [F (4,300) = 2.96, p<0.05] (Figure 1c). Posthoc
151 analysis showed a significantly higher locomotor count with the 20µg dose in
152 comparison to control, (Figure 1d; p<0.05). There was a significant effect of time [F

153 (11,300) = 11.8, $p < 0.001$], and no time x treatment interaction was found [F
154 (44,300) = 1.04, NS].

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

160

161 **Discussion**

162

163 Amantadine, a weak NMDA receptor antagonist is used clinically to treat the
164 symptoms of Parkinson's Disease. Its sister compound, memantine has been studied
165 in clinical trials for Parkinson's disease with some limited success [18], particularly
166 in the treatment of dementia in Parkinsons disease [19]. The polyamines are positive
167 modulators of NMDA receptor function in vivo [20], and as such, antagonists of
168 polyamine binding sites may have therapeutic potential as motor stimulants, possibly
169 without many of the disabling side effects of the more direct competitive and open
170 channel antagonists of the NMDA receptor [21]. Ifenprodil has been shown to have
171 anti-parkinsonian actions in reserpinised rat, 6-hydroxydopamine-lesioned primate
172 and MPTP-lesioned primate models [7, 8, 22]. This anti-parkinsonian action is
173 thought to occur through inhibition of overactive NR2B-containing NMDA receptors
174 [7]. However, it is also known that ifenprodil and its sister compound eliprodil
175 possess a degree of calcium and sodium channel antagonist potential [23, 24], inhibit
176 inwardly rectifying potassium channels [25] and can also block the NMDA receptor
177 evoked release of neuromodulators in the striatum [26]. There is also evidence that

178 eliprodil suppresses the uptake and enhances the efflux of dopamine in striatal tissues
179 [27]. In addition, ifenprodil-like GluN2B antagonists have a high degree of
180 interaction with α 1-adrenergic receptors, which have been suggested to contribute to
181 adverse side effects of these compounds [28]. The extent to which the interactions
182 with GluN2B subunits or sites other than GluN2B subunits contribute to the overall
183 effect of ifenprodil-like GluN2B antagonists *in vivo* is, as yet, unclear.

184

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

197

198 N¹Dansylspermine is a potent polyamine competitive antagonist acting via the
199 positive polyamine modulatory binding site on the NMDA receptor [31]. In naïve
200 mice, N¹Dansylspermine caused reduced locomotor activity. Sedation has previously
201 been reported following i.c.v. administration of 1,10-diaminodecane and
202 diethylenetriamine, two polyamine analogues with some antagonist activity [32]. In

203 this study, N¹Dansylspermine had a mild, but significant stimulatory effect in
204 reserpinised mice, indicating that an interaction with the polyamine binding site on
205 the GluN2B subunit can reduce hypokinesia, albeit mildly.

206

207 **Conclusions**

208 The GluN2B antagonist, Ro25,6981 and the competitive polyamine antagonist,
209 N¹Dansylspermine, both mildly reduced hypokinesia in the reserpinised mouse model
210 of Parkinson's Disease. These findings suggest that antagonism of the polyamine
211 binding site on the GluN2B subunit can reduce hypokinesia, albeit only to a limited
212 extent. It may be worthwhile to assess the effect of repeated administration of
213 polyamine antagonists in a more dopamine-specific neurotoxic or genetic model of
214 Parkinson's disease, but it may be that their therapeutic potential in Parkinson's
215 disease is limited.

216

217

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220 Hertfordshire and the kind provision of N1-acetylspermine by Prof. Graham Shaw,
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222

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224

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- 315

317

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

326

327 Figure 2: Effect of Ro 25,6981 on locomotor activity in naïve mice over time (Figure
328 2a; n=6 per treatment). Locomotor activity was recorded every 10 minutes for up to 2
329 hours. Figure 2b shows total locomotor activity (LMA) in the 2 hour observation
330 window in naïve mice administered a range of doses of Ro 25,6981. Figures 2c and
331 2d show the effect of Ro 25,6981 in reserpinised mice (n=6 per treatment). Data is
332 expressed as mean locomotor count \pm sem; ****p<0.0001, 2-way ANOVA with
333 Bonferroni post-hoc analysis.

334

335



