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The NMDA receptor NR2B subtype selective antagonist Ro 25-6981 aggravates paroxysmal dyskinesia in the dt^{sz} mutant

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Abstract

Previously, enhanced levels of spermine which stimulates *N*-methyl-D-aspartate (NMDA) receptors, particularly those containing the NR2B subunit, were found in brains of dt^{sz} mutant hamsters, a model of paroxysmal dyskinesia in which dystonic episodes occur in response to stress. Therefore, the effects of the NR2B selective NMDA receptor antagonist Ro 25-6981 ([*R*-(*R*,*S*)]-α-(4-hydroxyphenyl)-β-methyl-4-phenyl-methyl)-1-piperidine-propanol] on severity of dystonia were investigated in the dt^{sz} hamster. Ro 25-6981 failed to exert antidystonic effects, but even caused a moderate aggravation at higher doses (10.0, 12.5 mg/kg). This result indicates that overstimulation of receptors that include the NR2B subunit by polyamines is not involved in the dystonic syndrome. NR2B-selective NMDA receptor antagonists seem not to provide a novel approach in the treatment of hereditary paroxysmal dyskinesias.

Keywords: Glutamate; Movement disorder; Paroxysmal choreoathetosis

1. Introduction

NR2B selective *N*-methyl-D-aspartate (NMDA) receptor antagonists have been shown to reduce levodopa-induced dyskinesias in parkinsonian monkeys, indicating that those compounds may be useful agents for the treatment of Parkinson's disease (Blanchet et al., 1999). The effects of NR2B selective NMDA receptor antagonists have so far not been examined in inherited paroxysmal dyskinesias, such as in primary paroxysmal non-kinesiogenic dystonic choreoathetosis (brief: paroxysmal dystonia), a subtype of dystonia in which dystonic episodes occur in response to stress (Demirkiran and Jankovic, 1995). Dystonia, regarded as a basal ganglia disorder, is an often-intractable syndrome characterized by involuntary contractions of opposing muscles (Fahn et al., 1998; Vitek and Giroux, 2000).

NMDA receptors are ligand-gated ion channels composed of NR1 and NR2 subunits with binding sites for a number of positive and negative modulators (Dingledine et al., 1999). Such modulators are the endogenous polyamines spermine and spermidine which activate NMDA receptors that incorporate NR2B subunits (Ramson and Stec, 1988). Previously, enhanced brain levels of spermine were determined in the dt^{sz} mutant hamster, a well-defined animal model of paroxysmal dystonia (Richter et al., 1998; Richter and Löscher, 1998). There is evidence that enhanced glutamatergic activity contributes to the occurrence of dystonic episodes in mutant hamsters, although γ-aminobutyric acid (GABA)ergic disinhibition seems to be the culprit in this animal model (Nobrega et al., 1997, 2002; Gernert et al., 2000; Richter and Löscher, 2002). As shown in previous experiments, various competitive and non-competitive NMDA receptor antagonists which block both NR1/NR2A and NR1/NR2B receptor subtypes (e.g., memantine, dizocilpine and DL-[*E*]-2-amino-4-methyl-5-phosphono-3-pentenoic acid) exerted beneficial effects in mutant hamsters (Richter and Löscher, 1998). However, the antidystonic effects were in part only moderate and particularly non-competitive inhibitors provoked marked adverse effects (Richter et al., 1991; Richter and Löscher, 1997). NR2B selective antagonists are thought to show a better profile than high affinity channel blockers and competitive NMDA receptor antagonists (Parsons, 2001).

To examine whether NR2B-selective antagonists may provide advantages in view of the therapeutic index, the

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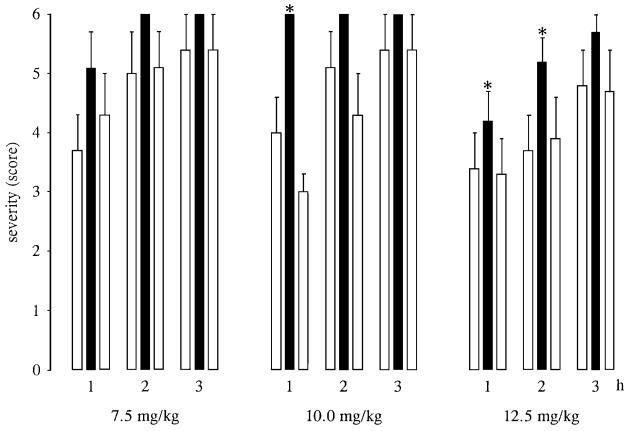

Fig. 1. Effect of Ro 25-6981 (7.5, 10.0 and 12.5 mg/kg, i.p.) on severity of dystonia in mutant hamsters (max-period, age: 35–39 days). The white bars in each set of three bars indicate the control values obtained two days before (pre-drug control) drug administration (first white bar) and two days after (post-drug control) drug administration (second white bar). The black bar refers to the day of drug administration in the same animal groups. The individual maximum severity of dystonia is usually reached within 3 h after induction of dystonia by triple stimulation including the injection of drugs or vehicle. The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd hour post-injection of vehicle or Ro 25-6981, reflecting the progression of dystonia in dt^{sz} hamsters during control recordings and after treatment with the active compound. Asterisks indicate significant aggravation of dystonia in comparison to the pre- and post-drug control (*$P<0.05$). Data are shown as means ± S.E. of seven (7.5), eight (10.0 mg/kg) or nine (12.5 mg/kg) animals. Absence of S.E. bars indicates that all hamsters had reached the same severity.

effects of Ro 25-6981 ([*R*-(*R*,*S*)]-α-(4-hydroxyphenyl)-β-methyl-4-phenyl-methyl)-1-piperidine-propanol] on severity of dystonia in mutant hamsters was tested in the present study. Ro 25-6981 can be regarded as a highly selective non-competitive NR2B-subunit NMDA receptor antagonist (Fischer et al., 1997; Lynch et al., 2001).

2. Materials and methods

The three groups of 7–9 dt^{sz} mutant hamsters used for the present study were obtained by selective breeding as described previously (Löscher et al., 1989; Richter and Löscher, 1998). In these mutant hamsters, dystonic attacks can be induced by handling or mild environmental stimuli. The dystonic attacks were induced by a triple stimulation technique (Löscher et al., 1989), i.e., stressful stimuli consisting of (1) taking the animal from its home cage and placing it on a balance, (2) an intraperitoneal injection (injection volume: 5 ml/kg) of vehicle (sterile water) or of Ro 25-6981, and (3) placement of the animal in a new plastic cage. Thereafter, dt^{sz} hamsters develop a sequence of abnormal movements and postures, allowing to rate the severity of dystonia by the following score-system (Löscher et al., 1989; Richter and Löscher, 1998): stage 1, flat body posture; stage 2, disturbed gait with hyperextended fore-paws; stage 3, hyperextended hindlimbs so that the animals appear to walk on tiptoes; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; stage 6, immobilisation in a twisted, hunched posture with hind- and forelimbs tonically extended forward. Since the individual maximum stage of dystonia is usually reached within 3 h, the hamsters were observed for 3 h after triple stimulation. During this period, the severity of dystonia, the latencies to the different stages and the side effects were noticed. Pre- and post-drug control trials were undertaken 2 days before and 2 days after drug testing. Ro 25-6981, kindly provided by Hoffmann-La Roche (Basel, Switzerland), was dissolved in sterile water. The solutions were freshly prepared prior to the experiments.

Dystonia in mutant hamsters shows an age-dependent time course (e.g., Richter and Löscher, 1998). The experiments were done in mutant hamsters at an age of maximum expression of dystonia between 30 and 40 days (max-period, suitable to detect beneficial drug effects) and also in older animals, which had passed the age of maximum severity (age: 50–60 days; post-max period, suitable to detect prodystonic effects).

The significance of differences in severity of dystonia and in latencies to onset between control and drug trials was calculated by the Friedman test and post-hoc by the Wilcoxon signed rank test for paired replicates.

3. Results

Ro 25-6981 accelerated the progression of dystonia, i.e., the severity of dystonia was significantly increased during the first (10 and 12.5 mg/kg) and second hour (12.5 mg/kg) after the induction of a dystonic attack (Fig. 1). Ro 25-6981 tended to reduce the latency to onset of dystonia, but a significant decrease was only observed after administration of 10 mg/kg in mutant hamsters at the most sensitive age (Table 1). In older dt^{sz} hamsters, which had passed the age of maximum severity (post-max period), Ro 25-6981 did not exert prodystonic effects even at a higher dose of 15 mg/kg (Fig. 2, Table 1).

Table 1
Effects of Ro 25-6981 on the latency of onset of dystonia

Dose (mg/kg)	Age (days) at drug trial	Latency (min)			*n*
		Pre-drug	Drug	Post-drug	
7.5	35	13.0 ± 2.1	6.7 ± 1.0	10.7 ± 1.7	7
10.0	39	11.7 ± 2.2	6.1 ± 1.2[a]	11.7 ± 1.6	8
12.5	37	10.7 ± 1.2	7.4 ± 0.6	12.1 ± 1.1	9
10.0	53	15.1 ± 2.5	11.6 ± 2.3	16.3 ± 1.6	8
15.0	58	15.7 ± 1.6	13.6 ± 3.6	14.4 ± 1.9	9

Latency was determined as the time to the first unequivocal signs of the dystonic attacks (stage 2). Data are shown as means ± S.E. of the number of animals indicated (*n*). Significant differences to pre-drug and post-drug controls are marked by superscript "a" ($P<0.05$).

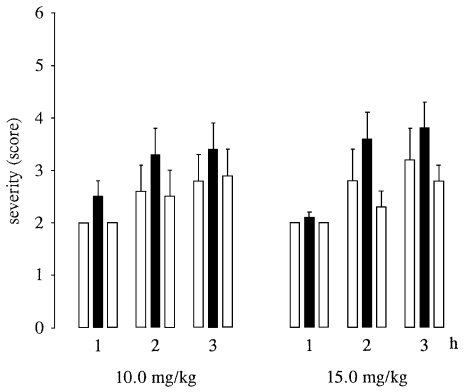

Fig. 2. Effect of Ro 25-6981 (10 and 15 mg/kg, i.p.) on severity of dystonia in mutant hamsters, which had passed the age of maximum severity of dystonia (post-max period; age: 53–58 days). The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd hour after administration of Ro 25-6981. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Data are shown as means ± S.E. of eight (10 mg/kg) or nine (15 mg/kg) dt^{sz} hamsters. For further explanation, see Fig. 1.

At a dose of 7.5 mg/kg, Ro 25-6981 did not provoke any behavioural effects. Moderate hyperlocomotion was caused by 10 and 12.5 mg/kg during the first hour after administration. Mutant hamsters treated with a dose of 15 mg/kg showed hyperlocomotion, which lasted for about 90 min. Salivation could be observed in four hamsters of the group treated with the high dose.

4. Discussion

The present data clearly show that the NR2B selective antagonist Ro 25-6981 failed to improve the dystonic syndrome in mutant hamsters, while previous examinations demonstrated significant antidystonic efficacy of various glutamate receptor antagonists which block both NR2A- and NR2B-containing NMDA receptors (Richter et al., 1991; Richter and Löscher, 1997, 1998). Together these results suggest that particularly overstimulation of NR2A-containing NMDA receptors is involved in the manifestation of a dystonic episode in mutant hamsters.

Polyamines activate NMDA receptors that incorporate NR2B, but not those which include the NR2A receptor subunit (Zhang et al., 1994; Gallagher et al., 1997). Since Ro 25-6981 unexpectedly aggravated dystonia in mutant hamsters at an age of most marked expression of the dystonic syndrome, it may be concluded that enhanced stimulation of the polyamine binding site of the NMDA receptor by increased spermine levels, previously found in brains of dt^{sz} mutants at an age of 32 days (Richter et al., 1998), is not critically involved in the dystonic syndrome.

This is in line with the observation that ifenprodil aggravated dystonia in mutant hamsters (Richter et al., 1998). Ifenprodil blocks the polyamine binding site, but is less selective in inhibiting NMDA receptors composed of NR1A and NR2B subunits than Ro 25-6981 (Fischer et al., 1997; Dingledine et al., 1999). Previously, the prodystonic effects of ifenprodil have been suggested to be related to other mechanisms of action (Richter et al., 1998), e.g., an increase of striatal dopamine efflux (Woodward and Harms, 1992). Indeed, the prodystonic effects of ifenprodil were more marked than those of Ro 25-6981 and were not restricted to the age of most marked expression of dystonia. During the postnatal development, the ratio of NR2A/NR2B increases in most regions of the rat brain (Wenzel et al., 1997). Thus, the disappearance of prodystonic effects of Ro 25-6981 in older dt^{sz} hamsters could be related to an ontogenetic decrease of NR2B-containing receptors.

NR2B-selective NMDA receptors antagonists may be useful agents for the treatment of Parkinson's disease because of the reduced risk to induce iatrogenic dyskinesias (Blanchet et al., 1999), but as indicated by the present data, these compounds seem not to provide a novel approach in the treatment of hereditary paroxysmal dyskinesias.

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References

Blanchet, P.J., Konitsiotis, S., Whittemore, E.R., Zhou, Z.L., Woodward, R.M., Chase, T.N., 1999. Differing effects of *N*-methyl-D-aspartate receptor subtype selective antagonists on dyskinesias in levodopa-treated 1-methyl-4-phenyl-tetrahydropyridine monkeys. J. Pharmacol. Exp. Ther. 290, 1034–1040.

Demirkiran, M., Jankovic, J., 1995. Paroxysmal dyskinesias: clinical features and classification. Ann. Neurol. 38, 571–579.

Dingledine, R., Borges, K., Bowie, D., Traynekis, S.F., 1999. The glutamate receptor ion channels. Pharmacol. Rev. 51, 1–61.

Fahn, S., Bressman, S.B., Marsden, C.D., 1998. Classification of dystonia. Adv. Neurol. 78, 1–10.

Fischer, G., Mutel, V., Trube, G., Malherbe, P., Kew, J.N.C., Mohacsi, E., Heitz, M.P., Kemp, J.A., 1997. Ro 25-6981, a highly potent and selective blocker of *N*-methyl-D-aspartate receptors containing the NR2B subunit. Characterization in vitro. J. Pharmacol. Exp. Ther. 283, 1285–1292.

Gallagher, M.J., Huang, H., Grant, E.R., Lynch, D.R., 1997. The NR2B-specific interactions of polyamines and protons with the *N*-methyl-D-aspartate receptor. J. Biol. Chem. 272, 24971–24979.

Gernert, M., Hamann, M., Bennay, M., Löscher, W., Richter, A., 2000. Deficit of striatal parvalbumin-reactive GABAergic interneurons and decreased basal ganglia output in a genetic rodent model of idiopathic paroxysmal dystonia. J. Neurosci. 20, 7052–7058.

Löscher, W., Fisher Jr., J.E., Schmidt, D., Fredow, G., Hönack, D., Iturrian, W.B., 1989. The *sz* mutant hamster: a genetic model of epilepsy or of paroxysmal dystonia? Mov. Disord. 4, 219–232.

Lynch, D.R., Shim, S.S., Seifert, K.M., Kurapathi, S., Mutel, V., Gallagher, M.J., Guttmann, R.P., 2001. Pharmacological characterization of

interactions of RO 25-6981 with the NR2B (epsilon 2) subunit. Eur. J. Pharmacol. 416, 185–195.

Nobrega, J.N., Richter, A., Jiwa, D., Raymond, R., Löscher, W., 1997. Alterations in *N*-methyl-D-aspartate receptor binding in dystonic hamster brains. Brain Res. 744, 161–165.

Nobrega, J.N., Raymond, R., Barlow, K., Hamann, M., Richter, A., 2002. Changes in AMPA receptor binding in an animal model of inborn paroxysmal dystonia. Exp. Neurol. 176, 371–376.

Parsons, C.G., 2001. NMDA receptors as targets for drug action in neuropathic pain. Eur. J. Pharmacol. 429, 71–78.

Ramson, R.W., Stec, N.L., 1988. Cooperative modulation of [^{3}H]MK801 binding to the *N*-methyl-D-aspartate receptor-ion channel complex by l-glutamate, glycine, and polyamines. J. Neurochem. 51, 830–836.

Richter, A., Löscher, W., 1997. Dextrorphan, but not dextromethorphan, exerts antidystonic effects in mutant dystonic hamsters. Brain Res. 745, 336–338.

Richter, A., Löscher, W., 1998. Pathophysiology of idiopathic dystonia: findings from genetic animal models. Prog. Neurobiol. 54, 633–677.

Richter, A., Löscher, W., 2002. Animal models of paroxysmal dystonia. Adv. Neurol. 89, 443–451.

Richter, A., Fredow, G., Löscher, W., 1991. Antidystonic effects of the NMDA receptor antagonists memantine, MK-801 and CGP 37849 in a mutant hamster model of paroxysmal dystonia. Neurosci. Lett. 133, 57–60.

Richter, A., Morrison, L.D., Löscher, W., 1998. Pharmacological and neurochemical examinations of polyamines in genetically dystonic (*dt*^{sz}) hamsters. Naunyn-Schmiedeberg's Arch. Pharmacol., Suppl., R99.

Vitek, J.L., Giroux, M., 2000. Physiology of hypokinetic and hyperkinetic movement disorders: model for dyskinesia. Ann. Neurol. 47, 131–140.

Wenzel, A., Fritschy, J.M., Mohler, H., Benke, D., 1997. NMDA receptor heterogeneity during postnatal development of the rat brain: differential expression of the NR2A, NR2B, and NR2C subunit proteins. J. Neurochem. 68, 469–478.

Woodward, J.J., Harms, J., 1992. The putative polyamine antagonists ifenprodil and SL 82.0715 enhance dopamine efflux from rat striatal slices independent of NMDA receptor activation. Eur. J. Pharmacol. 210, 265–270.

Zhang, L., Zheng, X., Pauqard, M.-C., Wang, A.P., Santchi, L., Friedman, L.K., Zukin, B.S., Bennett, M.V.L., 1994. Spermine potentiation of recombinant *N*-methyl-D-aspartate receptors is affected by subunit composition. Proc. Natl. Acad. Sci. 91, 10883–10887.