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Short communication

# Effects of the kynurenine 3-hydroxylase inhibitor Ro 61-8048 after intrastriatal injections on the severity of dystonia in the $dt^{sz}$ mutant

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**Abstract**

Striatal dysfunctions seem to play a key role in the pathophysiology of dystonia in the $dt^{sz}$ mutant hamster, a model of paroxysmal non-kinesigenic dyskinesia, in which stress precipitates dystonic episodes. Previous examinations have shown changes in kynurenic acid levels and antidystonic effects of the kynurenine 3-hydroxylase inhibitor 3,4-dimethoxy-*N*-[4-(3-nitrophenyl)thiazol-2-yl]benzenesulfon-amide (Ro 61-8048) after systemic treatment in $dt^{sz}$ hamsters. In the present study, intrastriatal injections of Ro 61-8048 (60–80 μg/hemisphere) significantly reduced the severity of dystonia in $dt^{sz}$ hamsters, suggesting that kynurenine 3-hydroxylase inhibitors may be interesting candidates for managing dyskinesias which are related to striatal dysfunction.


*Keywords:* Basal ganglia; Dyskinesia; Kynurenic acid; Kynurenine hydroxylase; Movement disorder; Striatum

## 1. Introduction

Kynurenic acid is an endogenous, astrocyte-derived antagonist of all ionotropic glutamate receptors with preferential affinity for the glycine site of the *N*-methyl-D-aspartate (NMDA) receptor (Schwarcz and Pellicciari, 2002). Furthermore, kynurenic acid can reduce the extracellular levels of glutamate and dopamine in the striatum, suggesting an important role in basal ganglia function (Moroni et al., 2005; Wu et al., 2007). In fact, changes in the kynurenine pathway were found in neurodegenerative basal ganglia disorders (Stone, 2001; Nemeth et al., 2006) and recently also in patients with focal dystonia (Hartai et al., 2007).

Dystonia, a common movement disorder characterized by sustained muscle contractions frequently causing twisting and repetitive movements or abnormal postures, is regarded as a basal ganglia disorder. Although the pathogenesis differs in various types of dystonia and dystonia-associated dyskinesias, several lines of evidence indicate that striatal alterations are involved in the dystonic syndrome (Zhuang et al., 2004). In the $dt^{sz}$ mutant hamster, an animal model of paroxysmal dystonia with striatal dysfunctions (Richter, 2005), increased kynurenic acid levels in dystonic brains were interpreted as an upregulation in response to an enhanced glutamatergic activity (Richter et al., 1996), because the kynurenine 3-hydroxylase inhibitor 3,4-dimethoxy-*N*-[4-(3-nitrophenyl)thiazol-2-yl]benzenesulfon-amide (Ro 61-8048) exerted antidystonic efficacy after intraperitoneal injections (Richter and Hamann, 2003). Ro 61-8048 exerts high activity as an inhibitor of kynurenine 3-hydroxylase (Carpenedo et al., 1999; Urenjak and Obrenovitch, 2000) and has been shown to significantly increase the extracellular kynurenic acid concentrations in different brain regions of mutant hamsters at antidystonic effective doses (Richter and Hamann, 2003). The aim of the present study was to examine if improvement of dystonia after systemic treatment with Ro 61-8048 is mediated by its effect within the striatum.

## 2. Methods

### 2.1. Animals

The present experiments were carried out in groups of homozygous $dt^{sz}$ mutant hamsters which were obtained by selective

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breeding as previously described (Richter and Löscher, 1998). All hamsters were born and kept under the same controlled and constant environmental conditions. The experiments were done in compliance with the German Animal Welfare Act (G 0160/05).

### 2.2. Induction of dystonic attacks and severity score of dystonia

The $dt^{sz}$ mutant hamster shows the characteristics of primary paroxysmal non-kinesigenic dyskinesia (in brief: paroxysmal dystonia) in humans (Richter, 2005). In this type of dystonia, episodes of generalized dystonic and choreoathetotic movements can be provoked by stress and last up to several hours. In mutant hamsters, dystonic attacks can be reproducibly induced by a triple stimulation technique, i.e., stressful stimuli consisting of (1) taking the animal from its home cage and placing it on a balance, (2) injection of saline/vehicle (or of drugs), and (3) placement of the animal in a new plastic cage. Thereafter, $dt^{sz}$ hamsters develop a sequence of abnormal movements and postures. The severity of dystonia can be rated by the following score-system (Richter and Löscher, 1998): stage 1, flat body posture; stage 2, facial contortions, rearing with forelimbs crossing, disturbed gait with hyperextended forepaws; 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. The individual maximum stage of dystonia is usually reached within 3 h after the induction of dystonia. Thereafter, the hamsters recover within 2–5 h.

In the present study, all animals were examined for the presence of dystonia after weaning at the age of 21 days by the triple stimulation procedure 3 times per week until the animals exhibited constant individual severity scores and latencies to onset of unequivocal dystonic symptoms (stage 2) before the implantation of guide cannulae for microinjections.

### 2.3. Surgery and microinjections

Permanent stainless-steel guide cannulae (length: 12.2 mm, inner diameter: 0.45 mm) were chronically implanted into the left and right dorsal striatum in groups of 6–9 mutant hamsters at an age of 30–32 days for bilateral microinjections. In anaesthetized animals (pentobarbital 60 mg/kg), bilateral guide cannulae were implanted into the striatum according to the following coordinates (relative to bregma in mm): AP +1.5, L +/− 2.3, V −2.7. The guide cannulae were held in place with anchor screws and dental acrylic cement on the skull surface. Two to 3 days after surgery, the microinjections into the striatum of freely moving hamsters were performed using an injection cannula (length of 13.2 mm, inner diameter of 0.2 mm), which was inserted through the guide cannula into the left and right striatum (V: −3.7 mm to bregma). The drug-solutions or vehicle (for pre- and post-drug recordings) were bilaterally delivered at a rate of 0.1 µl/min. The injection cannula was removed 5 min after the administration.

### 2.4. Pharmacological treatments

The drug effects on severity of dystonia were examined in groups of 6–9 dystonic hamsters at an age of maximum severity of dystonia (33–42 days). Dystonic attacks were induced by the procedure of triple stimulation, as described above, including injections of vehicle (control trials) or Ro 61-8048 (3,4-

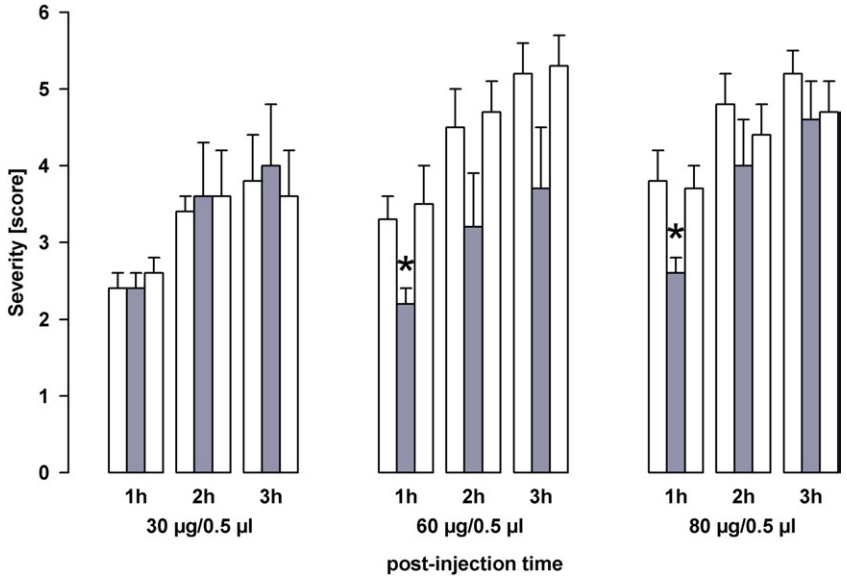


Fig. 1. Effects of intrastriatal injections of the kynurenine 3-hydroxylase inhibitor Ro 61-8048 (30, 60 and 80 µg/hemisphere) on severity of dystonia in mutant hamsters. The white bars in each set of three bars indicate the control values obtained 2 days before (pre-drug control) drug administration (first white bar) and 2 days after (post-drug control) drug administration (second white bar). The grey bar refers to the day of drug administration in the same animal groups. The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd hour post-injections (p. inj.) of vehicle or Ro 61-8048, reflecting the progression of dystonia in $dt^{sz}$ hamsters after treatment with the active compound and during control recordings. Asterisks indicate significant improvements of dystonia in comparison to the pre- *and* post-drug control (*$P<0.05$). Data are shown as means+S.E. of 5 (30 µg), 6 (60 µg) and 9 (80 µg) dystonic hamsters.

dimethoxy-*N*-[4-(3-nitrophenyl)thiazol-2-yl]benzenesulfonamide) into the left and right striatum per microinjections. Ro 61-8048, kindly provided by Hoffmann-La Roche (Basel, Switzerland), was freshly dissolved in isotonic saline (pH 7.7) prior the experiments. Solubility of Ro 61-8048 at appropriate pH values was limited to a concentration of 80 μg/0.5 μl. The injection volume was 0.5 μl/hemisphere. For pre- and post-drug control recordings the animals received the same volume of vehicle. Since the individual maximum stage of dystonia (score rating system see above) is usually reached within 3 h, the hamsters were observed for 3 h. Two days before drug testing, a control trial was undertaken by injections of the vehicle before the latencies to the different stages and the severity of the dystonic attacks were noted after placing the animals in the new cage (pre-drug control). Two days later, the drug was administered in the same group of animals and the latencies and severity were noted. Furthermore, animals were observed for central adverse effects, e.g., locomotor activity. As described for pre-drug-controls, a control trial with vehicle was done 2 days after drug treatment (post-drug control). The examiner rating the severity of dystonia was blind to the treatment condition of the animals. Hamsters that differed in the maximum severity of dystonia reached during the 3-h-observation period in the pre-drug and post-drug control trials by more than two stages were omitted from the drug evaluation (4 out of 24). All control and drug trials were done at the same time of the day between 9:00 and 12:00 a.m. After the experiments, the positions of the tip of the guide cannulae were determined as previously described (Sander and Richter, 2007).

### 2.5. Statistics

The significance of differences in severity of dystonia and in latencies to onset of dystonia between control trials (pre- and post-drug) and drug trial in the same group of animals was calculated by the Friedman test and, if there was found a significant difference (at least $P<0.05$), the Wilcoxon signed rank test for paired replicates was used.

## 3. Results

Ro 61-8048 significantly reduced the severity of dystonia during the first hour after bilateral intrastriatal injections of 60 and 80 μg per hemisphere (Fig. 1) and tended to delay the onset of dystonia (Table 1). 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. At a lower dose of 30 μg, Ro 61-8048 failed to exert any effects on the severity of dystonia, but significantly increased the latency to onset of dystonic attacks. Ro 61-8048 caused a moderate hypolocomotion from 5 to 25 min after injections, followed by a hyperlocomotion which disappeared about 60 min after administration. At the higher doses of 60 and 80 μg, the hamsters exhibited a moderate ataxia which lasted for about 30 min.

Table 1
Effects of intrastriatal injections of Ro 61-8048 on latency to onset of dystonia in $dt^{sz}$ mutant hamsters

| Dose | Latency (min) | | | (*n*) |
|---|---|---|---|---|
| (μg/hemisphere) | Pre-drug | Drug | Post-drug | |
| 30.0 | 6.0±0.7 | 18.2±2.5[a] | 4.2±0.9 | 5 |
| 60.0 | 5.8±0.5 | 12.2±2.5 | 6.2±0.5 | 6 |
| 80.0 | 6.1±0.8 | 13.2±3.1 | 7.4±1.4 | 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 [a]($P<0.01$).

## 4. Discussion

The antidystonic effects shown in this study after intrastriatal injections of the kynurenine 3-hydroxylase inhibitor Ro 61-8048 are in line with previous findings of beneficial effects of this compound after systemic treatment in mutant hamsters (Richter and Hamann, 2003). The present data suggest that improvement of dystonia after systemic treatment with Ro 61-8048 is at least in part mediated by striatal effects. As previously observed after systemic administration (Richter and Hamann, 2003), there was a fast onset of action after striatal injections of Ro 61-8048. However, the reduction of the severity of dystonia and the behavioral effects lasted for only 1 h after intrastriatal microinjections, while systemic treatment improved dystonia during the whole 3-h-observation period. In view of the mode of action of Ro 61-8048, the time-course of its effects after striatal injections was an unexpected finding.

As shown by Moroni et al. (2005), a single injection of Ro 61-8048 (40 mg i.p.) provoked a slowly appearing, long lasting (at least 4 h) increase in kynurenic acid concentrations that reached a maximum after 2 h in the rat striatum. Previous studies have shown that Ro 61-8048 induces two- to threefold increases of kynurenic acid in brain homogenates of $dt^{sz}$ hamsters 90 min after intraperitoneal injections of the antidystonic effective dose of 100 mg/kg (Richter and Hamann, 2003). The striatal extracellular kynurenic acid levels were found to progressively increase from about 3 to 8 nM within the first hour and a peak concentration of 31 nM was reached 3 h after intraperitoneal administration of Ro 61-8048 in rats (Urenjak and Obrenovitch, 2000). Since there is no invasion phase after intrastriatal injections, peak levels of kynurenic acid as well as a following decline are probably reached earlier than after systemic treatment. Nevertheless, the fast disappearance of antidystonic effects, observed in the present study after intrastriatal injections of 60 and also of 80 μg, cannot be explained by the present data.

Carpenedo et al. (2002) suggested that Ro 61-8048 has direct antiglutamatergic effects that are unrelated to a rise in kynurenic acid which could explain the fast onset of antidystonic efficacy and of behavioral effects. However, this is probably not relevant for the antidystonic effects, because various NMDA receptor antagonists, including the glycine/NMDA receptor ligand (+)-HA-966, failed to reduce the severity of dystonia after striatal injections in mutant hamsters (Sander and Richter, 2007). Thus,

it is unlikely that the antidystonic efficacy of Ro 61-8084-induced increases of kynurenic acid is solely related to anti-glutamatergic effects. Recently, intrastriatal infusion of very low concentrations of kynurenic acid has been shown to reduce extracellular glutamate and also dopamine levels in the rat striatum by inhibition of alpha7 nicotinic acetylcholine receptors on glutamatergic afferents (Rassoulpour et al., 2005; Wu et al., 2007). This mechanism might be important for the improvement of levodopa-induced dyskinesias in parkinsonian monkeys (Samadi et al., 2005) and also for the antidystonic efficacy of Ro 61-8048. There is evidence that increased striatal dopamine levels are critically involved in the induction of dystonia in the hamster model (Hamann and Richter, 2004; Rehders et al., 2000).

The present data together with previous studies suggest that kynurenine 3-hydroxylase inhibitors may be interesting candidates for managing dyskinesias which are related to striatal dysfunction.

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