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Short communication

Antidystonic efficacy of γ-aminobutyric acid uptake inhibitors in the dt^{sz} mutant

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Abstract

In the dt^{sz} mutant hamster, a model of paroxysmal dyskinesia in which dystonic episodes occur in response to stress, previous studies suggested that retarded development of γ-aminobutyric acid (GABA)ergic inhibition plays a critical role in the pathogenesis. In the present study, we therefore examined the effects of selective GABA uptake inhibitors on severity of dystonia in dt^{sz} hamsters. R(-)*N*-(4,4-di(3-methylthien-2-yl)-but-3-enyl) nipecotic acid hydrochloride (tiagabine, 5–20 mg/kg i.p.) and 1-[2-[[(diphenylmethylene) imino]oxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride (NNC-711, 1–10 mg/kg i.p.) significantly reduced the severity of dystonia. These data suggest that GABA uptake inhibitors may provide novel therapeutic approaches for paroxysmal dyskinesias.

Keywords: Dyskinesia; GABA (γ-aminobutyric acid); Movement disorder

1. Introduction

The re-uptake of γ-aminobutyric acid (GABA) from the synaptic cleft represents an important mechanism in the regulation of GABAergic activity. Extracellular GABA is rapidly removed via different subtypes of high-affinity, sodium-dependent GABA transporters located on presynaptic terminals and surrounding astroglial cells (Kanner, 1994; Kubova, 1999). The GABA transporter subtype 1 (GAT-1) is the most abundant one in the mammalian brain, accounting for approximately 70% of GABA uptake (Borden, 1996; Kubova, 1999). GABAergic activity is known to be enhanced by highly selective GAT-1 inhibitors, such as the antiepileptic drug tiagabine (Borden et al., 1994; Krogsgaard-Larsen et al., 2000).

Decreased GABAergic inhibition appears to be involved in several neurological disorders, such as in epilepsy (Rogawski and Löscher, 2004). Dependent on the phenotypic and genotypic subtype, idiopathic dystonias are probably related to various brain abnormalities as reflected by the distinct response to drugs (Fahn, 1995). However, beneficial effects after acute intake of benzodiazepines in different types of dystonia and decreased intra- and subcortical inhibition suggest that GABAergic disinhibition may be critically involved in the pathophysiology of this movement disorder (Fahn, 1995; Levy and Hallett, 2002). The dystonic syndrome, regarded as a basal ganglia disorder, is characterized by contractions of opposing muscles leading to twisting movements and abnormal postures. In paroxysmal non-kinesiogenic dyskinesias (briefly: paroxysmal dystonia), dystonic episodes (often associated with choreoathetosis) occur in response to stress (Nardocci et al., 2002). In the dt^{sz} mutant hamster, an animal model of this type of paroxysmal dystonia (Richter and Löscher, 1998), previous neurochemical, immunhistochemical and electrophysiological investigations clearly indicated that a retarded development of the GABAergic inhibition is important in the dt^{sz} mutant (for review: Richter and Löscher, 1998; Richter, 2005).

The previous observations in dystonian patients and in the hamster model of paroxysmal dystonia prompted us to examine if NNC-711 (1-[2-[[(diphenylmethylene)imino]oxy] ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride) and tiagabine, two highly selective and potent GAT-1 inhibitors (Borden et al., 1994), exert antidystonic effects in the dt^{sz} mutant hamster and might be candidates for the treatment of paroxysmal dystonia.

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2. Materials and methods

Groups of 6–10 dt^{sz} mutant hamsters, used for the present study, were obtained by selective breeding as described previously (Richter and Löscher, 1998). The experiments were done in mutant hamsters at an age of maximum expression of dystonia between 32 and 42 days. The animals were kept under controlled and constant environmental conditions (21–23 °C, 13-h light cycle). The hamsters had ad libitum access to standard diet and water. The experiments were done in compliance with the German Animal Welfare Act. In mutant hamsters, dystonic attacks can be induced by mild stress, such as handling. The dystonic attacks were induced by a triple stimulation technique (Richter and Löscher, 1998), 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 body weight) of vehicle (NNC-711: saline; tiagabine: 0.3% Tween) or of the GAT-1 inhibitors, and (3) placement of the animal in a new plastic cage. The dt^{sz} hamsters develop a sequence of abnormal movements and postures, allowing to rate the severity of dystonia by the following score-system (Richter and Löscher, 1998): stage 1, flat body posture; stage 2, 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. 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. The latency to onset of dystonia was determined as the time to the first unequivocal signs of the dystonic attacks (stage 2). The rater of the severity of dystonia was blind to the treatment condition of the animals. The locomotor activity was determined by a score system, as used in previous studies (e.g., Hamann and Richter, 2002). Other adverse effects were not quantified. Pre- and post-drug control trials were undertaken 2 days before and 2 days after drug testing. All control and drug trials were done at the same time of the day between 9:00 and 12:00 a.m. NNC-711 (Sigma, Germany) was dissolved in saline and tiagabine (Sigma, Germany) was dissolved in 0.3% Tween 80 (Sigma, Germany).

The significance of differences in severity of dystonia and in latencies to onset (stage 2) 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

Tiagabine delayed the progression of dystonia at doses of 5 mg/kg and 20 mg/kg i.p., reflected by a significant reduction of the severity of dystonia during the first or second hour after administration (Fig. 1). At a dose of 20 mg/kg, tiagabine delayed the progression and also decreased the maximum severity of dystonia reached at the end of the observation period (3 h), i.e., all mutant hamsters showed only moderate dystonic symptoms after treatment. Tiagabine did not exert significant effects on the latency to onset of dystonia (not illustrated), indicating a retarded onset of action of tiagabine. Central

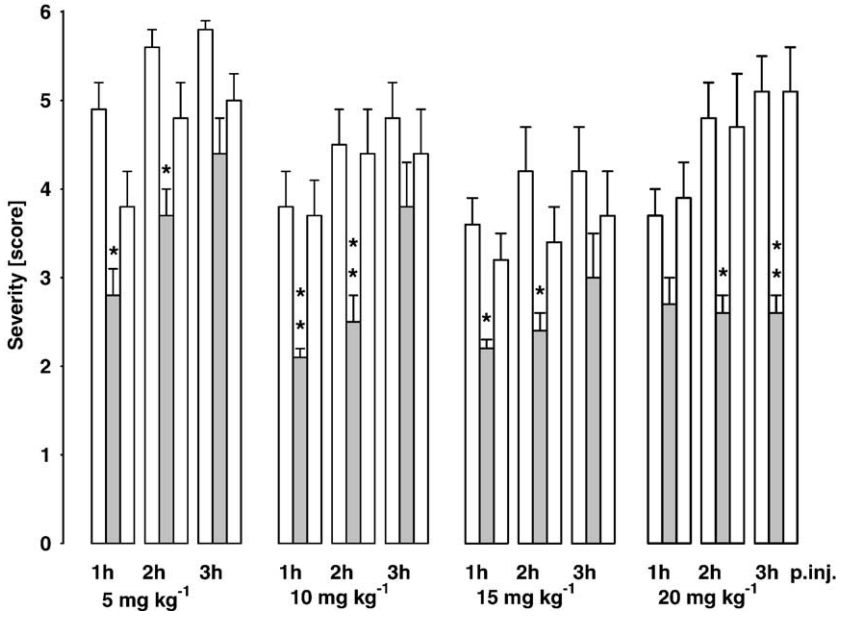

Fig. 1. Effect of tiagabine (5, 10, 15 and 20.0 mg/kg i.p.) on severity of dystonia in dt^{sz} mutant hamsters. The white bars in each set of three bars indicate the control values obtained when animals were injected with vehicle two days before (pre drug control) drug administration (first white bar) and two 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 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 h post injection (p. inj.) of vehicle or tiagabine, reflecting the progression of dystonia in dt^{sz} hamsters during control recordings and after treatment with the active compound. Asterisks indicate significant improvement of dystonia in comparison to the pre- *and* post-drug control (*$P<0.05$; **$P<0.01$). Data are shown as means+S.E.M. of 9 (5, 15, 20 mg/kg) or 10 (10.0 mg/kg) animals.

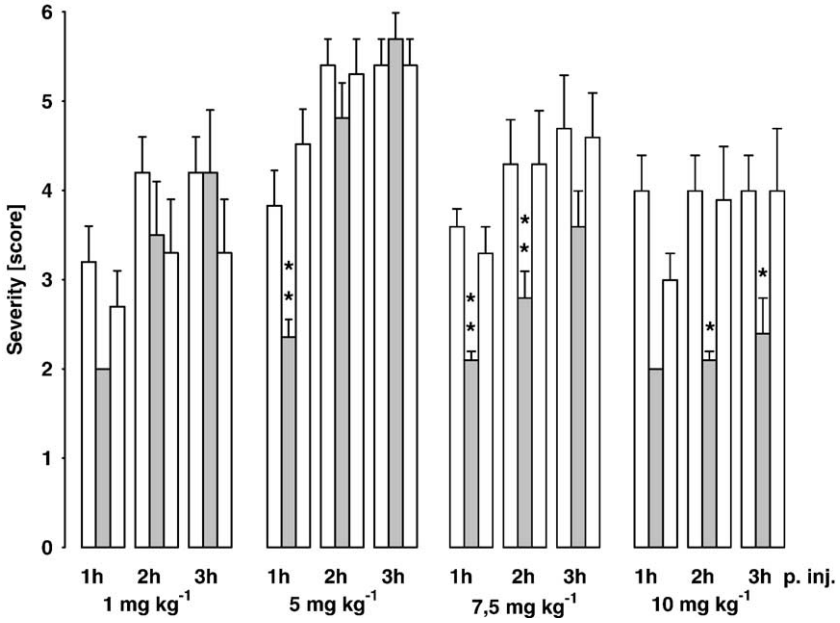

Fig. 2. Effect of NNC-711 (1, 5, 7.5 and 10 mg/kg i.p.) on severity of dystonia in dt^{sz} mutant hamsters. The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd h post injection (p. inj.) of vehicle (white bars) or NNC-711 (grey bars). Asterisks indicate significant reductions of the severity of dystonia in comparison to the pre- *and* post-drug control (*$P<0.05$; **$P<0.01$). Data are shown as means+S.E.M. of 6 (1 mg/kg), 7 (10 mg/kg), 8 (5 mg/kg) or 9 (7.5 mg/kg) animals.

adverse effects were moderate to unequivocal hyperlocomotion and at higher doses of 10–20 mg/kg moderate to unequivocal ataxia. These adverse effects occurred after 10 to 20 min and lasted for 60 to 150 min after injection of the different doses.

NNC-711 did not exert significant effects at a dose of 1 mg/kg, but retarded the progression of dystonia at doses of 5 and 7.5 mg/kg i.p. (Fig. 2). A significant reduction of the maximum severity of dystonia became evident after treatment with 10 mg/kg NNC-711 (similar to tiagabine at a dose of 20 mg/kg). NNC-711 failed to exert dose-dependent effects on the latency to onset of dystonic episodes at the tested doses (not illustrated). Adverse effects, observed at doses of 5–10 mg/kg NNC-711, were comparable to those of tiagabine (see above).

4. Discussion

The present study demonstrates for the first time significant antidystonic effects of selective GABA uptake inhibitors in an animal model of paroxysmal dystonia. These data are in line with previous observations of beneficial effects of GABApotentiating drugs in the dt^{sz} mutant hamster (Richter, 2005). In comparison to previously examined compounds which increase brain GABA levels, i.e., gabapentin and aminooxyacetic acid (Richter and Löscher, 1998, 1999), tiagabine and NNC-711 exerted higher antidystonic efficacy. At the GAT-1, NNC-711 shows higher potency than tiagabine (Borden et al., 1994). In accordance, NNC-711 improved dystonia and caused adverse effects at lower doses than tiagabine in the present study.

Both GAT inhibitors, tiagabine and NNC-711, did not completely prevent the occurrence of dystonic episodes in the dt^{sz} mutant probably because of a delayed onset of action, as indicated by the lack of significant effects on the latency to onset of dystonia. Indeed, the mechanism of action let assume a retarded onset of action and that long-term treatment with GAT inhibitors may be more effective than acute trials. Examinations in animal models of epilepsy have shown that chronic treatment with tiagabine produces tolerance to the sedative and ataxic effects, but not the anticonvulsant effects (Suzdak, 1994). In patients with epilepsy, long-term safety studies of tiagabine showed mild to moderate adverse effects, such as dizziness, asthenia and nervousness, and no evidence of tolerance for anticonvulsant efficacy (LaRoche and Helmers, 2004; Schachter, 2001). Antiepileptic drugs, such as tiagabine, are commonly prescribed for nonepileptic conditions (Rogawski and Löscher, 2004). However, there are obviously no case reports about empirical trials with GAT inhibitors in patients with dystonia or dyskinesias, although benzodiazepines are commonly used for acute treatment during periods of aggravation of dystonia (Fahn, 1995). Benzodiazepines, also effective in the dt^{sz} mutant (Richter and Löscher, 1998), are less suitable for long-term treatment because of the well-known development of tolerance.

In accordance with the present finding of beneficial effects of GAT-1 inhibitors, a deficit of striatal GABAergic interneurons was found to play a critical role in the dystonic syndrome of mutant hamsters (Gernert et al., 2000; Hamann and Richter, 2002). The GAT-1 subtype is predominant in the striatum and is located on GABAergic interneurons (Augood et al., 1995; Borden, 1996). GAT-1 inhibitors have been shown to increase extracellular GABA levels in the striatum (Anderson and DiMicco, 1992). Interestingly, a significant decrease of striatal GABA levels was recently detected in patients with focal dystonia by using a novel noninvasive magnetic resonance spectroscopy method (Levy and Hallett, 2002). Together with pathophysiological findings in dystonian patients and the dt^{sz} mutant, the present data suggest that GAT-1 inhibitors, such as

tiagabine, might represent interesting candidates for the long-term therapy of dystonias and dyskinesias.

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