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The carbonic anhydrase inhibitor acetazolamide exerts antidystonic effects in the dt^{sz} mutant hamster

Angelika Richter*, Melanie Hamann

Institute of Pharmacology and Toxicology, School of Veterinary Medicine, FU Berlin, Koserstrasse 20, 14195 Berlin, Germany

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Abstract

Previous studies suggested an involvement of γ-aminobutyric acid (GABA)-mediated excitation by an enhanced efflux of bicarbonate ions in addition to retarded development of GABAergic inhibition in the syndrome of dt^{sz} mutant hamsters, a model of paroxysmal dyskinesia in which dystonic episodes occur in response to stress. Acetazolamide blocks bicarbonate regeneration in neurons and can thereby reduce GABA-mediating excitation without affecting GABA-mediated inhibition. In the present study, the effects of acetazolamide (15–60 mg/kg, i.p.) on severity of dystonia were therefore examined in dt^{sz} hamsters. Acetazolamide significantly reduced the severity of dystonia at a dose of 60 mg/kg. These data are in line with several case reports from patients with paroxysmal dystonia, suggesting that acetazolamide can be useful in the treatment of this movement disorder. The mechanism of the antidystonic efficacy of acetazolamide has to be examined by further studies.

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

1. Introduction

The carbonic anhydrase inhibitor acetazolamide is known to improve periodic neurological disorders such as epilepsy and episodic ataxia (Gordan, 1998; Resor et al., 1995). There are also case reports about beneficial effects of acetazolamide in different types of paroxysmal dyskinesias, e.g., in patients with paroxysmal non-kinesigenic dyskinesia (in brief: paroxysmal dystonia) in which episodes of dystonic and choreoathetotic movements last up to several hours and can be provoked by stress (Bressman et al., 1988). Acetazolamide has, so far, not been examined in animal models of paroxysmal dyskinesias.

In the dt^{sz} mutant hamster, an animal model of paroxysmal dystonia, basal ganglia dysfunctions have been shown to be critically involved (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, 2002). The severity of dystonia shows an age-dependent time-course with a maximum at an age of 30–40 days of life. Then, the severity decreases until a complete disappearance of the stress-inducible disorder occurs at an age of about 10 weeks (e.g., Richter and Löscher, 1998). While acute treatment with various GABA-potentiating drugs, including muscimol, benzodiazepines, gabapentin and phenobarbital exerted antidystonic efficacy, chronic administration of phenobarbital worsened dystonia in the dt^{sz} mutant and caused a notable delay of the age-dependent regression of dystonia (Richter and Löscher, 1999, 2000, 2002). In view to different observations, e.g., a rapid decline of the severity of dystonia when phenobarbital was withdrawn, its prodystonic effect has been explained by a GABA-mediated excitation under pathological conditions in dt^{sz} hamsters.

The main inhibitory transmitter GABA becomes under different circumstances exciting on neurons, such as in the

* Corresponding author. Tel.: +49 30 838 54317; fax: +49 30 838 53112.
E-mail address: richtera@zedat.fu-berlin.de (A. Richter).

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immature nervous system or during intense activation of the $GABA_A$ receptor by barbiturates. Efflux of intracellular bicarbonate, which is formed by carbonic anhydrase, through $GABA_A$ activated channels is a major factor underlying depolarising responses by GABA or barbiturates (Staley et al., 1995). Inhibition of bicarbonate regeneration by acetazolamide has been shown to block $GABA_A$ receptor-mediated depolarisation without a reduction of GABA-mediated hyperpolarisation of neuronal membranes. This may be an important mechanism for the beneficial effects of acetazolamide in episodic neuronal disorders (Staley et al., 1995; Rivera et al., 1999). With regard to previous observations in mutant hamsters (see above), the effects of acetazolamide on the severity of paroxysmal dystonia were examined in the present study.

2. Materials and methods

Groups of 9–11 dt^{sz} mutant hamsters used for the present study were obtained by selective breeding as described previously (Löscher et al., 1989). The experiments were done in mutant hamsters at an age of maximum expression of dystonia between 30 and 40 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 (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 (cremophore 10%) or of acetazolamide, 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, 2002): 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. Locomotor activity was determined by a score system as used in previous studies (e.g., Hamann and Richter, 2002). Pre- and post-drug control trials were undertaken 2 days before and 2 days after drug testing. Acetazolamide (Sigma, Steinheim, Germany) was suspended in 10% cremophore. 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

Acetazolamide exerted only moderate antidystonic effects at doses of 15 and 30 mg/kg, i.p. (Fig. 1). Thus, a significant reduction of the severity of dystonia was restricted to the first (15 mg/kg) or second hour (30 mg/kg) after administration, suggesting a delayed progression of dystonia. The onset of a dystonic attack was however not significantly changed after treatment of 15 or 30 mg/kg (Table 1). With regard to a lack of significant increases of the latency to onset, the statistical significance during the first h after administration of 15 mg/kg is probably accidental. Unequivocal beneficial effects of acetazolamide became evident at a higher dose of 60 mg/kg i.p. As shown in Fig. 1, acetazolamide significantly reduced the maximum severity of dystonia reached at the end of the observation period (3 h). Furthermore, the latency to onset of dystonia was significantly increased at this higher dose (Table 1). Central adverse effects were moderate (30 mg) to unequivocal hypolocomotion (not quantified) which lasted for 2 h

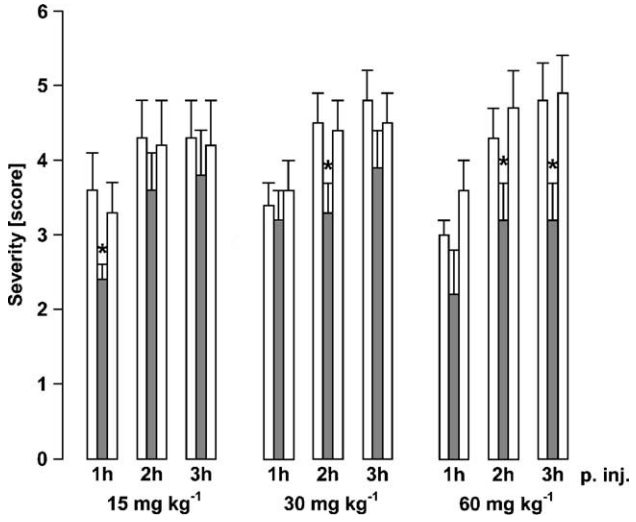

Fig. 1. Effect of acetazolamide (15.0, 30.0 and 60.0 mg/kg, i.p.) on severity of dystonia in mutant hamsters. The white bars in each set of three bars indicate the control values obtained 2 days before (pre-drug control) drug administration (first white bar) and 2 days after (post-drug control) drug administration (second white bar). The grey bar refers to the day of drug administration in the same animal groups. The 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 acetazolamide, 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 (*$P<0.05$). Data are shown as means+S.E. of 9 (15.0 mg/kg), 11 (30.0 mg/kg) or 10 (60.0 mg/kg) animals.

Table 1
Effects of acetazolamide on the latency to onset of dystonia

Dose (mg/kg)	Age (days) at drug trial	Latency (min)			(*n*)
		Pre-drug	Drug	Post-drug	
15.0	39	6.2±0.8	14.1±4.0	10.7±1.8	9
30.0	36	9.8±1.3	11.3±2.0	8.1±2.4	11
60.0	35	10.0±2.0	31.1±12.1[a]	5.2±1.0	10

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

(30 mg) or 3 h (60 mg/kg). At a dose of 15 mg/kg, acetazolamide did not cause any central adverse effects.

4. Discussion

Acetazolamide clearly improved paroxysmal dystonia in mutant hamsters at a dose of 60 mg/kg, which has been found to be effective in animal models of epilepsy and episodic ataxia (Hamada et al., 2001; Herson et al., 2003). Acetazolamide is a well-tolerated adjunctive agent in the pharmacotherapy of epilepsy (Reiss and Oles, 1996). Apart from a case report about aggravation of paroxysmal exertion-induced dyskinesia (Guimaraes and Vale Santos, 2000), several case reports suggested that acetazolamide may be suitable for the treatment of paroxysmal non-kinesigenic dystonia (Bressman et al., 1988). This is supported by the present data.

The pathophysiology of this often intractable movement disorder is not well understood. It has been hypothesized that channelopathies are important in the pathogenesis of inherited paroxysmal dyskinesias (Nardocci et al., 2002). In dt^{sz} mutant hamsters, sodium channel function seems to be unaltered, while there is clear evidence for an age-dependent GABAergic disinhibition of striatal efferent pathways probably related to a deficit of GABAergic interneurons (Gernert et al., 2000; Siep et al., 2002; Richter and Löscher, 2002). GABA controls developmental expression of the K^+–Cl^- co-transporter 2 (KCC2), a neuron-specific chloride exporter (Köhling, 2002; Payne et al., 2003). The role of the KCC2 in the pathophysiology of paroxysmal dyskinesias is unexplored. KCC2 supports a chloride gradient across the membrane which enables a chloride influx, thereby a hyperpolarisation of membranes when $GABA_A$ receptors are activated (Rivera et al., 1999). A low expression of KCC2, found in brain tissue of an animal model of epilepsy and immature neurons, is related to $GABA_A$ receptor-mediated excitation (Köhling, 2002; Payne et al., 2003). In view of a retarded development of the GABAergic system in the dt^{sz} mutant, indicated by a deficit of GABAergic interneurons in the striatum (Gernert et al., 2000), it may be speculated that the expression of KCC2 is also reduced in these animals. A resulting imbalance towards enhanced GABA-mediated depolarisation can be obviously intensified by chronic treatment with phenobarbital, while hyperpolarisation is enhanced by acute administration of GABAmimetics (Staley et al., 1995). This could explain previous pharmacological observations with phenobarbital, i.e., an improvement of dystonia after acute administration but an aggravation during chronic treatment in mutant hamsters (Richter and Löscher, 2002), and the present finding of antidystonic effects of acetazolamide, because this carbonic anhydrase inhibitor can reduce $GABA_A$ receptor-mediated excitation by a decrease of intracellular bicarbonate anions. This mechanism has been suggested to be essential for the therapeutic effects of acetazolamide in other episodic disorders (Herrero et al., 2002).

Thus, the present finding of antidystonic effects of acetazolamide should initiate further examinations of KCC2 expression and $GABA_A$ receptor-mediated chloride currents in mutant hamsters. The present data indicate that acetazolamide and possibly other carbonic anhydrase inhibitors are useful agents in the therapy of paroxysmal dystonia.

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