Neuroscience 157 (2008) 188–195

EXTRACELLULAR AMINO ACID LEVELS IN THE STRIATUM OF THE *dt*^{sz} MUTANT, A MODEL OF PAROXYSMAL DYSTONIA

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Abstract—The pathophysiology of idiopathic dystonia is still unknown, but it is regarded as a basal ganglia disorder. Previous studies indicated an involvement of a striatal GABAergic disinhibition and a cortico-striatal glutamatergic overactivity in the manifestation of stress-inducible dystonic episodes in the *dt*^{sz} hamster, a model of idiopathic paroxysmal dystonia. These investigations were carried out postmortem or in anesthetized animals. In the present study, *in vivo* microdialysis in conscious, freely-moving *dt*^{sz} and non-dystonic control hamsters was used to examine the levels of GABA, aspartate, glutamate, glutamine, glycine and taurine in each animal during following conditions: (1) at baseline in the absence of dystonia, (2) during an episode of paroxysmal dystonia precipitated by stressful stimuli, (3) during a recovery period and (4) at baseline after complete recovery. In comparison to non-dystonic controls, which were treated in the same manner as the dystonic animals, no differences could be detected under basal conditions. The induction of a dystonic episode in mutant hamsters led to higher contents of glycine in these animals in comparison to stressed but non-dystonic controls. Significant changes of glycine levels within the animal groups were not detected. The levels of the excitatory amino acids glutamate, glutamine and aspartate as well as the levels of the inhibitory amino acids GABA and taurine did not differ between the animal groups or between the periods of measurement. The higher levels of glycine might contribute to the manifestation of paroxysmal dystonia in *dt*^{sz} hamsters, although unaltered glutamate, glutamine and aspartate levels do not support the hypothesis of a critical involvement of a cortico-striatal overactivity. It seems that a deficiency of GABAergic interneurons, found by previous immunohistochemical examinations, does not lead to reduced extracellular GABA levels in the striatum.

Key words: basal ganglia, caudate putamen, hamster, microdialysis, movement disorders, dyskinesia.

Dystonias are characterized by sustained muscle co-contractions and frequently cause twisting, repetitive movements or abnormal postures (Fahn, 1988). Genetic analyses, experiments in animal models and imaging studies in patients indicated striatal disruptions of neurotransmitter communication, including the inhibitory amino acid GABA and the dopaminergic and cholinergic systems (Breakefield et al., 2008; Levy and Hallett, 2002). The presumably heterogeneous pathogenesis of different forms of primary dystonia is mirrored by contradictory pharmacological and neurochemical findings across different subtypes of this neurological syndrome. GABA-potentiating substances like benzodiazepines are one of the most effective therapeutics in patients with generalized dystonia (Jancovic, 2006). Although the excitatory amino acid glutamate plays a critical role in regulating the physiological processes of movements, studies investigating its pathophysiological role are rare (Richter and Löscher, 1998). Neither in dystonic patients nor in different animal models of dystonia are data on measurements of extracellular levels of amino acids and metabolites within the striatum by *in vivo* microdialysis available so far.

Several lines of evidence suggested that changes of inhibitory and excitatory amino acids play a role in the pathophysiology of the dystonic syndrome in the *dt*^{sz} mutant hamster, an animal model of paroxysmal non-kinesigenic dyskinesia (PNKC, briefly: paroxysmal dystonia). Suitable animal models of inborn dyskinesias, which are useful for giving insights into the underlying mechanisms of this movement disorder and for preclinical drug testing, are rare (for review: Richter and Löscher, 1998; Raike et al., 2005; Richter, 2005). The *dt*^{sz} hamster shows all clinical and pharmacological characteristics of PNKC in humans, in which dystonia is a predominant symptom. In this subtype of dystonia, episodes of generalized dystonia can be provoked by stress and last up to several hours (Nardocci et al., 2002; Raike et al., 2005; Richter, 2005).

Previous neurochemical, immunohistochemical and electrophysiological investigations clearly demonstrated striatal alterations in the *dt*^{sz} mutant hamster. The striatum plays a pivotal role in the processing of neuronal activity by a circuit involving the cortex, the basal ganglia, and the thalamus. The major input to the striatum derives from excitatory afferents from the cortex. As shown by electrophysiological studies, GABAergic interneurons represent the major inhibitory source within the striatum, but the extracellular amount of GABA provided by striatal GABAergic interneurons in comparison to GABAergic spiny projection neurons, which regulate the striatal output, is not known so far (Plenz, 2003; Tepper et al., 2004). In the *dt*^{sz} mutant hamster, a reduced density of striatal GABAergic interneurons can explain moderate decreases of GABA levels in tissue homogenates, an enhanced activity of GABAergic projection neurons and a reduced neuronal activity in a basal ganglia output structure in the *dt*^{sz} hamster (Löscher and Hörstermann, 1992; Gernert et al., 1999, 2000; No-

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Abbreviations: ANOVA, analysis of variance; HPLC, high-pressure liquid chromatography; NMDA, *N*-methyl-D-aspartate; PNKC, paroxysmal non-kinesigenic dyskinesia.

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doi:10.1016/j.neuroscience.2008.08.059

brega et al., 2002; Köhling et al., 2004; Hamann et al., 2005; Sander et al., 2006). Furthermore, a cortico-striatal glutamatergic overactivity may contribute to the manifestation of dystonic episodes (Nobrega et al., 2002; Köhling et al., 2004). These previous examinations of excitatory and inhibitory amino acids were done postmortem or in anesthetized hamsters. As shown by previous microdialysis studies, striatal monoamine levels were unchanged in the absence of dystonia, but extracellular dopamine concentrations increased during a dystonic episode in mutant hamsters (Hamann et al., 2004). Thus, the question arises if dystonic episodes are also accompanied by changes in extracellular amino acid concentrations. In the present study, we therefore investigated the extracellular levels of excitatory and inhibitory amino acids in awake, freely-moving animals under basal conditions and during the expression of a dystonic episode.

EXPERIMENTAL PROCEDURES

Animals

The present experiments were carried out in groups of 10 dt^{sz} mutant Syrian golden hamsters at an age of 35–42 days and 10 age- and sex-matched non-dystonic control hamsters of an outbred line. The animals were obtained by selective breeding as described in detail elsewhere (Richter and Löscher, 1998). All hamsters were born and kept under the same controlled and constant environmental conditions (housing in standard makrolon cages, ambient temperature 24±1 °C, 13-h/11-h light/dark cycle). Standard Altromin® (Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) diet and tap water were allowed *ad libitum*. The experiments were approved by the responsible governmental agency in Berlin (number of authorization: G 0160/05) and conducted in compliance with international ethical standards and the German Animal Welfare Act, equivalent to the NIH Guide for the Care and Use of Laboratory Animals. The number of animals used in the present study and their suffering were minimized as far as possible.

Induction of dystonic attacks and severity-score of dystonia

In mutant hamsters, dystonic attacks can be reproducibly induced by stress, such as handling and tactile stimuli. After the stressful stimuli, dt^{sz} hamsters develop a sequence of abnormal movements and postures. 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 three times per week until the animals exhibited constant individual severity scores. The triple stimulation technique is a standardized procedure of mild stressful stimuli and consists of (1) taking the animal from its home cage (prepubertal litters of hamsters were housed together) and placing it on a balance, (2) injection of saline and (3) placement of the animal in a new clean and empty cage (one animal per cage) and continuous stressing of the animals via gentle tactile stimuli. The severity of dystonia can be rated by the following score-system (Richter and Löscher, 1998): score 1, flat body posture; score 2, facial contortions, rearing with forelimbs crossing, disturbed gait with hyperextended forepaws; score 3, hyperextended hind limbs so that the animals appear to walk on tiptoes; score 4, twisting movements and loss of balance; score 5, hind limbs hyperextended caudally; score 6, immobilization in a twisted, hunched posture with hind and forelimbs tonically extended forward. The movement abnormalities usually start within 5–10 min after the stressful stimuli and the individual maximum score of dystonia is usually reached within 3 h after the hamsters were placed in the new cage. Therefore, the animals are generally observed and further stressed by tactile stimuli continuously over a time period of 3 h and the latency to the occurring of the different severity scores is noticed. If a mutant hamster exhibits dystonia, it usually takes 2–5 h before dt^{sz} hamsters, which are awake, completely recover from abnormal movements. The condition of doze and sleep, comparable to patients, is able to abolish dystonic episodes more rapidly in mutant hamsters within 20–30 min, as seen in the present study.

The present measurements of amino acid levels were done between 35 and 42 days of life, an age of maximum severity of dystonia. The control hamsters were stimulated in the same manner as the mutant hamsters during the experiments as well as prior to the microdialysis, i.e. these animals were also tested after weaning at the age of 21 days by the triple stimulation procedure three times per week (see above).

Implantation of guide cannulae for microdialysis

The dystonic syndrome in mutant hamsters is not lateralized. Thus, guide cannulae (CMA 11/polyurethane, Carnegie Medicine, Stockholm, Sweden) were unilaterally implanted into the right striatum. With intent to target the motor striatum, the tip of the guide cannula was implanted into the dorsal striatum of hamsters anesthetized with pentobarbital (70 mg/kg i.p.) according to the following coordinates (relative to bregma): rostral +1.5, lateral −2.2 and ventral −2.2 mm. These coordinates were chosen according to the atlas of Morin and Wood (2001) and were comparable to those used for previous striatal microdialysis experiments in mutant hamsters (Hamann and Richter, 2004). The cannula was held in place with additional anchor screws and dental acrylic cement (Paladur®, Heraeus Kulzer, Germany) on the skull surface. To avoid obstruction, the guide cannula was equipped with a dummy cannula until the insertion of the microdialysis probe.

Microdialysis procedure

After a post-surgery recovery period of at least 4 days, microdialysis experiments were performed in dt^{sz} mutant hamsters and non-dystonic control hamsters. Prior to the experiments, the hamsters had been adapted to the freely moving system, which consisted of a plastic cylinder with counterbalancing arm carrying a two-channel swivel (CMA 120, Carnegie Medicine). The microdialysis probe (CMA/11, 2-mm cuprophane membrane, cutoff 6 kDa; Carnegie Medicine) was lowered gently through the guide cannula and the hamster was put into the cage system for freely moving animals. This procedure was carried out very carefully to avoid induction of a dystonic episode in mutant hamsters, so that none of the dt^{sz} hamsters developed a dystonic episode after insertion of the cannula. Food and water were available *ad libitum*. Fourteen hours to 16 h after insertion, perfusion of the probe was started with Thomaejonin® (DelatSelect GmbH, Munich, Germany; mM 140 Na^+, 2.5 Ca^{2+}, 4.0 K^+, 1 Mg^{2+} and 106 Cl^-, pH was adjusted at 7.0). The inflow was driven by a CMA/100 microinjection pump (Carnegie Medicine; flow rate: 2 μl/min). Each 60 μl outflow sample was collected within 30 min intervals in a polypropylene tube. In order to allow appropriate analyses (e.g. filling of the 50 μl loop of the injection valve), shorter intervals of sampling were not possible. For analysis 50 μl of each sample were used. The temperature of the dialysates was adjusted to 4 °C during sampling by using a refrigerated fraction collector (CMA/170, Carnegie Medicine). After 2.5 h of perfusion to stabilize baseline, three samples were collected over a subsequent period of 90 min, during which the animals were not disturbed (basal period 1). In the following 60 min during which the hamsters were stressed by tactile stimuli, two samples were collected (stress period; mild handling stress over 60 min in contrast to a usually 3 h stimulation period in other pharmacological experiments as described above). In this phase of microdialysis, the dt^{sz} hamsters developed motor abnormalities not later than about 5 min after first