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Research Report

Age-related changes in parvalbumin-positive interneurons in the striatum, but not in the sensorimotor cortex in dystonic brains of the *dt*^{sz} mutant hamster

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ABSTRACT

In the *dt*^{sz} hamster, a model of paroxysmal dystonia, an age-dependent increase in the activity of striatal projection neurons has been hypothesized to be based on a deficit of striatal parvalbumin-immunoreactive (PV$^+$) interneurons at an age of most marked expression of dystonia (30–40 days of life). In the present study, the spontaneous age-dependent remission of paroxysmal dystonia in older *dt*^{sz} hamsters (age>90 days) was found to coincide with a normalization of the density of striatal PV$^+$ interneurons. Furthermore, the arborization of these interneurons was lower in 31 day old *dt*^{sz} hamsters, but was even higher in *dt*^{sz} mutant at an age of >90 days than in control animals. Double-labeling with bromodeoxyuridine failed to show a retarded proliferation, while the number of interneurons with strong expression of PV mRNA was lower in young mutant hamsters. As shown by unaltered density of PV$^+$ interneurons in sensorimotor cortex of 31 day old *dt*^{sz} hamsters, PV containing interneurons are not reduced throughout the whole brain at the sensitive age. The present data suggest that a retarded postnatal maturation of striatal PV$^+$ interneurons plays a critical role in paroxysmal dystonia.

1. Introduction

In the neostriatum, GABAergic medium-sized aspiny interneurons which co-express the calcium-binding protein parvalbumin (PV) provide the main source of inhibition and are involved in the synchronization of GABAergic spiny projection neurons (Koos and Tepper, 1999; Plenz, 2003; Ramanathan et al., 2002). Despite their low number, these interneurons powerfully control the striatal output to the globus pallidus (indirect pathway) and of the direct pathway, i.e., to the entopeduncular nucleus (EPN; the globus pallidus internus in primates) and to the substantia nigra (Bolam et al., 2000; Kawaguchi et al., 1995). Striatal neurons receive extensive glutamatergic afferents from the cerebral

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Abbreviations: PV$^+$, parvalbumin-immunoreactive

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cortex which contains a high density of PV positive (PV^+) interneurons.

With regard to their function within the basal ganglia for induction and suppression of movements, striatal interneurons seem to deserve attention in research of motor disturbances. Dystonias, a group of serious movement disorders characterized by involuntary contractions of opposing muscles, and paroxysmal dyskinesias which include dystonic symptoms, are thought to result from reduced basal ganglia output (Fahn et al., 1998; Vitek and Giroux, 2000; Wichmann and DeLong, 1996). However, the pathogenesis of dystonia is not well understood. Previous studies in the dt^{sz} mutant hamster, one of the few suitable animal models of inborn dyskinesias/dystonias (Richter, 2005), revealed a deficiency of different types of striatal GABAergic interneurons which co-express PV, calretinin or nitric oxide oxidase (Gernert et al., 2000; Hamann et al., 2005; Sander et al., 2006). Neurochemical and pharmacological findings in mutant hamsters are in line with this structural defect (Hamann and Richter, 2002; Richter, 2005). No other histopathological changes were found in these animals (Hamann et al., 2006; Nobrega et al., 1999; Richter and Löscher, 1998; Wahnschaffe et al., 1990).

The dt^{sz} mutant hamster shows the phenotype of primary paroxysmal non-kinesiogenic dystonia (briefly: paroxysmal dystonia; Nardocci et al., 2002; Richter and Löscher, 1998, 2002). In paroxysmal dystonia, episodes of generalized dystonia and choreoathetosis can be provoked by stress, last up to several hours, and often improve by treatment with GABA-potentiating drugs (Nardocci et al., 2002). In the dt^{sz} mutant, paroxysmal dystonia is age-dependent with a maximum severity at an age of 30–40 days, followed by a slow decline of the severity. A complete remission of stress-inducible dystonic symptoms occurs at an age of about 10 weeks (Richter and Löscher, 1998, 2002). This age-dependent time-course allows examinations of the relevance of changes for the occurrence of dystonia, i.e., alterations detected at an age of maximum severity should be reduced or be disappeared in older animals after spontaneous remission of stress-inducible attacks.

In line with the current concept of the pathophysiology of human dystonia (Vitek and Giroux, 2000; Wichmann and DeLong, 1996), significantly decreased discharge rates and irregular discharge patterns of entopeduncular neurons were found in mutant hamsters at the most sensitive age (Gernert et al., 2000, 2002). The remission of paroxysmal dystonia was accompanied by a normalization of the entopeduncular activity in dt^{sz} mutants (Bennay et al., 2001; Gernert et al., 2002). There is evidence that the reduced entopeduncular activity is related to an age-dependent increase in striatal output (Gernert et al., 1999).

In the present study, we examined if the disappearance of dystonia and of disturbed activity of striatal projection neurons coincide with a normalization of the density of striatal PV^+ interneurons and of their arborization. Furthermore, PV containing interneurons of the sensorimotor cortex were analyzed to examine whether an abnormal density occurs also in other brain regions of the dt^{sz} mutant. The sensorimotor cortex was chosen because a decreased GABAergic inhibition in this afferent structure of the striatum has been suggested to be involved in the pathophysiology of human dystonia (Tinazzi et al., 2003).

2. Results

2.1. Density of striatal and cortical PV^+ interneurons

All mutant hamsters, used for the present experiments, exhibited severe dystonia at an age of 21 and 30 days, while no motor impairments could be provoked in control animals. The older dt^{sz} hamsters (92–98 days), used for the present experiments, had lost their dystonic symptoms at an age of 80 days.

The PV^+ cells in the striatum of dt^{sz} mutant hamsters and non-dystonic control hamsters showed the morphological characteristics of medium-sized aspiny GABAergic interneurons with varicose dendrites (see Fig. 1 and Fig. 2A; Cowan et al., 1990; Kawaguchi et al., 1995). Previously, we have shown a significantly decreased density of PV^+ neurons (−26%; $p=0.0006$) within the whole caudate–putamen (1218±68 in mutant hamsters vs. 1533±79 neurons/mm^3 in control animals) as well as in all striatal subregions of mutant hamsters at an age of most marked expression of dystonia in comparison to age-matched controls (Fig. 2B and Table 1; Gernert et al., 2000). The analysis in >90 day old animals revealed that the density of PV^+ interneurons in the caudate–putamen of dt^{sz} hamsters was comparable to age- and sex-matched non-dystonic control hamsters (Fig. 2, Table 1). As determined by behavioral testing, mutant hamsters at an age of 80 days had completely lost their susceptibility for showing stress-inducible dystonic episodes (not illustrated). After remission of paroxysmal dystonia, the density within the striatum was 2103±113 in dt^{sz} hamsters and did not significantly differ from data in age-matched controls (1709±255 neurons/mm^3, $p=0.189$). In comparison with 31 day old mutant hamsters, the density within the whole caudate–putamen was significantly higher in mutant hamsters at an age of >90 days ($p=0.001$), while no age-dependent changes were found in control hamsters ($p=0.396$; Fig. 2). The density of

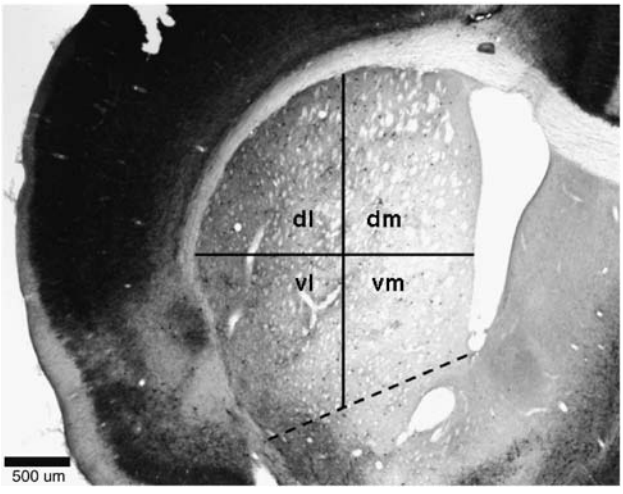

Fig. 1 – Parvalbumin immunoreactive (PV^+) interneurons in coronal sections in a control hamster (age > 90 days). PV^+ cells were counted within the sensorimotor cortex and in different subregions of the striatum, including the indicated quadrants, i.e., dorsolateral (dl), dorsomedial (dm), ventrolateral (vl), ventromedial (vm). Scale bar 500 μm.