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Research Report

The number of nociceptors in the trigeminal ganglion but not proprioceptors in the mesencephalic trigeminal tract nucleus is reduced in dystonin deficient *dystonia musculorum* mice

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ABSTRACT

The trigeminal ganglion (TG) and mesencephalic trigeminal tract nucleus (Mes5) were investigated in wild type and dystonia musculorum (*dt*) mice to study the effect of *dystonin* deficiency on primary sensory neurons in the trigeminal nervous system. At postnatal day 14, the number of TG neurons was markedly decreased in *dt* mice when compared to wild type mice (43.1% reduction). In addition, *dystonin* disruption decreased the number of sensory neurons which bound to isolectin B4, and contained calcitonin gene-related peptide or high-affinity nerve growth factor receptor TrkA. Immunohistochemistry for caspase-3 demonstrated that *dystonin* deficiency induced excess cell death of TG neurons during the early postnatal period. In contrast, Mes5 neurons were barely affected in *dt* mice. These data together suggest that *dystonin* is necessary for survival of nociceptors but not proprioceptors in the trigeminal nervous system.

Dystonin [also known as Bullous Pemphigoid Antigen 1 (Bpag1)] is a member of the plakin family of high molecular weight cytoskeletal linker proteins (Young and Kothary, 2007). The *Dst* gene is expressed by the developing cranial and spinal sensory ganglia (Bernier et al., 1995) and neural isoforms of dystonin/Bpag1 are predicted to link actin filaments to microtubules. Thus, the dystonin isoforms are considered to play a role in cytoskeleton organization during axonogenesis (Dalpé et al., 1998; Leung et al., 1999). Targeted and spontaneous mutation of the dystonin locus in mice [*dystonia musculorum* (*dt*) mice] results in dystonic movement and severe ataxia after birth (Bernier et al., 1995). In these mice, the motor system has not yet been shown to have neuronal degeneration. However, sensory nerve fibers are reduced and numerous axonal swellings are detected in the remaining fibers (Bernier et al., 1995).

In the spinal nervous system, proprioceptors are located in the dorsal root ganglia (DRG). These neurons have large cell bodies and innervate the musculature. In the trigeminal ganglion, however, muscular proprioceptors are very rare. Trigeminal proprioceptors are primarily located in the mesencephalic trigeminal tract nucleus (Mes5). Mes5 neurons innervate masticatory muscles and periodontal ligaments. Primary proprioceptors in the spinal and trigeminal

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nervous systems contain parvalbumin (PV), a member of calcium-binding proteins (Celio, 1990; Ichikawa et al., 1994; 1999; Matsuo et al., 2000). In the DRG and trigeminal ganglion (TG), primary nociceptors mainly have small cell bodies, and contain calcitonin gene-related peptide (CGRP) and high-affinity nerve growth factor (NGF) receptor TrkA (Silverman and Kruger, 1989; Ichikawa et al., 1994; Averill et al., 1995; Krekoski et al., 1996). Isolectin B4 (IB4)-binding small neurons are also considered to be nociceptors in the ganglia (Bergman et al., 1999; Carlsten et al., 2001). Such nociceptors are not located to the Mes5. Previous studies have demonstrated that peripheral nerve injury induces various changes in the peripherally axotomized primary sensory neurons. Neonatal nerve transection in rats induces primary neuronal death causing reduction in number of DRG and TG neurons (Henderson et al., 1993; Cheema et al., 1994). The nerve injury in adult animals does not cause massive neuronal loss. However, the proportion of sensory neurons expressing IB4-binding, CGRP and TrkA are markedly decreases (Bergman et al., 1999; Krekoski et al., 1996). On the other hand, the proportion of neurons containing galanin (GAL) and neuropeptide Y (NPY) is significantly increased by the injury (Verge et al., 1995). These changes are considered to be due to depletion of neurotrophic substances following the injury.

In the DRG, *dystonin* mutation decreases the number of large neurons which innervate the musculature and small neurons which bind to IB4 (Carlsten et al., 2001). It is likely that failure in axonal transport of neurotrophic substances, as the consequence of microtubule network perturbation, resulted in excessive death of the proprioceptors and nociceptors in *dt* mice (Carlsten et al., 2001; De Repentigny et al., 2003). However, the proportion of CGRP-containing DRG neurons increases in *dt* mice (mean ± S.E.M. = 30.4 ± 2.15%) compared to wild type mice (mean ± S.E.M. = 23.3% ± 0.96%) (Carlsten et al., 2001). In addition, the distribution of CGRP-containing nerve fibers is similar in wild type and *dt* mice. Therefore. CGRP-containing neurons are considered to be barely affected by *dystonin* mutation. Thus, the effect of *dystonin* mutation on sensory neurons is probably correlated to their cell size, function, neurochemical substances and/or neurotrophin dependency. In the sensory ganglia, development of CGRP-containing and IB4-binding neurons is considered to depend on NGF and glial cell derived neurotrophic factor (GDNF), respectively (Klein, 1994; Molliver et al., 1997; Zwick et al., 2002). However, primary proprioceptors in the spinal and trigeminal nervous systems have different neurotrophin dependency. The survival of proprioceptors in the DRG is dependent upon neurotrophin-4 (NT-4) (Klein, 1994; Matsuo et al., 2000). Both NT-4 and brain-derived neurotrophic factor is necessary for development of muscular and periodontal proprioceptors in the Mes5 (Fan et al., 2000; Matsuo et al., 2000). However, little is known about the effect of *dystonin* deficiency on TG and Mes5 neurons. In addition, the expression of GAL or NPY in *dt* mice has not been reported.

In this study, the number of TG and Mes5 neurons and the expression of neurochemical substances were examined in *dt* mice. Caspase-3 (CAS-3) expression was also studied to demonstrate the cell death of TG neurons during the early postnatal period (Sugimoto et al., 2004).

1. Results

1.1. *Nissl stain in the TG at P14*

The TG contained abundant neuron profiles in Nissl-stained sections of wild type mice (Fig. 1A). In *dt* mice, however, the number of neuron profiles markedly decreased (Fig. 1B). The mean ± S.D. of the total number of neuron profiles in every tenth of serial sections of the TG was 4377.5 ± 872.2 (n = 4) in wild type mice and 2490.5 ± 245.32 (n = 4) in *dt* mice (43.1% reduction). The difference between wild type and *dt* mice was statistically significant ($p < 0.01$, Student's t-test).

1.2. *IB4-binding and neurochemical substances in the TG at P14*

In wild type mice, the TG contained abundant IB4-binding neuron profiles (Fig. 1C). The strong reaction was localized to granules around the nucleus and membranes of the cytoplasm. IB4-binding neurons had small cell bodies (Fig. 1C). In *dt* mice, however, IB4-binding neuron profiles dramatically decreased (78.1% reduction) (Fig. 1D and Table 1). The localization of IB4-binding within neuronal cell bodies was similar in wild type and *dt* mice.

Many CGRP- and TrkA-immunoreactive neurons profiles were observed in wild type mice (Figs. 1E, G). These neurons had small to medium-sized cell bodies. In *dt* mice, however, the number of such neuron profiles significantly decreased (28% and 37.7% reduction for CGRP- and TrkA-immunoreactive neuron profiles, respectively) (Figs. 1F, H and Table 1.

In wild type mice, the TG contained a few GAL-immunoreactive neuron profiles (Fig. 1I). These neurons had small cell bodies. In *dt* mice, however, GAL-immunoreactive neuron profiles dramatically increased (7.7-fold increase) (Figs. 1J, Table 1). Such neurons were small to medium-sized in the mutant TG.

NPY-immunoreactive neurons could not be detected in the TG of wild type mice (Fig. 1K). In *dt* mice however, many NPY-immunoreactive neuron profiles appeared (Fig. 1L, Table 1). They had medium-sized cell bodies in the ganglion.

All of these types were evenly distributed in the TG of wild type and *dt* mice.

1.3. *The Mes5 at P14*

Numerous PV-immunoreactive neuron profiles were observed in the Mes5 of both wild type and *dt* mice (Figs. 2A, B). The mean ± S.D. of the total number of immunoreactive neuron profiles in every fourth of serial sections of the Mes5 was 107.5 ± 29.0 (n = 4) in wild type mice and 90.3 ± 12.8 (n = 4) in *dt* mice.

1.4. *CAS-3 in the TG*

In the TG of wild type mice, many CAS-3-immunoreactive neuron profiles were observed at P0 (Table 2). However, the