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Research Report

Prolonged generalized dystonia after chronic cerebellar application of kainic acid

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ABSTRACT

Dystonia has traditionally been considered as a basal ganglia disorder, but there is growing evidence that impaired function of the cerebellum may also play a crucial part in the pathogenesis of this disorder. We now demonstrate that *chronic* application of kainic acid into the cerebellar vermis of rats results in a prolonged and generalized dystonic motor phenotype and provide detailed characterization of this new animal model for dystonia. *c-fos* expression, as a marker of neuronal activation, was increased not only in the cerebellum itself, but also in the ventro-anterior thalamus, further supporting the assumption of a disturbed neuronal network underlying the pathogenesis of this disorder. *Preproenkephalin* expression in the striatum was reduced, but *prodynorphin* expression remained unaltered, suggesting secondary changes in the indirect, but not in the direct basal ganglia pathway in our model system. *Hsp70* expression was specifically increased in the Purkinje cell layer and the red nucleus. This new rat model of dystonia may be useful not only for further studies investigating the role of the cerebellum in the pathogenesis of dystonia, but also to assess compounds for their beneficial effect on dystonia in a rodent model of prolonged, generalized dystonia.

1. Introduction

Dystonia is a common and frequently disabling disorder. It is typically defined as a syndrome of sustained muscle contraction, leading to involuntary twisting and abnormal posture.

The pathophysiology of dystonia is poorly understood. Animal models of dystonia are therefore of considerable interest because they provide experimental paradigms for elucidating the mechanisms underlying this movement disorder (Calderon et al., 2011). They can be divided into those that mimic the dystonic phenotype and those that parallel the genetic abnormalities detected in human patients (Breakefield et al., 2008; Jinnah et al., 2005; Raike et al., 2005). Perhaps surprisingly, none of the different

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DYT1-related mouse models develops an overt dystonic phenotype (reviewed in Breakefield et al., 2008).

Dystonia has traditionally been considered to be a basal ganglia disorder, but there is growing evidence that impaired function of the cerebellum may also play a crucial role. Functional imaging studies revealed increased resting metabolic activity in the cerebellum of both manifesting and non-manifesting *DYT1* carriers (Eidelberg et al., 1998). The defective protein in the dystonic *(dt)* rat, caytaxin, is highly expressed in cerebellar neurons during development and cerebellectomy eliminates the motor syndrome observed in these animals (LeDoux, 2011; LeDoux et al., 1993; 1995). Pizoli and co-workers tested the hypothesis that abnormal cerebellar signaling pathways can induce dystonia by microinjecting the excitatory glutamate receptor agonist kainic acid into the cerebellar vermis of normal mice (Pizoli et al., 2002). Kainic acid injection led to a dystonic phenotype in a dose-dependent manner, ranging from dystonic attacks lasting for 2–15 s after a low dose injection of kainic acid to tensely held dystonic postures for 2–20 min at higher doses.

The main aim of our study was to determine whether chronic application of kainic acid results in prolonged manifestation of a dystonic phenotype and thus mimics more closely the clinical phenotype of generalized dystonia observed in human patients. We also hypothesized that kainic acid injection into the cerebellar vermis may lead to (indirect) neuronal activation in more distant brain areas which would be in keeping with the current neuronal network hypothesis of dystonia (Neychev et al., 2011). The cerebellum and the basal ganglia are interconnected (Bostan and Strick, 2010; Perciavalle et al., 1989; Snider et al., 1976). Striatal expression of *preproenkephalin* (*penk*), a marker of the indirect basal ganglia pathway, is markedly lower in the dtsz hamster after a dystonic episode (Nobrega et al., 2004). We therefore hypothesized that cerebellar injection of kainic acid may also result in a decreased activation of the indirect pathway with reduced expression of *penk* but no effect on *prodynorphin* (*pdyn*) expression, a marker of direct basal ganglia pathway activation. The Delta 302/303 in-frame deletion in the *DYT1* gene results in upregulation of heat shock proteins such as Hsp70 in vitro (Baptista et al., 2003). *Hsp70* expression is also upregulated in the dystonic rat (Xiao et al., 2007), suggesting that *Hsp70* upregulation may be a shared pathogenic mechanism or marker in different types of dystonia. Thus, we finally determined whether *Hsp70* may also be upregulated in our kainic-acid induced rat model of dystonia.

2. Results

2.1. *Continuous kainic acid injection leads to sustained and generalized dystonia*

Rats receiving a single kainic acid injection into the cerebellar vermis reliably and reproducibly displayed a dystonic phenotype 10 min after injection. The motor phenotype was present for approximately 100 min. Continuous kainic acid application using osmotic mini-pumps resulted in a sustained motor phenotype for more than 48 h. The dystonia was more pronounced in the hind limbs than in the forelimbs, with either the whole trunk and all four limbs being involved or only the hindlimbs which were then held up tonically against the trunk. Additional craniocervical abnormalities were observed, suggesting the presence of blepharospasm and torticollis (see Video). Dystonia typically increases with action and decreases with rest in human patients with primary dystonia. It was therefore of interest to note that the dystonic phenotype in the kainic acid-injected rats was also attenuated or disappeared during resting. It was possible at any time during the 48 h chronic injection period to re-provoke dystonic postures by sudden noise, fright or gentle touch of the paws (see Video). Post-mortem HE- and Nissl-staining revealed only minor tissue damage in the cerebellar vermis at the infusion site (Figs. 1A, B). The increase in the severity of the dystonic phenotype was most marked in the first 16 h, but there was then a further increase until it reached a plateau after 32 h (Fig. 1C).

In a subset of animals EEG was performed in parallel both after a single injection and during continuous application of kainic acid to exclude the possibility of the dystonic phenotype being due to epileptiform discharges. The EEG recordings remained normal in both experimental settings (Fig. 1D).

2.2. *Increased and specific expression of c-fos in distinct brain regions*

c-fos expression was analyzed in the thalamus, hippocampus, brain stem, dorsal and ventral striatum, primary and secondary motor cortex, prefrontal cortex, substantia nigra, cerebellar cortex and deep cerebellar nuclei. *c-fos* expression was assessed 24 h after induction of the dystonic phenotype. Continuous application of kainic acid into the vermis induced c-*fos* expression within the cerebellum in all three layers of the cerebellar cortex and in deep cerebellar nuclei (DCN, Figs. 2B, D). In contrast, expression was below the detection limit in sham infused animals (Figs. 2A, C). Interestingly, c-*fos* expression was also enhanced in the ventral anterior thalamic nucleus (PFC, Fig. 2F). In contrast, c-*fos* mRNA expression was not changed in the hippocampus (data not shown) as observed after kainic acid-induced seizures (Morgan et al., 1987). It also remained unchanged in all other brain regions analyzed.

2.3. *Differential effect on direct and indirect basal ganglia pathways*

Expression of *pdyn* was similar in vehicle (100%±13.4) and kainic acid (75%±10.5, $p=0.4$) infused animals 24 h after onset of the dystonic phenotype (Figs. 3A–C). In contrast, *penk* expression was markedly down-regulated (34.7%±12.2 in kainic acid injected animals vs 100%±3.8 in vehicle-infused animals, $p<0.05$; Figs. 3A, D, E).

2.4. *Increased expression of Hsp70 in cerebellar Purkinje cell layer and the red nucleus*

Hsp70 expression was up-regulated in the Purkinje cell layer of the entire cerebellum (Figs. 4A, B). *Hsp70* was also up-regulated in the magnocellular part of the red nucleus, the first output target of the DCN (235.71%±30.3% compared to controls, $p<0.05$; Figs. 4C, D), but not elsewhere.