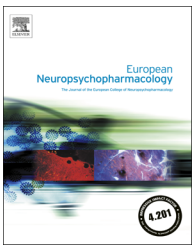
European Neuropsychopharmacology (2014) 24, 1896–1906



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Dysregulated intracellular signaling in the striatum in a pathophysiologically grounded model of Tourette syndrome

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KEYWORDS
Tourette syndrome;
Histamine;
Dopamine;
Basal ganglia;
Striatum

Abstract
Tic disorders produce substantial morbidity, but their pathophysiology remains poorly understood. Convergent evidence suggests that dysregulation of the cortico-basal ganglia circuitry is central to the pathogenesis of tics. Tourette syndrome (TS), the most severe end of the continuum of tic disorders, is substantially genetic, but causative mutations have been elusive. We recently described a mouse model, the histidine decarboxylase (*Hdc*) knockout mouse, that recapitulates a rare, highly penetrant mutation found in a single family; these mice exhibit TS-like phenomenology. These animals have a global deficit in brain histamine and a consequent dysregulation of DA in the basal ganglia. Histamine modulation of DA effects is increasingly appreciated, but the mechanisms underlying this modulation remain unclear; the consequences of modest DA elevation in the context of profound HA deficiency are difficult to predict, but understanding them in the *Hdc* knockout mouse may provide generalizable insights into the pathophysiology of TS. Here we characterized signaling pathways in striatal cells in this model system, at baseline and after amphetamine challenge. *In vivo* microdialysis confirms elevated DA in *Hdc*-KO mice. We find dephosphorylation of Akt and its target GSK3β and activation of the MAPK signaling cascade and its target rpS6; these are characteristic of the effects of DA on D2- and D1-expressing striatal neurons, respectively. Strikingly, there is no alteration in mTOR signaling, which can be regulated by DA in both cell types. These cellular effects help elucidate

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http://dx.doi.org/10.1016/j.euroneuro.2014.10.007
0924-977X/

striatal signaling abnormalities in a uniquely validated mouse model of TS and move towards the identification of new potential therapeutic targets for tic disorders.

1. Introduction

Tic disorders affect approximately 5% of the population; Tourette syndrome (TS), which lies at the most severe end of the spectrum of tic disorders, has a prevalence of 0.3–1% (Robertson et al., 2009; Williams et al., 2013). Severe cases can cause profound morbidity. The pathophysiology of TS is not well understood, which has retarded the development of new treatments (Williams et al., 2013). Progress has been delayed by the complexity of TS genetics, which has not converged on any clear etiology (Davis et al., 2013; State, 2011), and by the lack of validated, pathophysiologically-grounded animal models in which to test specific hypotheses and generate new molecular insights (Pittenger, 2014).

We recently described a novel animal model of the pathophysiology of TS (Castellan Baldan et al., 2014), based on a rare mutation in the *histidine decarboxylase* (*Hdc*) gene found in a unique family with an exceptionally high incidence of tics (Ercan-Sencicek et al., 2010). Subsequent genetic studies have suggested that dysregulation of HA signaling contributes to TS beyond this index family (Fernandez et al., 2012; Karagiannidis et al., 2012). *Hdc* knockout animals exhibit potentiated tic-like stereotypies when stimulated with D-amphetamine; this is mitigated by systemic injection of the D2 antagonist haloperidol, which is often efficacious in patients with tics, and by direct infusion of HA into the brain. Both knockout animals and patients carrying the *Hdc* mutation also exhibit a deficit in prepulse inhibition, providing further face validity for the model (Castellan Baldan et al., 2014).

Convergent evidence implicates dysfunction of the cortico-basal ganglia circuitry in the pathophysiology of TS (Williams et al., 2013). In particular, dopaminergic excess in this circuitry has been suggested by PET imaging studies (Singer et al., 2002; Wong et al., 2008); the therapeutic efficacy of D2 antagonists (Bloch, 2008) supports the causal importance of this DA excess in the etiology of tics. We therefore focused on the striatal circuitry, and in particular on DA modulation of this circuitry, in the *Hdc* knockout mouse model. *In vivo* microdialysis revealed dysregulated DA; immunohistochemistry showed elevated expression of the immediate early gene *Fos*, which is regulated by DA in the principle cells of the striatum, the medium spiny neurons (MSNs). Dopamine D2/D3 receptors were elevated in the substantia nigra of both mice and patients carrying a mutated *Hdc* gene, further supporting *in vivo* dysregulation of DA and providing additional translational validation of the model (Castellan Baldan et al., 2014).

Histamine is produced by *Hdc*-expressing cells in the posterior tuberomammilary nucleus of the hypothalamus; these neurons project broadly throughout the brain (Panula and Nuutinen, 2013). The striatum receives a substantial projection from these histaminergic cells (Haas et al., 2008) and contains a disproportionately high amount of HDC protein (Krusong et al., 2011). MSNs express histamine receptors H1R, H2R, and H3R (Haas et al., 2008; Pillot et al., 2002), and histamine modulates the synaptic responses and electrical properties of MSNs in acute brain slices (Ellender et al., 2011). The H3 receptor, in particular, has recently been revealed as a potentially important regulator of signal transduction in MSNs (Moreno et al., 2011, 2014; Panula and Nuutinen, 2013). The interaction between HA and DA in the modulation of striatal responses is not well understood.

Signaling within MSNs, in response to activity, DA, and other regulators, is complex (Greengard, 2001). Striatal MSNs are separable into those expressing the D1 DA receptor, which project to the substantia nigra pars reticulata (the striatonigral or direct pathway) and those expressing the D2 receptor, which project to the globus pallidus (the striatopallidal or indirect pathway). The dynamic balance between these two pathways is thought to be critical to normal striatal function; it has been hypothesized that imbalance – excessive direct pathway activation and indirect pathway inhibition – is central to the pathogenesis of TS (Albin and Mink, 2006; Baym et al., 2008; Williams et al., 2013). In D1-expresing MSNs, DA leads to elevation of cAMP, activation of PKA and MAPK (ERK) signaling, and activation of DARPP-32 (Bateup et al., 2008; Bertran-Gonzalez et al., 2008; Nishi et al., 2011). In D2-expressing MSNs, DA has the opposite effects on cAMP, PKA, and DARPP-32 (Bateup et al., 2008; Bertran-Gonzalez et al., 2008); it also inhibits Akt via β-arrestin, reducing the phosphorylation of GSK and thereby activating it (Beaulieu et al., 2005). All of these signaling pathways can regulate critical downstream events, including translation and gene expression. For example, the translational regulator ribosomal protein S6 (rpS6) is phosphorylated and activated by the ERK signaling pathway via the kinase ribosomal S6 kinase (RSK1/2) (Frodin and Gammeltoft, 1999), and by the mTOR pathway via its regulation of S6 kinase (S6K) (Magnuson et al., 2012).

Our previous studies establish the *Hdc* knockout mouse as an informative, pathophysiologically-grounded model of at least a rare genetic form of TS (Castellan Baldan et al., 2014). Leveraging of this finding towards generalizable insights into the pathophysiology of TS and, ultimately, towards the development of new treatments requires characterization of how MSN signaling is dysregulated in these mice. The *Hdc* knockout mouse has elevated DA levels, which may increase tonic D1 and D2-regulated signaling, but it also has reduced striatal HA (Castellan Baldan et al., 2014); given the interaction between HA and DA in the regulation of MSN signaling (Bertran-Gonzalez et al., 2008; Moreno et al., 2011, 2014; Panula and Nuutinen, 2013), it is unclear how these two neurochemical abnormalities might interact in altering MSN signal transduction pathways.

We have previously demonstrated that the immediate early gene *Fos*, which is a downstream target of convergent DA-regulated signaling pathways in D1-expressing MSNs, is upregulated in the *Hdc* knockout mouse (Castellan Baldan et al., 2014). Here we examined this and other MSN