Review

The neurobiological basis for novel experimental therapeutics in dystonia

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ABSTRACT

Dystonia is a movement disorder characterized by involuntary muscle contractions, twisting movements, and abnormal postures that may affect one or multiple body regions. Dystonia is the third most common movement disorder after Parkinson’s disease and essential tremor. Despite its relative frequency, small molecule therapeutics for dystonia are limited. Development of new therapeutics is further hampered by the heterogeneity of both clinical symptoms and etiologies in dystonia. Recent advances in both animal and cell-based models have helped clarify divergent etiologies in dystonia and have facilitated the identification of new therapeutic targets. Advances in medicinal chemistry have also made available novel compounds for testing in biochemical, physiological, and behavioral models of dystonia. Here, we briefly review motor circuit anatomy and the anatomical and functional abnormalities in dystonia. We then discuss recently identified therapeutic targets in dystonia based on recent preclinical animal studies and clinical trials investigating novel therapeutics.

1. Introduction

Dystonia, the third most common movement disorder after tremor and Parkinson’s disease (Defazio, 2010), is characterized by involuntary muscle contractions that cause twisting movements and postures (Albanese et al., 2013). The causes of dystonia are diverse. It may occur as a sporadic (idiopathic) or inherited disorder (Schwarz and Bressman, 2009; Tanabe et al., 2009) and can sometimes occur as a result of brain injury (Frei et al., 2004; Krauss and Jankovic, 2002; Lo et al., 2005). The clinical features of dystonia are also heterogeneous. In some patients, dystonia affects only a small number of muscles, such as those of the hand in writer’s cramp or those of the neck in torticollis. In others, muscles throughout the body are involved. Although dystonia is not lethal, it is debilitating. Indeed, standardized quality of life scores for dystonia fall into the same range as Parkinson’s disease, stroke and multiple sclerosis (Camfield et al., 2002). Despite the prevalence and detrimental impact on quality of life, patients have very limited treatment options and struggle to find off-label alternatives that are rarely efficacious. Therefore, this review examines promising drug targets for dystonia, and presents a summary of recent preclinical, animal studies and clinical trials supporting their efficacy.

2. Motor circuit anatomy

The scarcity of small molecule drugs for the treatment of dystonia is directly attributable to the lack of clearly validated therapeutic targets; dystonia is not typically associated with degeneration or evident neuropathological abnormalities and the underlying cellular mechanisms are largely unknown. However, convergent evidence from clinical investigation and experimental models supports the view that dystonia is a circuit disorder, involving both the basal ganglia-thalamo-cortical and cerebello-thalamo-cortical pathways. Thus, a better understanding of how these networks function both normally and in dystonia is critical for discovering new therapeutics.

2.1. The cortico-basal ganglia-thalamo-cortical pathway

The basal ganglia are subcortical nuclei involved in diverse functions, including motor control and motor learning, executive function, and emotion. The basal ganglia include the striatum (caudate-putamen and nucleus accumbens), globus pallidus, subthalamic nucleus (STN), substantia nigra, and pedunculopontine nucleus (PPN). In brief, information flows through the basal ganglia back to the cortex through...
two pathways with opposing effects on movement execution: the “direct pathway” and the “indirect pathway” (Albin et al., 1989; DeLong, 1990). The original model of basal ganglia organization was based on experimental and clinical evidence of opposite functional effects of the direct and indirect projections on the output structures, which facilitate or inhibit movements, respectively (Gerfen, 2000). This model, which is supported by evidence from genetic, lesion and optogenetic studies, proposes that during normal behavior the direct pathway promotes a specific motor program while the indirect pathway inhibits competing motor programs (Bateup et al., 2010; Durieux et al., 2009; Kravitz et al., 2010). However, this model has undergone significant revisions to reflect more recent evidence demonstrating that direct and indirect pathways are not as segregated as originally proposed (Cui et al., 2013; Tecuapetla et al., 2016).
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The striatum is the input nucleus of the basal ganglia. The striatum receives dopaminergic (DA) afferents from neurons originating in the substantia nigra pars compacta (SNC) and ventral tegmental area. The striatum also receives glutamatergic input from the cerebral cortex and thalamus. Dopaminergic and glutamatergic input to the striatum is integrated by GABAergic spiny projection neurons (SPNs), the major output neurons of the striatum. SPNs compose ≥95% of neurons in the striatum (Kawaguchi, 1993; Tepper et al., 2010). SPNs of the indirect striatopallidal pathway innervate the external segment of the globus pallidus (GPe) and express the dopamine (DA) D2 receptor. SPNs of the direct striatonigral pathway express the D1 DA receptor, and project directly to the internal segment of the globus pallidus (GPI) and the substantia nigra pars reticulata (SNr). The GABAergic GPI and SNr neurons send inhibitory input to the ventral and intralaminar thalamic nuclei. Thalamic glutamatergic neurons then provide excitatory input to the motor cortex, which projects to spinal motor neurons or provides feedback to the striatum. In the indirect pathway, striatopallidal neurons provide inhibitory GABAergic input to GABAergic GPe neurons, which in turn provide inhibitory input to the STN. GABAergic neurons in the STN provide excitatory input to the GPI and SNr, which inhibit the thalamus and cortex (Albin et al., 1989; Parent and Hazrati, 1997). The remaining ~5% of striatal neurons are represented by several different classes of interneurons, including: cholinergic interneurons (ChIs), GABAergic fast-spiking interneurons (FSIs), and other peptidergic interneurons (Kawaguchi, 1997). Some striatal interneurons, such as ChIs and FSIs, respond to dopaminergic signaling and reciprocally modulate both SPNs and other interneuron populations (Gittis et al., 2010; Gritton et al., 2019; Tepper et al., 2008).

2.2. The basal ganglia connections with the cerebello-thalamo-cortical pathway

The cerebellum forms complex, large-scale connections with other brain regions and has a well-characterized role in motor learning and control. The cerebellar cortex has a trilaminar cytoarchitecture composed of the granular layer, the molecular layer, and the Purkinje cell layer. In the granular layer, mossy fiber afferents from extracerebellar regions activate granule cells and Golgi cells. Granule cells form ascending axons, called parallel fibers, which extend within the molecular layer. In the molecular layer, parallel fibers innervate inhibitory stellate and basket interneurons as well as Purkinje cells. Purkinje cells are also activated by climbing fibers, which originate in the inferior olives. The Purkinje cells project to the deep cerebellar nuclei, which in turn convey cerebellar output to other brain regions.

Anatomical and functional data support several bidirectional cerebellar-thalamo-cortical circuits connecting the cerebellar cortex to motor, sensory, or associative cortical areas (Gornati et al., 2018; Krienen and Buckner, 2009; Schmahmann et al., 2019). Recent work uncovered an anatomical substrate between the basal ganglia and cerebellum, suggesting these structures may form an integrated functional network. Bidirectional communication occurs through a disynaptic projection from the dentate nucleus of the cerebellum to the striatum (Hoshi et al., 2005) and a disynaptic projection from the subthalamic nucleus of the basal ganglia to the cerebellar cortex (Bostan et al., 2010). This network is topographically organized, whereby the motor, cognitive and affective territories of the basal ganglia, cerebellum and cerebral cortex are interconnected. It is now accepted that the basal ganglia, cerebellum, and cerebral cortex form an integrated network, and this basal ganglia-to-cerebellar pathway plays a role in dystonic movements (Fig. 1) (Bostan et al., 2018; Neychev et al., 2008; Shakkottai et al., 2017).

3. Anatomical basis of dystonia

Historically, dystonia has been viewed as a disease of the basal ganglia due to the wealth of evidence implicating this region. In patients with hemidystonia, focal brain lesions were identified in the basal ganglia or associated regions (Bhatia and Marsden, 1994). Further, comorbidity of dystonia with other diseases of the basal ganglia, such as Parkinson’s disease (Tolosa and Compta, 2006) and Huntington’s disease (Louis et al., 1999), suggested involvement of the basal ganglia in the pathology of dystonia.

Human imaging studies have also implicated the basal ganglia in dystonia. In summary, functional magnetic resonance imaging studies of dystonia patients that reveal changes in basal ganglia activity generally show an increase in activation, particularly in the caudate, putamen and globus pallidus (Blood et al., 2004; Haslinger et al., 2010; Schneider et al., 2010). Voxel-based morphometry studies reveal alterations in volume of basal ganglia nuclei. In these studies, regional volume was increased in the caudate and decreased in the putamen of patients with cervical dystonia and blepharospasm (Obermann et al., 2007), though others have observed opposing trends within these regions (Draganski et al., 2009). Within the globus pallidus, regional volume is generally increased (Draganski et al., 2009; Draganski et al., 2003; Egger et al., 2007). Positron emission tomography (PET) imaging studies demonstrate a reduction in striatal D2 DA receptor availability in patients with DYT1 dystonia, writer’s cramp and spasmodic dysphonia (Asanuma et al., 2005b; Berman et al., 2013; Simonyan et al., 2013), and an increase in the availability of striatal D1 DA receptors in individuals with laryngeal dystonia and focal hand dystonia (Simonyan et al., 2017), which may contribute to an imbalance in the direct and indirect basal ganglia pathways. [18F]-fluorodeoxyglucose-PET imaging demonstrate that glucose metabolism is increased within the caudate and putamen in patients with cervical dystonia and DYT1 dystonia (Carbon et al., 2004; Galardi et al., 1996), though others have noted a reduction in activity in the putamen in dopa-responsive dystonia (DRD) patients (Asanuma et al., 2005b). These findings have been reviewed extensively (Asanuma et al., 2005a; Jinnah et al., 2017; Lehericy et al., 2013; Neychev et al., 2011; Simonyan, 2018) and point to the many associations between basal ganglia dysfunction and dystonia. Targeted interventions to basal ganglia nuclei for the treatment of dystonia such as pallidotomy (Lozano et al., 1997) and deep-brain stimulation of the GPI and STN (Ostrem et al., 2017; Ostrem and Starr, 2008) are also compelling evidence.

Although abundant evidence supports the role of the basal ganglia in dystonia, other regions, including the cerebellum, are also involved (Bologna and Berardelli, 2018; Neychev et al., 2011; Shakkottai et al., 2017). For example, cerebellar lesions occur in patients with cervical dystonia (LeDoux and Brady, 2003). Further, post-mortem analysis of brains from patients with cervical dystonia revealed loss of Purkinje cells, increased gliosis, and inclusions of torpedo bodies within Purkinje cells (Prudente et al., 2013). Additionally, there is an increase in metabolic activity in the cerebellum in myoclonus dystonia and cervical dystonia as assessed by PET imaging (Carbon et al., 2013; Eidelberg et al., 1998), and fMRI studies show alterations in cerebellar activation in individuals with dystonia (Filip et al., 2017; Prudente et al., 2016). Moreover, evidence from animal models also implicates the cerebellum in dystonia. Dystonia in both tottering mice (Campbell et al., 1999) and the Dt rat (LeDoux, 2011) arises from cerebellar dysfunction. Additionally, cerebellar knockdown of Tor1a, the gene associated with DYT1 dystonia, results in dystonic movements in wild-type mice, while striatal knockdown does not induce dystonia (Fremont et al., 2017). Dystonia can also be induced in normal mice by pharmacological manipulation of cerebellar signaling using glutamatergic agonists (Fan et al., 2018; Pizoli et al., 2002) or the sodium-potassium pump blocker ouabain (Calderon et al., 2011).

Although the basal ganglia and the cerebellum have been the primary focus of dystonia research, other regions are implicated in dystonia. For example, brainstem lesions are sometimes associated with dystonia, and thalamic stimulation or ablation has been used to treat hand dystonia (Shimizu et al., 2018). Involvement of these other regions has been reviewed elsewhere (Jinnah et al., 2017; Neychev et al., 2011).
4. Existing treatments for dystonia

Current treatments for dystonia include oral pharmaceuticals, botulinum toxin, and surgical procedures (Cloud and Jinnah, 2010; Thenganatt and Jankovic, 2014). Here, the focus is on oral pharmaceuticals. Trihexyphenidyl (THP), a nonselective muscarinic acetylcholine receptor (mAChR) antagonist is the only widely used oral pharmaceutical that has been studied in a double-blind controlled trial for dystonia. This clinical trial demonstrated that THP was effective at alleviating dystonia in approximately 70% of patients (Burke et al., 1986). While THP is an effective treatment, it is often poorly tolerated due to significant side effects, including: cognitive impairments, memory loss, nightmares, dry mouth, constipation, blurred vision, and urinary retention (Bymaster et al., 2003; Jabbari et al., 1989; Lumsden et al., 2016). These side effects are often exacerbated by the high doses of THP needed to treat dystonia (Lang, 1986). Other nonselective mAChR antagonists have been used clinically, including atropine (Lang et al., 1982), procyclidine (Paulson, 1960), benztropine (Lang et al., 1982; Stern and Anderson, 1979), and biperiden (Cloud and Jinnah, 2010).

Despite the clear involvement of dopaminergic transmission in the pathophysiology of dystonia (Karimi and Perlmutter, 2015; Thompson et al., 2011), DA agonists, including direct D1/D2 receptor agonists and indirect agonists such as l-DOPA and amphetamine, are generally not effective treatments for dystonia. The exception is the use of l-DOPA to treat patients with DRD, a form of dystonia caused by mutations in genes involved in catecholamine synthesis. However, there are some reports that suggest DA receptor agonists may be effective in subsets of dystonia patients in addition to those with DRD (Fan et al., 2018).

Other pharmaceuticals have been used to treat dystonia, with varying degrees of success. Baclofen, a presynaptic GABA_B receptor antagonist, is sometimes used in dystonia. However, a meta-analysis of clinical trials and case reports found that baclofen improved symptoms in only 20% of patients with dystonia (Greene et al., 1988). Other pharmaceuticals used to treat dystonia are: benzodiazepines, muscle relaxants, and anti-epilepsy medications, although there is limited evidence for the efficacy of any of these for the treatment of dystonia (Thenganatt and Jankovic, 2014).

5. New experimental therapeutic and pharmacological targets

In light of the limited and sometimes ineffective treatments for dystonia, there is significant interest in developing new therapeutics for the treatment of dystonia. There are several broad approaches to identifying new therapeutics: refining existing therapeutics to improve efficacy and limit off-target effects, identifying pharmacological targets that are implicated in multiple forms of dystonia, or investigating pharmacological targets that are implicated in other movement disorders, such as l-DOPA induced dyskinesias (LIDs). Below, we review recent preclinical and clinical work to identify new therapeutics and therapeutic targets. To date, most preclinical studies have focused on therapeutic targets in the basal ganglia. Although the cerebellum is clearly implicated (Tewari et al., 2017), less is known about targets in this region. For a summary of recent preclinical and clinical studies see Table 1.

5.1. Antimuscarinic receptor antagonists

While nonselective mAChR antagonists are effective in many forms of dystonia, they are often poorly tolerated due to debilitating side effects. Several studies have attempted to improve the therapeutic potential of mAChR antagonists by identifying the specific mAChR subtype(s) that mediate the therapeutic effects of THP and other nonselective mAChR antagonists. These studies have been facilitated by the recent development of truly selective mAChR antagonists and modulators (Bender et al., 2018; Lewis et al., 2008; Marlo et al., 2009; Wood et al., 2017).

In general, mAChR antagonists are thought to improve dystonia by modulating ACh at the striatum. Chls and cholinergic afferents from the PPN and laterodorsal tegmental nuclei provide ACh to the striatum, although most research has focused on Chls (Brindlecombe et al., 2018; Dautan et al., 2016). Chls constitute less than 1% of all neurons in the striatum, but they have broad axonal arborizations that cover most of the striatum (Gonzales and Smith, 2015). Chls are tonically active and are modulated by dopaminergic and glutamatergic afferents as well as GABAergic neurons in the striatum (Cai and Ford, 2018; Dawson et al., 1990; Kosillo et al., 2016; Tepper et al., 2008; for review see: Bonsi et al., 2011). ACh modulates striatal activity via both nicotinic acetylcholine receptors (nAChR) and mAChR. In the striatum, nAChR are expressed on presynaptic terminals of nigral dopaminergic afferents and glutamatergic cortical and thalamic afferents (Esley et al., 2008). mAChRs are divided into two broad classes: M1, M2, and M3 subtypes coupled to Gα_q, and M2 and M4 subtypes coupled to Gα_i/o. Corticostriatal terminals express M2, M4, and mAChR that modulate glutamate release (Girasole and Nelson, 2015; Pancani et al., 2014). Direct SPNs express M1, and M4 mAChR, while indirect SPNs express only M1 mAChR (Alcantara et al., 2001; Harrison et al., 1996; Hernandez-Flores et al., 2015). Both mAChR subtypes on SPNs modulate corticostriatal plasticity. In contrast, M2, and M4 mAChR are restricted to DA neurons and modulate DA release (Foster et al., 2014). Chls express M2 and M4 mAChR autoreceptors that reduce tonic firing and inhibit ACh release (Bonsi et al., 2008; Dawson et al., 1990; Girasole and Nelson, 2015; Threlfell et al., 2010).

To date, two mechanisms have been proposed to mediate the therapeutic effects of THP in dystonia, although these mechanisms are not mutually exclusive. A recent study found THP normalizes DA release in a mouse knockin model of DYT1 dystonia. This effect is likely mediated by M2 and/or M4 mAChRs on Chls and depends on nAChR to indirectly mediate the effect of THP on DA release (Downs et al., 2019). However, it is worth noting that this study did not specifically rule out other mAChR subtypes that may mediate the increase in DA release after THP administration. Indeed, previous studies have shown that M1, M4, and M6 mAChRs modulate DA release in striatal sections (Zhang et al., 2002). THP may also produce therapeutic effects by normalizing corticostriatal plasticity in dystonia. Previous studies have identified abnormal corticostriatal long-term depression (LTD) as a common pathology in Dyt1 mouse models (Maltese et al., 2018; Martella et al., 2009, 2014). Abnormal LTD is thought to be mediated by Chl dysfunction, which has been consistently identified in Dyt1 knockin mice (Scarduzio et al., 2017; Sciamanna et al., 2012). THP restores normal patterns of corticostriatal LTD (Dang et al., 2012; Martella et al., 2014) and this effect is mimicked by the M1 mAChR selective antagonist VU0255035 (Maltese et al., 2014). Alternatively, M1 mAChR antagonists may simply block the excitatory effects of M1 mAChR on striatal spiny projection neurons to ameliorate dystonia. However, THP may improve dystonia by other mechanisms, which may involve other mAChR subtypes and other brain regions. A study in a mouse model of DRD found that THP improves dystonic movements, although it is unclear if its therapeutic effects are restricted to the striatum or involve other brain regions (Rose et al., 2015). mAChRs are expressed throughout the basal ganglia and other areas of the brain (Weiner et al., 1990), and, accordingly, the therapeutic actions of THP may not be restricted to the striatum. One study in the dt^d^ hamster demonstrated that M1- and M4-preferring mAChR antagonists improved dystonia, although the mechanism of action was less clear given the limited mAChR subtype selectivity of the compounds used (Hamann et al., 2017). Therapeutics targeting specific mAChR subtypes may...
Table 1
Summary of recent preclinical and clinical work to identify new therapeutics and therapeutic targets for dystonia.

<table>
<thead>
<tr>
<th>Target Type</th>
<th>Type of study</th>
<th>Model/patient population</th>
<th>Compound</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimuscarinics</td>
<td>Clinical trial (randomized, double-blind, placebo)</td>
<td>31 adults (dystonia)</td>
<td>Trihexyphenidyl</td>
<td>Improved dystonia</td>
<td>(Burke et al., 1986)</td>
</tr>
<tr>
<td></td>
<td>Clinical trial (open label)</td>
<td>23 children (dystonia)</td>
<td>Trihexyphenidyl ethopropazine</td>
<td>Improved dystonia</td>
<td>(Fahn, 1983)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>Dyt1 knockin mice</td>
<td>Trihexyphenidyl</td>
<td>Normalized DA release</td>
<td>(Downs et al, 2019)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>Dyt1 knockin mice</td>
<td>Trihexyphenidyl U0255035 (M₃ antagonist)</td>
<td>Normalized corticostriatal plasticity</td>
<td>(Maltese et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>Trihexyphenidyl tropicamide</td>
<td>Improved dystonia</td>
<td>(Lees, 1984)</td>
</tr>
<tr>
<td></td>
<td>Case reports</td>
<td>2 adults (hemidystonia)</td>
<td>Nicotine (lozenges and transdermal patch)</td>
<td>Improved dystonia</td>
<td>(Vaughan et al., 1997)</td>
</tr>
<tr>
<td>Nicotinic receptors</td>
<td>Animal study</td>
<td>Dyt1 knockin mice</td>
<td>AZD4646 (non-desensitizing nAChR agonist)</td>
<td>No effect on DA release/overflow</td>
<td>(Zimmerman et al., 2017)</td>
</tr>
<tr>
<td>mGluR5 receptors</td>
<td>Animal study</td>
<td>Dyt1 knockin mice</td>
<td>ADX48621 (mGluR5 NAM)</td>
<td>Normalized corticostriatal plasticity</td>
<td>(Sciamanna et al., 2014)</td>
</tr>
<tr>
<td>A2A adenosine receptors</td>
<td>Animal study</td>
<td>Dyt1 knockin mice</td>
<td>KW6002 (A2A receptor antagonist)</td>
<td>Normalized corticostriatal plasticity</td>
<td>(Napolitano et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>Dyt1 knockin mice</td>
<td>SCH 58261 (A2A receptor antagonist)</td>
<td>Normalized corticostriatal plasticity</td>
<td>(Maltese et al., 2017)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>CPA (N(6)-cyclopentyladenosine (A1A receptor agonist)</td>
<td>Improved dystonia</td>
<td>(Richter and Hamann, 2001)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>Non-selective adenosine receptor antagonists (A1A receptor antagonists)</td>
<td>Worsened dystonia</td>
<td>(Richter and Hamann, 2001)</td>
</tr>
<tr>
<td>Endocannabinoid receptors (CB₁)</td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>WIN 55,212-2 (CB₁ agonist)</td>
<td>Improved dystonia</td>
<td>(Richter and Loscher, 1994)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>WIN 55,212-2 (CB₁ agonist)</td>
<td>Improved dystonia</td>
<td>(Richter and Loscher, 2002)</td>
</tr>
<tr>
<td></td>
<td>Clinical trial (randomized, double-blind, placebo controlled)</td>
<td>15 (primary dystonia)</td>
<td>Nabilone (CB₁/CB₂ agonist)</td>
<td>No improvement</td>
<td>(Fox et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Clinical trial (randomized, placebo controlled)</td>
<td>9 (cervical dystonia)</td>
<td>Dronabinol (CB₁/CB₂ agonist)</td>
<td>No improvement</td>
<td>(Zadikoff et al., 2011)</td>
</tr>
<tr>
<td>NMDA receptors</td>
<td>Clinical trial (open-label)</td>
<td>5 (undefined dystonia)</td>
<td>Camtadil (CB₁/CB₂ partial agonist)</td>
<td>Improved dystonia</td>
<td>(Consroe et al., 1986)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>NVP-AAM077 (NR2A antagonist)</td>
<td>Improved dystonia</td>
<td>(Avchalamov et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>Ro 25-6981 (NR2B antagonist)</td>
<td>Worsened dystonia</td>
<td>(Richter, 2003)</td>
</tr>
<tr>
<td>SV2A modulators</td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>Piracetam</td>
<td>Improved dystonia</td>
<td>(Loscher and Richter, 2000)</td>
</tr>
<tr>
<td></td>
<td>Animal study</td>
<td>dt¹ hamster</td>
<td>Breviaracetam</td>
<td>Improved dystonia</td>
<td>(Hamann et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Clinical trial</td>
<td>7 (cranial and oromandibular dystonia)</td>
<td>Levetiracetam</td>
<td>No improvement</td>
<td>(Park et al, 2017)</td>
</tr>
<tr>
<td></td>
<td>Clinical trial</td>
<td>10 (generalized/segmental dystonia)</td>
<td>Levetiracetam</td>
<td>No improvement</td>
<td>(Hering et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Case report</td>
<td>1 (generalized dystonia)</td>
<td>Levetiracetam</td>
<td>Significant improvement</td>
<td>(Sullivan et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Case report</td>
<td>1 (Meige’s syndrome)</td>
<td>Levetiracetam</td>
<td>Significant improvement</td>
<td>(Yardimci et al., 2006)</td>
</tr>
<tr>
<td>EF2α signaling</td>
<td>Animal study</td>
<td>Dyt1 knockin mice</td>
<td>Sahubral (EF2α dephosphorylation inhibitor)</td>
<td>Rescued ER function and abnormal torsinA localization</td>
<td>(Ritter et al., 2016)</td>
</tr>
<tr>
<td>BDNF signaling</td>
<td>Animal study</td>
<td>Dyt1 knockin mice</td>
<td>ANA-12 (TrkB inhibitor)</td>
<td>Normalized corticostriatal plasticity</td>
<td>(Maltese et al., 2018)</td>
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</table>
enhance the treatment of dystonia by minimizing off-target side effects. However, additional studies are needed to further characterize the role of each mAChR subtype in dystonia.

5.2. Nicotinic receptor agonists

Nicotinic receptor (nAChR) agonists have been proposed as novel therapeutics for dystonia based on evidence implicating abnormal striatal ACh signaling in animal models of dystonia (Eskow Jaunarajs et al., 2015), and from two case reports that show nicotine lozenges and patches are effective in treating dystonia (Lees, 1984; Vaughan et al., 1997). Nicotine is thought to improve dystonia by activating nAChRs on DA terminals in the striatum or DA cell bodies in the SNC, which increases the firing rate of DA neurons, increases striatal DA release, and changes the amplitude of DA release in response to tonic and phasic firing of DA neurons (Grenhoff et al., 1986; Rice and Cragg, 2004; Threlfell and Cragg, 2011). However, a recent study in a knockin mouse model of DT1 dystonia showed the non-desensitizing agonist, AZD1446, did not normalize or increase extracellular DA in this model (Zimmerman et al., 2017). In contrast to nAChR agonists, nAChR positive allosteric modulators may warrant further investigation because they might subtly increase DA release without desensitizing nAChRs.

5.3. Dopamine receptor agonists

DA modulates the activity of most striatal neurons including SPNs, the sole output neurons of the striatum. The two main classes of DA receptors are defined based on their ability to modulate adenylyl cyclase: D1-class DA receptors (D1, D5) which couple to Gαolf or Gαs, and D2-class DA receptors (D2, D3, D4) which couple to Gαi/o (Andersen et al., 1990; Kehabban and Calne, 1979; Niznik and Van Tol, 1992; Sibley and Monsma Jr., 1992; Tiberi et al., 1991). Within the striatum, D1 receptors are almost exclusively expressed on direct SPNs (Corvol et al., 2001; Zhuang et al., 2000). In contrast, D2 receptors are expressed on indirect SPNs and Chls, where they reduce neuronal firing, and on presynaptic DA terminals, where they inhibit DA release and regulate DA synthesis (Dawson et al., 1990; De Mei et al., 2009; Giros et al., 1989; Monsma Jr. et al., 1989; Usiello et al., 2000). D3, D4, and D5 receptors are also found in the basal ganglia at lower levels (Gainetdinov et al., 1996; Huntley et al., 1992; Missale et al., 1998; Rivera et al., 2002; Rondou et al., 2010).

Clinical research suggests a common cellular mechanism underlying various forms of dystonia is disrupted DA neurotransmission (Nemeth, 2002; Perlmutter and Mink, 2004; Wichmann, 2008). Reduced DA neurotransmission is associated with dystonia in inherited disorders such as DOPA-responsive dystonia (Furukawa et al., 1996; Ichinose et al., 1995, 2000), DA transporter deficiency syndrome (Kurian et al., 2011), amino acid decarboxylase deficiency (Brun et al., 2010), VMAT2 deficiency (Rilstone et al., 2013), and Lesch-Nyhan disease (Lloyd et al., 1981). Reduced DA neurotransmission is also observed in more common idiopathic dystonias such as writer’s cramp (Berman et al., 2013) and spasmodic dysphonia (Simonyan et al., 2013). Further, dystonia can occur in response to therapy with DA receptor antagonists (i.e. tardive dystonia) (Mahmoudi et al., 2014) and is often co-morbid with degenerative disorders that affect DA neurons such as Parkinson’s disease (Lopez-Ariztegui et al., 2009). These observations suggest that restoring striatal DA with direct or indirect DA antagonists should improve dystonia (Karimi and Perlmutter, 2015).

Paradoxically, DA agonists are seldom used to treat dystonia, with the exception of DRD. However, a recent meta-analysis suggests there is insufficient evidence in the literature to make definitive conclusions about the efficacy of DA agonists in dystonia (Fan et al., 2018). In fact, previous case reports and small clinical studies demonstrate that amphetamine (Myers and Loman, 1942), apomorphine (Micheli et al., 1982), bromocriptine (Stahl and Berger, 1981), and lisuride (Micheli and Fernandez Pardal, 1986) are effective in some patients with idiopathic dystonia. Apomorphine is a partial agonist at both D1 and D2 class receptors, whereas bromocriptine and lisuride are D2-like receptor agonists. D2-like receptors agonists are more commonly used to treat dystonia, whereas D1-like receptor-selective agonists are not yet available for use in humans, although they are effective in animal models of dystonia (Fan et al., 2018; Rose et al., 2015). It is thought that D2 and D1/D2 agonists improve dystonia by mimicking normal DA receptor activation of SPNs, which normalizes the activity of the direct and indirect pathways in the basal ganglia (Rose et al., 2015).

Recent studies in animal models of dystonia, especially Dyt1 mice, have suggested that D2 receptors on Chls may be a target for therapeutics. Chls exhibit abnormal responses to D2 receptor activation in several Dyt1 mouse models. While D2 receptor activation normally reduces the firing rate of Chls, D2 receptor activation increases Chl firing rate in Dyt1 mice (Napolitano et al., 2010; Sciamanna et al., 2012, 2014). This abnormal D2 receptor signaling is mediated by a switch from Gαi/o protein signaling to β-arrestin signaling (Bonisi et al., 2019; Scarduzio et al., 2017). Furthermore, a recent study found that overexpressing the striatal-specific RGS9-2 regulatory protein functionally inhibits β-arrestin and restores normal D2 receptor function in Chls of Dyt1 mice (Bonisi et al., 2019). Taken together, these studies suggest that D2 receptor agonists biased towards G-protein mediated signaling may be effective therapeutics in DT1 dystonia. These studies also identify RGS9-2, a target previously proposed for other disorders (Sjögren, 2017; Traylor et al., 2009), as a novel therapeutic target for dystonia. Future studies should evaluate the therapeutic efficacy of RGS9-2 potentiators in dystonia.

5.4. mGluRs negative allosteric modulators (NAMS)

The effects of glutamate are mediated by two families of receptors, metabotropic and ionotropic, which are categorized by their mode of signal transduction. Metabotropic glutamate receptors are subdivided into three groups: Group I (mGluRs 1 and 5), Group II (mGluRs 2 and 3), and Group III (mGluRs 4, 6, 7, and 8) (Conn and Pin, 1997). In general, Group I mGluRs are located postsynaptically and couple to Gαq/11, whereas Groups II and III are localized presynaptically and couple to Gαi/o (Niswender and Conn, 2010). Group I mGluRs have been most extensively evaluated for therapeutic efficacy in dystonia. Within the striatum, mGluR5 receptors are expressed on SPNs as well as FSIs, Chls, and low-threshold spike interneurons (Conn et al., 2005; Hubert et al., 2004). On SPNs, mGluR5 and NMDA receptors are tethered by scaffolding proteins and activation of mGluR5 receptors amplifies NMDA receptor-mediated currents (Conn et al., 2005; Pisani et al., 2001). On indirect SPNs, mGluR5 receptors physically interact with A2A adenosine receptors to synergistically promote MAPK signaling, thereby counteracting the effect of D2 receptor activation (Ferre et al., 2002; Nishi et al., 2003). Further, Group I mGluRs are expressed on cell bodies and terminals of dopaminergic neurons from the SNc (Conn et al., 2005; Hubert et al., 2001). Outside of the striatum, Group I mGluRs are expressed on GABAergic neurons located in the globus pallidus and SNr.

Given the broad distribution of mGluRs in the basal ganglia, mGluR5 negative allosteric modulators (NAMS) have been proposed as a new therapeutic in dystonia. One study in Dyt1 knockin mice showed that the mGluR5 NAM ADX48621 restores normal responses to D2 receptor activation of striatal Chls (Sciamanna et al., 2014). Neither mGluR5 NAMS nor antagonists have been tested in dystonia patients, but tests in both animal models of LIDs and patients with LIDs have shown promising results (Bezard et al., 2014; Dekundy et al., 2011; Tison et al., 2016). Future studies examining metabotropic glutamate receptors in dystonia will be aided by the recent discovery of a number of subtype-selective mGluR negative and positive allosteric modulators (Bollinger et al., 2017; Nichols et al., 2016; Panarese et al., 2019; Reed et al., 2019).
5.5. NMDA receptor antagonists

Ionotropic glutamate receptors are subdivided into three classes named according to their affinity for their selective agonists: α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), kainic acid or kainate (KA), and N-methyl-D-aspartate (NMDA) (Traynelis et al., 2010). NMDA receptor antagonists have been most extensively studied for treatment of dystonia. NMDA receptor complexes are composed of NR1, NR2A-D and NR3A-B subunits (Kew and Kemp, 2005). The NR1 subunit is expressed ubiquitously throughout the brain, whereas the NR2 subunits have regional patterns of distribution and confer distinct electrophysiological and pharmacological properties to NMDA receptor complexes (Traynelis et al., 2010). In the adult brain, striatal SPNs are enriched for NR2A and NR2B subunits (Dunah and Standaert, 2003; Goebel and Poosch, 1999; Kosinski et al., 1998; Landwehrmeyer et al., 1995; Monyer et al., 1994; Smeal et al., 2008; Standaert et al., 1994). In contrast, the globus pallidus and STN primarily express NR2A and NR2D subunits (Wenzel et al., 1997; Wenzel et al., 1996).

NMDA receptor antagonists, such as amantadine, are sometimes used to treat dystonia (Borison, 1983; Gilbert, 1971). The use of amantadine is limited due to significant side effects including: constipation, cardiovascular dysfunction, hallucinations, delirium, and anxiety (Perez-Lloret and Rascal, 2018). In Dyr1 knockin mice, there is a selective increase in NR2A, but not NR2B, subunits at striatal post-synaptic sites (Malaste et al., 2018). In the dtz hamster, an NR2A-prefering antagonist improved dystonia, while NR2B-prefering antagonist worsened dystonia (Avchalumov et al., 2014; Richter, 2003). Additional studies in the dtz hamster found that antagonists with equal affinity for NR2A/NR2B produced moderate improvement (Sander and Richert, 2007). Interestingly, this is in agreement with studies in rodent models of LDs, in which NR2A-prefering antagonists were effective in reducing abnormal movements (Gardoni et al., 2012), while NR2B-prefering antagonists exacerbated abnormal movements (Nash et al., 2004). These studies suggest that more selective NR2A-prefering antagonists, or even NAMs, might be effective therapeutics for dystonia and could reduce some of the negative side effects that limit the use of nonselective NMDA receptor antagonists. NMDA receptor antagonists have not been tested in dystonia patients. However, one clinical trial found that the NR2B selective NMDA receptor antagonist MK-0657 did not improve dyskinesias or motor symptoms in patients with Parkinson’s disease, which agrees with preclinical studies (Herring et al., 2017).

5.6. Adenosine A2A receptor antagonists

Adenosine acts on two different G-protein coupled receptors within the basal ganglia: A1 and A2A. A1 adenosine receptors are located pre-synaptically on corticostriatal afferents, and postsynaptically on striatonigral and striatopallidal SPNs as well as Chls (Alexander and Reddington, 1989; Fastbom et al., 1987; Ferre et al., 1996; Preston, 2000). Activation of Gαq/11-coupled A1 adenosine receptors decreases cAMP levels, increases K+ conductance, and decreases transient Ca2+ levels (Ambrosio et al., 1996), thereby decreasing the likelihood of neurotransmitter release from presynaptic terminals (Fredholm, 1995). A2A adenosine receptors are selectively expressed on striatopallidal SPNs and Chls (Fink et al., 1992; Preston et al., 2000; Schiffmann et al., 1991). Activation of Gαq/11-coupled A2A adenosine receptors increases cAMP levels, activating PKA and MAPK signaling (Schulte and Fredholm, 2003). Interestingly, A2A adenosine receptors also form heteromeric complexes with D2 receptors (Canals et al., 2003; Ferre et al., 2008; Fuxx et al., 2005; Hillion et al., 2002) and mGlR5s (Ferre et al., 2002; Morelli et al., 2007).

Studies in animal models suggest A2A adenosine receptor antagonists may be effective therapeutics. A2A adenosine receptor antagonists restore normal patterns of corticostriatal plasticity in Dyr1 (Napolitano et al., 2010) and Dyr11 mice (Malaste et al., 2017). In contrast, studies in the dtz hamster showed that A1- and A2A-selective adenosine receptor antagonists improved dystonia, while nonselective adenosine receptor antagonists worsened dystonia. A2A-selective adenosine receptor antagonists had no effect on dystonia severity in these hamsters (Richter and Hamann, 2001). Clinical trials using adenosine receptor compounds have not been conducted in dystonia patients. However, A2A adenosine receptor antagonists have been investigated in animal models of Parkinson’s disease and produce limited improvement in dyskinesia symptoms in clinical trials (LeWitt et al., 2008; Nunez et al., 2018; Wang et al., 2017).

5.7. Cannabinoid receptor antagonists

Most research has focused on type 1 (CB1) and type 2 (CB2) cannabinoid receptors. Both receptors are coupled to Gαq/11 and are activated by retrograde signaling of the endocannabinoids arachidonolide ethanolamide (anandamide or AEA) and 2-arachidonoyl glycercol (2-AG) (Freund et al., 2003; Kreitzer and Malenka, 2005; Mechoulam and Parker, 2013; Tanimura et al., 2010). The CB1 receptor is densely expressed in the striatum (Fusco et al., 2004; Herkenham et al., 1991; Matyas et al., 2006). CB1 receptors are localized to corticostriatal terminals, where they modulate cortical excitation of SPNs (Gerdeman and Lovinger, 2001; Wu et al., 2015). Additionally, CB2 receptors are robustly expressed on SPN axon terminals and have also been observed on striatal parvalbumin-containing GABA interneurons (Hohmann and Herkenham, 2000; Uchigashima et al., 2007). Unlike the CB1 receptor, the CB2 receptor was historically considered a peripheral cannabinoid receptor. However, the CB2 receptor is also expressed in the striatum and globus pallidus, albeit at significantly lower levels of expression than the CB1 receptor (Callen et al., 2012; Gong et al., 2006; Lanciego et al., 2011). A recent study found that CB2 receptors are expressed on striatal DA terminals and decrease striatal DA release through retrograde endocannabinoid signaling (Foster et al., 2016).

There has been significant interest in endocannabinoids in the treatment of movement disorders (Kluger et al., 2015; Koppel, 2015; Lim et al., 2017; Peres et al., 2018; Saft et al., 2018). Two studies in the dtz hamster model of dystonia found that the CB1/CB2 receptor agonist WIN 55,212-2 alleviated dystonia, and this effect was blocked by a CB1 receptor-specific antagonist (Richter and Loscher, 1994; Richter and Loscher, 2002). While animal studies have been generally positive, the few clinical trials and case reports in dystonia have been mixed. No improvement was observed in response to the CB1/CB2 receptor agonist nabilone (Fox et al., 2002), and another small placebo-controlled trial found no improvement for cervical dystonia in response to the CB1/CB2 agonist dronabinol (Zadikoff et al., 2011). However, an earlier study found significant improvement with the CB1/CB2 partial agonist cannabidiol in a small open-label trial (Consroe et al., 1986). Given the limited size of past clinical trials and inconsistency in results, further exploration of endocannabinoids for the treatment of dystonia is warranted.

5.8. Synaptic vesicle protein 2A (SV2A) modulators

SV2A modulators, including levetiracetam, were originally developed for epilepsy, but studies in rodents and humans have suggested therapeutic potential in dystonia. Levetiracetam is specific to the synaptic vesicle associated protein SV2A, which is found in all neurons in the CNS (Bajjalieh et al., 1993). Levetiracetam is thought to modulate the function of SV2A to reduce neurotransmitter release (Lynch et al., 2004). Several different SV2A modulators, including levetiracetam, improve dystonia in the dtz hamster model (Hamann et al., 2008; Loscher and Richter, 2000). However, the exact mechanism of action of levetiracetam in dystonia is unknown. One possibility is that SV2A modulators reduce cortical excitability, because an increase in cortical excitability is observed in multiple forms of dystonia in humans (Calabresi et al., 2016; Erro et al., 2018; Furuya et al., 2018; Hallett, 2004). Several different SV2A modulators, including levetiracetam, improve dystonia in the dtz hamster model (Hamann et al., 2008; Loscher and Richter, 2000). However, the exact mechanism of action of levetiracetam in dystonia is unknown. One possibility is that SV2A modulators reduce cortical excitability, because an increase in cortical excitability is observed in multiple forms of dystonia in humans (Calabresi et al., 2016; Erro et al., 2018; Furuya et al., 2018; Hallett, 2004).
5.10. Phosphodiesterase inhibitors (PDE10A)

Phosphodiesterases are responsible for terminating cellular cAMP and cGMP signaling. Accordingly, PDE inhibitors are expected to increase neuronal activity in the brain by enhancing cAMP and cGMP signaling. PDE10A is of specific interest to movement disorders because it is highly expressed throughout the basal ganglia, including direct and indirect pathway SPNs (Svedberg et al., 2019). A recent study in a transgenic mouse model of DYT1 dystonia found inverse changes in PDE10A expression in direct and indirect pathway SPNs (D’Angelo et al., 2017). PDE10A expression was decreased in direct SPNs, but increased in indirect SPNs. These changes are consistent with increased activation of the direct striatonigral pathway and reduced activity in the indirect striatopallidal pathway. There are no clinical trials of PDE10A inhibitors in dystonia. However, PDE10A inhibitors improve motor abnormalities in a mouse model of Huntington’s disease and a non-human primate model of LIDs (Beaumont et al., 2016; Beck et al., 2018). Future studies are needed to determine if PDE10A inhibition modifies dystonia.

5.11. Brain derived neurotrophic growth factor (BDNF)

Brain derived neurotrophic growth factor (BDNF) is a widely distributed neurotrophin in the mammalian brain. BDNF binds and activates TrkB to regulate a variety of cellular processes related to neuronal and glial development and synaptic plasticity (Huang and Reichardt, 2001). Because of the importance of BDNF to neuronal plasticity, it is of significant interest in many neurodevelopmental diseases. A previous study has shown that, in early development, there is increased expression of BDNF in Dyt1 mice relative to WT littermates. This parallels the development of abnormal synaptic plasticity in Dyt1 mice and suggests a critical period for targeted intervention. In vivo administration of a TrkB inhibitor during this critical window rescued the abnormal synaptic plasticity, which identifies TrkB/BDNF signaling as a potential therapeutic target for DYT1 dystonia (Maltese et al., 2018). In the dt<sup>cm</sup> hamster, changes in BDNF mRNA or protein expression were not detected (Bode et al., 2017). There is some evidence to suggest a polymorphism in the proband region of the BDNF gene may confer risk for developing cervical dystonia and/or blepharospasm (Chen et al., 2013; Siokas et al., 2018). The SNP rs6265 (G/A) (AF50.1896) results in the substitution of Val in amino acid position 66 with Met (val66met), and healthy carriers of the val66met have been reported to show differences in brain structure and abnormal motor cortex plasticity (Cheeran et al., 2008; Pezawas et al., 2004). However, a direct relationship between the val66met variant, abnormal motor cortex plasticity, and dystonia has yet to be uncovered. Taken together, these human genetics and preclinical studies support further investigation of TrkB/BDNF signaling as a potential therapeutic target in dystonia, as proposed for other neurological and psychiatric disorders (Deng et al., 2016; Longo et al., 2013; Nagahara et al., 2011).

6. Future directions for novel experimental therapeutics in dystonia

Despite advances in our understanding of the genetic and anatomical bases of dystonia, pathogenesis-targeting or disease-modifying therapies remain limited. While the limited oral therapies for dystonia are sometimes effective, patients often discontinue treatment due to dose-limiting side-effects, insufficient efficacy, or both. Recent advances in our understanding of the pathophysiological mechanisms of dystonia have revealed new therapeutic targets that deserve careful validation for clinical use. One challenge for drug discovery in dystonia is the heterogeneity of etiologies and disease presentations in the clinical population. The availability of a multitude of animal models for different forms of dystonia with construct and predictive validity will be instrumental for identifying promising common molecular targets. This, in turn, will facilitate clinical trials by identifying the most appropriate patient populations in which to test new, targeted therapies based on shared biological processes and molecular targets. Overall, recent advances in both basic and clinical research provide promising new platforms for the development of novel therapeutics.

Declarations of Competing Interest

None.

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