Molecular Neurobiology

ISSN0893-7648/98/16(2): 135–147/$11.25

Mutational and Biochemical Analysis of Dopamine in Dystonia

Evidence for Decreased Dopamine D_2 Receptor Inhibition

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Abstract

The dystonias are a group of serious movement disorders characterized by involuntary muscle spasms of different parts of the body. We recently proposed that hypofunction of dopamine D_2 receptor-mediated inhibition of the indirect output pathway of the basal ganglia can result in dystonia. In this review, we discuss the results of a variety of genetic and biochemical studies in light of this hypothesis. Several forms of early-onset dystonia show distinct autosomal dominant, recessive, or X-linked genetic transmission patterns. Late onset forms of dystonia, though not showing clear Mendelian transmission patterns, also appear to be highly familial. Recently, several genetic-linkage locations have been identified for early-onset dystonia and for two of these loci, mutations decreasing dopamine synthesis have been demonstrated. Biochemical studies of monkeys and man also demonstrate that several types of dystonia occur in a dopamine-deficiency state. Similarly, mice strains developed to be deficient in several dopamine-pathway components have motor abnormalities consistent with dystonia. Hypofunction of the dopamine D_2 receptor-mediated inhibition of the indirect output pathway of the putamen may be a common feature of many of these heritable and secondary dystonic syndromes.

Index Entries: Dystonia; dopamine; putamen; tyrosine hydroxylase; GTP cyclohydroxylase; dopamine receptors.

Introduction

The dystonias are a group of syndromes characterized by involuntary muscle spasms of different parts of the body. Dystonias are frequently classified by age of onset (childhood vs adulthood) muscle groups affected (for example, dystonic hand cramp, cranial dystonia, or generalized dystonia) and cause (symptomatic vs idiopathic). A variety of conditions

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may produce symptomatic dystonias including strokes involving the putamen (Bhatia and Marsden, 1994), acute dopamine-receptor blockade by antipsychotic drugs (Garver et al., 1976; Kolbe et al., 1981; Rupniak et al., 1996) or levo-DOPA-associated dopamine excess in patients or animals with preexisting striatal injury (Mitchell et al., 1990). Recently we reported that 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which can cause parkinsonism (Burns et al., 1983; Ballard et al., 1985), also causes transient dystonia associated with striatal dopamine deficiency and decreased dopamine D_2-receptor number (Perlmutter et al., 1997; Todd et al., 1966). These observations about symptomatic dystonia suggest that hypofunction of D_2 receptor-mediated inhibition of the indirect-output pathway of the putamen may be a common mechanism in the pathophysiology of dystonia. In this review article we summarize recent findings on the mutational analysis of familial forms of human dystonia, the behavioral consequences of the production of inbred mouse strains lacking components of the dopamine-pathway system, and on biochemical findings in primates and humans regarding dopaminergic function in dystonia. These studies support the notion that a substantial fraction of idiopathic cases of dystonia are caused by genetic factors that decrease the function of dopamine D_2 receptor-mediated inhibition of pathways involving the putamen.

Several lines of evidence suggest that dysfunction of the putamen may cause dystonia. The putamen is part of a large number of interconnected nuclei termed the basal ganglia that express multiple neurotransmitters and comodulators that affect signaling through these regions (Gerfen, 1992; Alexander and Crutcher, 1990; DeLong, 1990). This complexity has led to several models of the function of basal ganglia pathways. One popular model describes multiple, cortical-striatal-pallido-thalamic-cortical loops (Fig. 1). The cortico-striate projection fibers of the motor loop predominantly target the putamen (Gerfen, 1992; Alexander and Crutcher, 1990; Albin et al., 1989; Gerfen

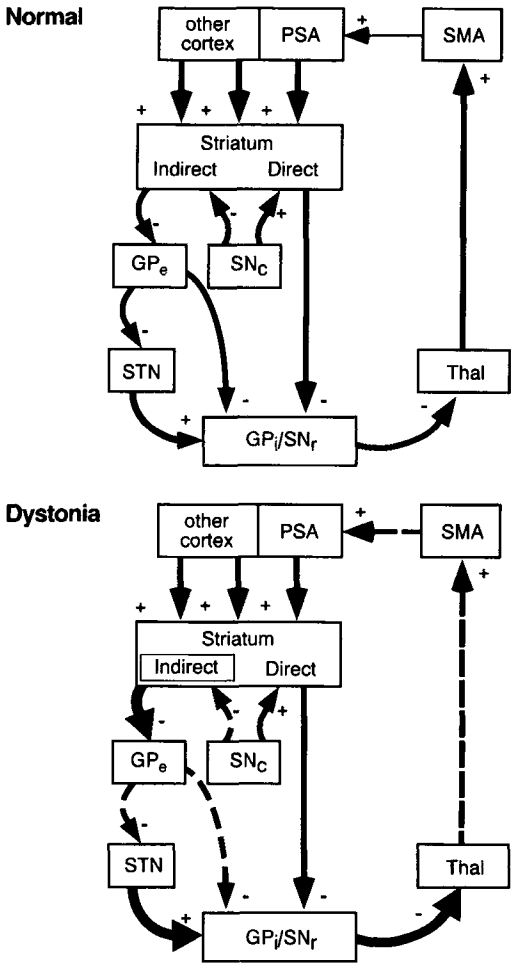

Fig. 1. Effect of decreased dopamine D_2-receptor inhibition of the indirect output pathway on activity of the cortico-striatal-pallido-thalamic-cortical motor loop. The upper figure shows the proposed motor loop under normal conditions (Gerfen, 1992; Alexander and Crutcher, 1990; Albin et al., 1989; Gerfen et al., 1990). The lower figure shows predicted changes in activity following decreased inhibitory dopaminergic input to the indirect output pathway (dashed arrow from SNc to striatum). Thicker arrows indicate increased and dashed arrows indicate decreased activity. Pluses indicate excitatory and minuses inhibitory connections. Abbreviations: GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SMA, supplementary motor area; PSA, primary sensorimotor area; STN, subthalamic nucleus; Thal, thalamus.

et al., 1990). From there, two major pathways lead to the major output nucleus of the basal ganglia, namely, the internal segment of the pallidum (GPi); the direct pathway via inhibitory GABAergic fibers connecting striatum and GPi, and the indirect pathway including the inhibitory GABAergic neurons from striatum to the external segment of the pallidum (GPe), inhibitory neurons projecting from GPe to subthalamic nucleus (STN) and excitatory neurons projecting from STN to GPi. Both the indirect and the direct pathways converge on GPi, which then sends inhibitory GABAergic neurons to the ventral anterior thalamus that then projects excitatory neurons to cortical areas including premotor and motor regions. There are multiple additional anatomical connections among the basal ganglia nuclei that may modify the response of these two pathways. Pharmacologically, postsynaptic dopamine D_2-like receptors predominantly localize to and inhibit the striato-pallidal neurons of the indirect pathway projecting to GPe, whereas postsynaptic dopamine D_1-like receptors predominately localize to and facilitate the neurons of the direct pathway that project from striatum to GPi (Gerfen, 1992; Gerfen et al., 1990; Keefe and Gerfen, 1995). The dopaminergic input to both the direct and indirect pathways in putamen is from dopamine-producing cells of the substantia nigra pars compacta. The direct pathway via two inhibitory connections from putamen to thalamus provides a net *positive* feedback, whereas the indirect pathway via two inhibitory, and excitatory and another inhibitory connections provides a net *negative* feedback to the thalamus and subsequently to cortical areas.

How these two pathways interact and how the basal ganglia control movement remain controversial (Mink and Thach, 1993; Mink, 1996). One view is that the balance of the two pathways allows initiation or inhibition of movement. Alternatively, the function of the indirect pathway may be to inhibit unwanted muscle contractions during an intentional movement, whereas the direct pathway focally permits the movement (Mink and Thach, 1993; Mink, 1996). In either event, there is differential dopaminergic control of the two pathways by specific dopamine-receptor classes (Gerfen, McGintry, and Young, 1991).

Therefore, dopamine and dopamine receptors play a key role in the modulation of the direct and indirect pathways. Dopamine is synthesized from dietary tyrosine via conversion to DOPA by the enzyme tyrosine hydroxylase (TH) and subsequent decarboxylation to dopamine by DOPA decarboxylase (DDC). The rate-limiting step in the production of dopamine is the activity of the enzyme tyrosine hydroxylase. TH activity is dependent on its phosphorylation state and on availability of the cofactor tetrahydrobiopterin which is synthesized by the GTP cyclohydroxylase I gene product (GCH). At synapses, dopamine interacts with at least five types of dopamine receptors that are the products of separate genes and are usually grouped into two gene families. The D_1-like family includes D_1 and D_5 receptors, which are probably exclusively expressed postsynaptically and are thought to be stimulatory. The D_2-like receptor gene family includes D_2, D_3, and D_4. In the basal ganglia D_2 and D_3 receptors may be expressed postsynaptically and presynaptically as autoreceptors on dopamine-producing cells and are thought to be inhibitory (Strange, 1993; Civelli, Bunzow, and Grandy, 1993; O'Hara et al., 1996). The D_4 receptor is expressed at very low levels in the normal basal ganglia. Postsynaptic D_1-like receptors appear to localize to and facilitate the neurons of the direct pathway. D_2 and D_3 receptors predominantly localize to and appear to inhibit the neurons of the indirect pathway of the basal ganglia. In primate brain the D_2 receptor is more abundant in regions of the basal ganglia associated with movement, whereas the D_3 receptor is preferentially expressed in regions associated with the limbic system.

There has been controversy regarding the coexpression of D_1-like and D_2-like receptors on individual neurons (Surmeier et al., 1993). Complete cellular separation of the D_1-like and D_2-like receptors, however, is not a pre-

requsite for the hypothesis of D_2-like receptor modulation of the indirect pathway and D_1-like receptor modulation of the direct pathway. This hypothesis only requires that there be preferential effects of these two receptor families for input to the direct and indirect pathways. As will be described next, we have experimental evidence that dystonia is associated with hypofunction of the dopamine D_2-like receptors linked to the indirect basal ganglia output pathway.

An Indirect Pathway Hyperfunction Model of Dystonia

Based on the results of MPTP lesioning studies of baboons (Todd et al., 1966) and imaging studies of human dystonia (Tempel and Perlmutter, 1990; Perlmutter et al., 1997) we have proposed that a common mechanism for the production of dystonia is a decrease in dopamine D_2-receptor inhibition of the indirect output pathway of the putamen (Perlmutter et al., 1997, 1993). As previously found by others (Bankiewicz et al., 1986; Guttman et al., 1990; Palombo et al., 1990; Joyce et al., 1986) unilateral intracarotid injection of MPTP in baboons results in the development of stable contralateral hemi-parkinsonism over the course of several months (mean onset of parkinsonian symptoms 49.8 d, mean for plateau of symptoms 159 d) (Perlmutter et al., 1997). However, we also observed the development of a transient contralateral hemidystonia within a few days of MPTP injection and prior to the development of any parkinsonian symptoms (mean onset of dystonic symptoms 4.9 d, mean for peak of symptoms 11.4 d). The dystonia phase was associated with a 98% reduction in ipsilateral putamen and caudate dopamine content and a 20–40% *bilateral* reduction in D_2-like receptor binding (Todd et al., 1966). The resolution of the dystonia phase correlated with an increase in D_2-like receptor binding. Interestingly, even though dopamine content decreased unilaterally, dopamine D_2-like receptor number changed bilaterally across the time-period studied.

Although we have not proven whether these transient changes in D_2-like receptor binding reflect decreases in pre- or postsynaptic receptors, or both, we propose that dystonia is associated with a net reduction in D_2-like receptor-mediated inhibition producing an increase in activity of the indirect striatal-pallidal output pathway, namely, increases in input to GP_i (Fig. 1). A decrease in postsynaptic D_2 receptors or a loss of presynaptic D_2 receptors accompanied by an overwhelming loss of striatal dopamine would produce the same net effect. For the models of basal ganglia function described above, a relative increase in activity to GP_i would produce an increase in inhibition of thalamus and subsequently reduced output to the supplementary motor area (SMA) and the primary sensorimotor area (PSA). Consistent with the hypothesis that relative hyperactivity of the indirect pathway results in dystonia, we have observed a 25% decrease in vibration induced cerebral blood flow to PSA (Tempel and Perlmutter, 1990, 1993) and SMA (Tempel and Perlmutter, 1993) in idiopathic dystonia patients. Interestingly, the decrease in stimulated blood flow was seen bilaterally even though most dystonia subjects had unilateral, focal dystonias (primarily writer's cramp).

To further characterize dopaminergic function in dystonic individuals, we have also estimated dopamine D_2-like receptor binding using [^{18}F]spiperone (Perlmutter, 1997). Consistent with our data for D_2-like receptor changes in MPTP-treated animals (Todd et al., 1996), there was a 29% reduction in [^{18}F]spiperone binding in individuals with idiopathic blepharospasm or oromandibular focal dystonias (Perlmutter et al., 1997). Similar to results on vibration induced cerebral blood flow changes (Tempel and Perlmutter, 1990, 1993) and D_2-like receptor changes in MPTP-induced dystonia (Perlmutter et al., 1997), the reductions in [^{18}F]spiperone binding were *bilateral*.

Several studies by others provide support for the hypothesis that decreased dopaminergic

function is an important component of the pathophysiology of dystonia. For example in idiopathic dystonia, [^{18}F]DOPA uptake in putamen may be reduced (Playford, 1993); cerebrospinal fluid homovanillic acid may be decreased (Ashizawa et al., 1980); and a single patient with idiopathic generalized dystonia was reported to have decreased striatal dopamine (Horneykiewicz et al., 1986). Another patient with a lesion on one side of the midbrain associated with hemiatrophy and hemidystonia subsequently developed hemiparkinsonism (Lang, 1995). Although the exact cause of this unusual syndrome is unclear, it suggests that striatal dopamine deficiency can lead to either dystonia or parkinsonism. Strikingly, the decreased vibration-induced cerebral blood flow changes in PSA we found in focal dystonia (Tempel and Perlmutter, 1990, 1993) could be normalized by levo-DOPA administration in one patient with DOPA-responsive dystonia (Perlmutter et al., 1997).

These studies support the hypothesis that decreased dopamine D_2-like receptor activity, whether secondary to decreased dopamine or to decreased postsynaptic receptor number or coupling, produces a relative increase in output activity of the indirect putamen pathway, eventual decreased activity of motor cortex and the development of muscle spasms. Decreased inhibition or altered modulation of the indirect pathway could be consistent with normal initiation of voluntary movement but loss of ability to inhibit unwanted involuntary movements in nearby parts of the body as the movement continues. This is typical of dystonia as involuntary postures and muscle spasms frequently occur only during a specific motor activity and not at rest. The involuntary spasms spread as the movement persists with the loss of "surround inhibition" mediated by the indirect pathway (Mink and Thach, 1993; Mink, 1996). Presumably, the specific site of putaminal dysfunction would influence which part of the body developed dystonia.

Of course, the results of individual studies could be interpreted differently to support other hypotheses about the pathophysiology of dystonia. As outlined below, however, several other investigations provide data compatible with genetic abnormalities leading to increased output of the indirect pathway and causing idiopathic dystonia.

Family and Linkage Studies of Dystonia

Several important genetic factors found to contribute to the risk of developing dystonia provide substantial evidence supporting our hypothesis about the pathophysiology of dystonia. Three types of studies have evaluated the importance of genetic factors in different types of dystonia: family, linkage, and candidate gene approaches. As shown in Table 1, four known chromosomal locations for clearly heritable forms of dystonia have been demonstrated. Three of these (chr 14q, chr 9q34, and chr xq13.1) were identified using genetic linkage approaches in relatively large families with Mendelian transmission patterns. For the chr14q chromosomal location for DOPA-responsive dystonia subsequent gene analysis has demonstrated a variety of causative mutations in the GTP cyclohydrolase I gene (GCH) (reviewed in Nagatsu and Ichinose [1996] and Williams [1995]). The gene product for the chr 9q34 idiopathic torsion dystonia locus (Ozelius et al., 1989, 1992, 1997) is an ATP-binding protein. The chr Xq13.1 adult-onset dystonia-parkinsonism gene is unknown (Haberhausen et al., 1995). In contrast, the chr 11p DOPA-responsive-dystonia locus was determined by direct mutational analysis of the candidate gene tyrosine hydroxylase (Knappskog, 1995). Subsequent chemical characterization of the protein products of the TH (Knappskog, 1995) and GCH (Nagatsu and Ichonese, 1996) mutations have demonstrated reduced enzymatic activities consistent with the responsiveness of these syndromes to levo-DOPA therapy (Table 2).

In addition to the results of linkage and candidate gene studies demonstrating the in-

Table 1
Known Chromosomal Locations of Dystonia Genes

Type of dystonia	Chromosomal location	Gene	Reference
DOPA-responsive dystonia	11p	Tyrosine hydroxylase	Knappskog et al., 1995
	14q	GTP cyclohydrolase I	Nagatsu and Ichinose, 1996
Idiopathic torsion dystonia	9q34	ATP-binding protein	Ozelius et al., 1989, 1992, 1997
X-linked adult onset Dystonia-Parkinsonism	Xq13.1	Unknown	Haberhausen et al., 1995

Table 2
Mutations Affecting Dopamine Function

Gene	Species	Type of mutation	Dopaminergic biochemical effect	Movement disorder	Reference
Dopamine receptors					
D1	Mouse	Knockout	No D1 receptors	↓ Rearing behavior	Drago et al., 1994
D2	Mouse	Knockout	No D2 receptors	Parkinsonism	Baik et al., 1995
D3	Mouse	Knockout	No D3 receptors	Hyperactive	Accili et al., 1995
D4	Human	Null	Unknown	None	Nöthen et al., 1994
	Human	Missense	Unknown	None	Liu et al., 1996
Catecholamine pathway enzyme					
Tyrosine hydroxylase (TH)					
	Mouse	Knockout	No catecholamines	(Perinatal death)	Zhou et al., 1995
	Mouse	Knockout	↓Dopamine	Hypoactive	Zhou and Palmiter, 1995
	Human	Missense	↓ TH activity	DOPA-responsive dystonia	Knappskog et al., 1995
	Human	Missense	↓ TH activity	DOPA-responsive parkinsonism	Ludecke et al., 1996
GTP cyclohydrolase I (GCH)					
	Human	Missense	No GCH activity	Severe hypotonia	Nagatsu and Ichinose, 1996
	Human	Various	≤ 20% GCH activity	DOPA-responsive dystonia	Nagatsu and Ichinose, 1996
Dopamine β hydroxylase (DBH)					
	Mouse	Knockout	No norepinephrine or epinephrine	None	Thomas and Palmiter, 1997
Other genes					
Dopamine transporter (DAT)					
	Mouse	Knockout	No DAT, ↑ Dopamine in synapse	Hyperactivity	Giros et al., 1996
Hypoxanthine phosphoribosyl transferase (HPRT)					
	Human	Various	60–90% ↓ Dopamine	Dystonia, chroreoathetosis	Jinnah et al., 1994
	Mouse	Knockout	20–50% ↓ Dopamine	None	Jinnah et al., 1994

volvement of genes of major effect in certain familial forms of dystonia, studies of the recurrent risk of dystonia among relatives of dystonic patients suggest that a significant proportion of cases of primary or idiopathic dystonia also involve prominent genetic factors. For example, two studies have determined the recurrence risk of dystonia in families in whom the probands (index cases) had torticollis or focal dystonias (Waddy et al., 1991; Stojanović, et al., 1995). Twenty to twenty-five percent of the probands had relatives with dystonia. Of particular interest, there was only a trend for relatives to have the same type of dystonia as the probands. This suggests that familial forms of dystonia can have various expressions in different family members. In both studies, traditional segregation analysis of the pattern of recurrence risk in relatives was consistent with the presence of an autosomal dominant gene or genes with reduced penetrance. Similarly, Micheli et al. (1994) have described two families identified through probands with focal dystonia which had marked variability of disease expression in relatives and Brashear et al. (Brashear et al., 1996) have described variable familial phenotypes for rapid-onset dystonia-parkinsonism. In the Ashkenazi Jewish population idiopathic torsion dystonia has been linked to the chromosome 9q34 region in the majority of affective families (Ozelius et al., 1989, 1992; Bressman et al., 1994a). However, in some Ashkenazi Jewish families with torsion-dystonia linkage to this region can be excluded. Moreover, Ashkenazi Jewish patients with focal hand dystonias (musician's cramp or writer's cramp) do not have the founder haplotype that has been found in greater than 90% of cases of torsion dystonia in this population (Gasser et al., 1996). Hence, in both the general population and in special populations such as the Ashkenazi Jews, there is evidence for marked genetic hetrogeneity of generalized dystonia and evidence for marked familiality of focal dystonias. As described above, within families there may be a variety of presentations of dystonia type. This appears to be especially true for adult onset dystonias.

There is mounting evidence that a significant fraction of idiopathic cases of early-onset and late-onset dystonia have significant genetic factors predisposing to illness. It should be emphasized, however, that in all cases described so far, it has been demonstrated that specific genetic factors are necessary but not necessarily sufficient for the development of dystonia. For example, mutations in the GTP cyclohydrolase gene may produce dystonia or severe hypotonia and mutations in the tyrosine hydroxylase gene may produce dystonia or parkinsonism (Table 2). Extensive analysis of the chromosome 9q34 idiopathic torsion dystonia locus and preliminary analyses of focal dystonia families are most compatible with their being an autosomal dominant mode of transmission with *low* penetrance. Whether the other necessary conditions for the development of dystonia or parkinsonism are genetic or environmental in origin are unknown. However, a variety of recent studies in rodents demonstrate that genetic abnormalities of dopamine pathway function can result in marked changes in movement.

Biochemical Analysis of Dystonia in Inbred Rodent Strains

Two inbred rodent strains which develop generalized dystonia have been described (Lorden et al., 1984; Löscher et al., 1989). The dystonic rat (dt) (Lorden et al., 1984) displays sustained twisting movements involving axial musculature that begin early in life and persist. The condition is inherited as an autosomal recessive mutation. The dystonic hamster (dt^{sz}) (Löscher et al., 1989) displays sustained-generalized dystonic movements and postures that occur spontaneously or during stress-inducing situations. In contrast to the rat model, the hamster dystonias are transient and spontaneously remit at approx 2 m of age (Löscher et al., 1989). Extensive histological, behavioral, and physiological analyses have been carried out on both these strains. In the dt

rat there are no significant changes in striatal D_2-like receptor binding or dopamine content (Beales et al., 1990; Lorden et al., 1988). However, there are marked changes in $GABA_A$-receptor binding and in glutamic acid decarboxylase activity in deep cerebellar nuclei. These findings are most consistent with the cause of the dystonic syndrome in the dt rat being secondary to cerebellar dysfunction rather than striatal dysfunction (Beales et al., 1990). Analyses of the dt^{sz} hamster have demonstrated modest decreases in dopamine content and more marked changes in norepinephrine and serotonin levels (Löscher et al., 1994). As discussed in the next section, changes in norepinephrine and serotonin are unlikely to be involved in the development of dystonia. These transmitter levels changes in the dt^{sz} hamster are associated with complex changes in dopamine receptor binding (Nobrega et al., 1996). These investigators found decreased D_1-like receptor binding in the stratum, olfactory tubercle, substantia nigra pars reticulata, and the core of the nucleus accumbens. They also found decreases in D_2-like binding in the striatum and the shell of the nucleus accumbens. Assuming that the decrease in D_2-like receptor binding represents decreases in autoreceptor function, these authors proposed the net result is an increase in the activity of dopamine postsynaptically (Nobrega et al., 1996). They further proposed that the decrease in D_1-like receptor binding may be on cholinergic interneurons rather than direct output pathway neurons. The net change for these two receptors then would lead to decrease inhibition of thalamic output and increased activity at motor areas. They proposed this as a mechanism for the generation of dystonia in the dt^{sz} hamster. This interpretation is not supported by the finding of reduced PSA and SMA responses in dystonic patients with focal symptomatology (Tempel and Perlmutter, 1990, 1993). Alternatively, these decreases in dopamine content and D_2-like receptor number may be similar to those seen in MPTP-treated baboons during the dystonic phase (Permutter et al., 1997; Todd et al., 1966) and result in increased activity of the indirect output pathway.

In summary, both these animal models share phenomenology with human dystonia syndromes. The biochemical findings for the dt rat are different than those of human studies. The proposed model for decreased D_2-like autoreceptor function and the interpretation of dopaminergic changes in the dt^{sz} hamster (Nobrega et al., 1996) would result in increased cortical motor activity which is at odds with findings in human dystonics (Tempel and Perlmutter, 1990, 1993), but would be consistent with increased blood flow found in more anterior SMA regions in response to a self-paced joystick task in dystonic patients (Ceballos-Baumann, 1995) If the decrease in D_2-like receptor binding in the dt^{sz} hamster is postsynaptic, then the combined decrease in dopamine content and D_2-like receptors would result in increased output of the indirect pathway. The decreased D_1-like receptor number might represent a compensatory change to balance output of the direct and indirect pathways. As described in the next section, animal strains developed to be deficient in particular gene products are broadly consistent with our proposed hypothesis.

Effects on Movement of Mutations Affecting Dopaminergic Function

Table 2 summarizes the biochemical and movement phenotypes of a variety of dopamine pathway mutations in mouse and man. These include mutations of dopamine receptor genes, catecholamine pathway enzymes and other related genes such as the dopamine transporter (DAT) and the hypoxanthine phosphoribosyl transferase (HPRT) genes. As pointed out by a number of authors, animal models of human diseases may show similar biochemical deficits without showing the same phenotype. Moreover, many mutations in rodents (such as null or knockout mutations of a given gene) may be more extreme than those

seen in human disease states. However, for many disorders there has been a remarkable correspondence between rodent results and those that have been demonstrated or theorized to occur in human disease states.

Of the five known dopamine receptor genes, mouse knockout models have been published for D_1, D_2, and D_3. In addition, single human individuals who are homozygous for a null mutation or for a missense mutation at the dopamine D_4 receptor gene have been identified (*see* Table 2). In the mouse knockout models for D_1, D_2, and D_3 receptors, biochemical analysis demonstrates the elimination of the receptors in the brains of mature animals. Interestingly, the behavior of none of these mouse models are consistent with dystonic movement problems. D_1 null animals show some reduction in rearing behavior but overall have little or no change in motor movement. D_2 null animals do show a parkinsonian-like state consistent with the effects of acute D_2-like receptor blockade and destructive lesions of the striatum. D_3 null mice are hyperactive with greatly increased rates of motor movements. The single human individuals who have been reported to have homozygous mutations of the dopamine D_4 receptor gene have no apparent movement problems. In these human cases, no biochemical analyses are available.

As described above, human missense mutations have been reported in DOPA-responsive dystonia for both the tyrosine hydroxylase and the GTP cyclohydrolase I genes. As discussed above for TH, missense mutations have been reported that result in dystonia or parkinsonism. There is little difference in the reported residual biological activity of the tyrosine hydroxylase protein for both types of mutations and it is unclear why decreased activity results in dystonia in one case and parkinsonism in another. A mouse null mutation for the TH locus has been reported in which no catecholamines (dopamine, epinephrine, or norepinephrine) can be detected in brain. These animals die in the perinatal period and no movement phenotype information has been reported. When catecholamine depletion in these animals is restricted to dopamine, animals are hypoactive, adipsic, and aphagic. Whether these behavioral abnormalities represent motor problems or motivational problems is unknown but all these characteristics respond to exogenous L-DOPA administration.

Human mutations in the GTP cyclohydrolase I gene result in severe hypotonia or DOPA-responsive dystonia. For the severe hypotonia syndrome, no GCH activity is detected in vitro whereas DOPA-responsive dystonia mutations demonstrate some (<20%) residual GCH activity. Since the effect of the GCH mutations to decrease tetrahydrobiopterin levels would affect other enzymatic systems in addition to TH, the development of severe hypotonia in the null mutation cases may be secondary to noncatecholaminergic effects. That the dystonia associated GCH mutations respond to L-DOPA therapy demonstrates that the dystonia is caused by effects on catecholamine systems. No animal models for GTP cyclodrolase I mutations have been described.

Since tyrosine hydroxylase activity is the rate-limiting step in the synthesis of the catecholamines, mutations that effect tyrosine hydroxylase activity will affect dopamine, epinephrine, and norepinephrine levels. Hence, in a strict sense, it can not be assumed that the effects of mutations in TH or GCH lead to dopamine deficiency. These alternatives can be distinguished by comparing the effects of mutations in TH or GCH and those of mutations in enzymes further down the pathway for the production of epinephrine and norepinephrine. One such enzyme, dopamine beta hydroxylase (DBH), has been investigated in mice using a knockout strategy. These animals lack norepinephrine and epinephrine. Whereas there are marked effects of the null mutation on energy conservation and the regulation of basal metabolic rate, no differences are found in movements. This suggests that the effects of mutations on tyrosine hydroxylase activity and movement problems can be attributed to the effects of decreased dopaminergic function.

Mouse knockout strains have also been developed for the DAT and the HPRT genes. The dopamine transporter is responsible for reuptake of dopamine in the synaptic cleft. Over or underactivity of this transporter could result in decreases or increases, respectively, in postsynaptic dopamine stimulation. Elimination of the dopamine transporter would be expected to increase synaptic dopamine. If elevated dopamine produces dystonia, one would expect that this mutation should also cause dystonia. Mice lacking a functional dopamine transporter gene show no dopamine transporter activity and have elevated dopamine concentrations synaptically. These animals demonstrate neither dystonia nor parkinsonism but similar to the dopamine D_3-receptor knockout mice, are hyperactive. Humans with various HPRT gene mutations develop dystonia and chorea (Lesch-Nyhan syndrome). Biochemical studies suggest that there is a 60–90% decrease in striatal dopamine in these individuals. Mouse models of HPRT deficiency demonstrate 20–50% decreases in striatal dopamine, but no effects on movement. Interestingly, dopamine synthesizing cell groups that do not project to the striatum appear to be relatively unaffected in these model systems suggesting a selective but secondary effect of the HPRT gene mutations on substantia nigra striatal projections.

In summary, a wide variety of mutational studies in mouse and man demonstrate that mutations of certain dopamine pathway genes result in movement problems. This is particularly true for the synthetic enzymes tyrosine hydroxylase and GTP cyclohydrolase I, the dopamine transporter, and the D_2 and D_3 dopamine receptors. Since the dopamine D_3 receptor can act as an autoreceptor to decrease dopamine synthesis and release (O'Hara et al., 1996; Tang et al., 1994), similar phenotypes of null mutations in mice for the dopamine transporter and the D_3 receptor genes are probably both because of increased synaptic levels of dopamine resulting in hyperactivity. The absence of effects of null mutations in the D_1 receptor and D_4 receptor genes argue against loss of function of pathways expressing these receptors being involved in the development of dystonia. One interpretation of the phenotypic effects of different mutations in TH and GCH genes and the effect of the null mutation in the dopamine D_2 receptor gene is that a moderate reduction in dopaminergic activity in D_2-mediating pathways can result in dystonia, whereas a severe reduction in function of D_2-associated pathways results in parkinsonism. As described above, many studies of dystonia patients are compatible with dystonia resulting from hypofunction of dopamine D_2-receptor inhibition of the basal ganglia indirect-output pathway.

Future Directions

More direct tests of the indirect pathway hyperfunction hypothesis for the origin of dystonia might be obtained through several experimental approaches. First, direct recording of the activities of direct and indirect pathway neurons in MPTP lesioned animals during the transient dystonic phase should offer a direct test of this model for secondary dystonia. Second, determination of whether D_2-like postsynaptic or autoreceptors are decreased would clarify the relationship between receptor changes and indirect-output pathway activity. Third, improvements in receptor imaging in vivo through the use of advanced imaging techniques and more specific receptor ligands should help differentiate which dopamine receptor classes are changed in human dystonia and whether subregion changes in putamen correlate with the type of dystonia. Finally, genetic analysis of dopamine pathways enzymes and receptors should be an efficient mechanism to identify putative mutations affecting the dopaminergic control of direct and indirect pathway function. New haplotype analyses derived from population biology approaches can allow more powerful tests of disease association with candidate genes in the absence of large families (Lobos and Todd, 1997).

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