JCI The Journal of Clinical Investigation

Infantile parkinsonism-dystonia: a dopamine "transportopathy"

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J Clin Invest. 2009;119(6):1455-1458. https://doi.org/10.1172/JCI39632.

Commentary

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Infantile parkinsonism-dystonia: a dopamine "transportopathy"

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The dopamine transporter (DAT) retrieves the neurotransmitter dopamine from the synaptic cleft at dopaminergic synapses. Variations in solute carrier family 6A, member 3 (*SLC6A3*/*DAT1*), the human gene encoding DAT, have been implicated in attention deficit hyperactivity and bipolar disorders, and DAT is a prominent site of action for drugs such as amphetamines and cocaine. In this issue of the *JCI*, Kurian et al. report that an autosomal recessive infantile parkinsonism-dystonia is caused by loss-of-function mutations in DAT that impair dopamine reuptake (see the related article beginning on page 1595). Though this might be predicted to result in dopamine excess in the synaptic cleft, it likely also causes depletion of presynaptic dopamine stores and possibly downregulation of postsynaptic dopamine receptor function, resulting in impairments in dopaminergic neurotransmission consistent with the clinical presentation. This is the first report of a genetic alteration in DAT function underlying a parkinsonian disorder.

The complex interconnections in the nervous system depend on synapses, specialized junctions at which a neuron contacts a target cell, most frequently another neuron. Synaptic transmission is mediated by chemical neurotransmitters in the synaptic cleft (~20–25 nm wide) and is regulated dynamically by presynaptic neurotransmitter release and subsequent reuptake as well as by other pre- and postsynaptic signaling mechanisms. Many neurotransmitter receptors are ligand-gated ion channels, including the predominant postsynaptic excitatory and inhibitory receptors in the central nervous system, which are gated by glutamate and GABA, respectively. Other neurotransmitter receptors are coupled through G proteins to intracellular second messenger systems, including receptors for the biogenic amine dopamine.

Dopaminergic neurons are found in relatively restricted areas in the brain, but prominently within the substantia nigra pars compacta, with projections to striatum, and in the ventral tegmental area of the midbrain, with projections to the cerebral cortex as well as to limbic and subcortical nuclei. Consistent with this distribution, dopamine exerts modulatory effects on human motor control, affect, behavior, and cognition (1, 2). Alterations in dopaminergic transmission have been implicated in a host of neuropsychiatric and movement disorders (3). These comprise both dopamine deficiency states such as Parkinson disease as well as states of dopamine excess, as proposed for Tourette syndrome and schizophrenia. Furthermore, a large number of commonly prescribed neuropsychiatric medications as well as drugs of abuse have major effects at these synapses. For instance, L-dopa used to treat Parkinson disease, methylphenidate and amphetamine used for attention deficit hyperactivity disorder, and cocaine all promote dopaminergic transmission, while neuroleptic medications commonly prescribed for schizophrenia and Tourette syndrome inhibit dopamine transmission.

The dopaminergic synapse comprises a number of signature elements, situated both pre- and postsynaptically (Figure 1A). Postsynaptic dopamine receptors can be divided into two major subtypes that are positively (D1 class) or negatively (D2 class) coupled through G proteins to regulate production of cAMP by adenylate cyclase. D1-class receptors are also coupled to phosphoinositide metabolism, and D2-class receptors are coupled to Ca^{2+}-dependent intracellular signaling cascades (4).

Conflict of interest: The author has declared that no conflict of interest exists.

Nonstandard abbreviations used: DAT, dopamine transporter; SLC6, solute carrier family 6; *SLC6A3*, SLC6A, member 3; TM, transmembrane domain.

Citation for this article: *J. Clin. Invest.* **119**:1455–1458 (2009). doi:10.1172/JCI39632.

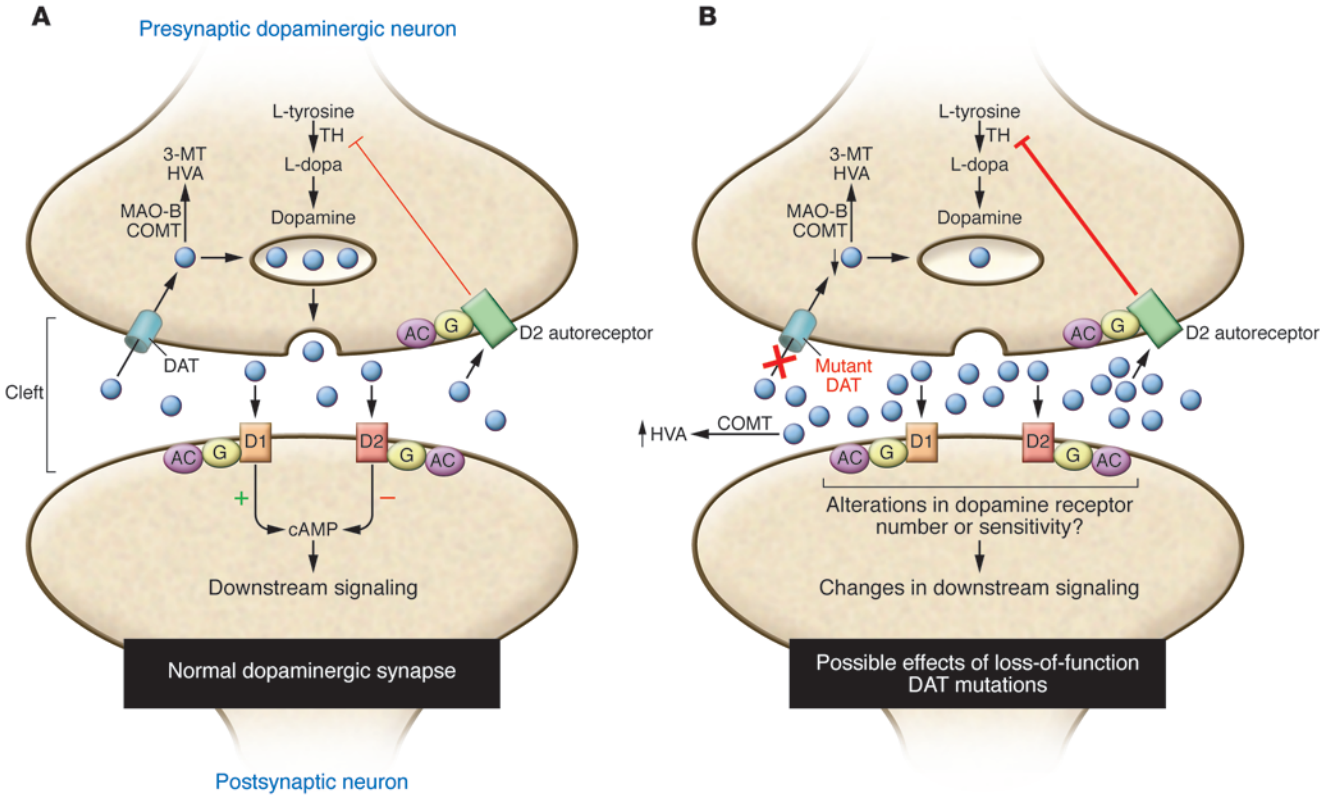

Figure 1

Diagram of a dopaminergic synapse illustrating possible effects of the loss of DAT function. (**A**) Presynaptic and postsynaptic neuron membranes and the synaptic cleft are indicated. D1-class and D2-class dopamine receptors are positively or negatively coupled to adenylate cyclase (AC) via G proteins (G). Coupling to other signaling pathways is not shown. Catechol *O*-methyltransferase (COMT) and monoamine oxidase B (MAO-B) are involved in the metabolism of dopamine (blue circles) to products such as homovanillic acid (HVA) and 3-methoxytyramine (3-MT). Dopamine in the cleft can bind presynaptically to D2 autoreceptors or the DAT, or postsynaptically to D1- and D2-class receptors. The DAT is predominately located perisynaptically. (**B**) In this issue of the *JCI*, Kurian et al. (14) show that SLC6A3/DAT1 loss-of-function mutations in individuals with infantile parkinsonism-dystonia result in inhibition of DAT-mediated dopamine reuptake activity. Increased time that dopamine is present in the synaptic cleft will result in dopamine degradation there, predominantly by COMT, as well as increased levels of the dopamine metabolite homovanillic acid in the cerebrospinal fluid. Overstimulation of D2 autoreceptors is predicted to inhibit the phosphorylation-dependent activation of tyrosine hydroxylase (TH), which is rate limiting for the production of dopamine. Mutations in the gene encoding TH have been implicated in other movement disorders such as L-dopa–responsive dystonia and infantile parkinsonism. Prolonged dopamine presence in the synaptic cleft may result in desensitization or downregulation of postsynaptic dopamine receptors, with alterations in downstream signaling.

Within the presynaptic terminal are enzymes for both the synthesis and breakdown of dopamine, and the presynaptic membrane harbors not only dopamine receptors but also a specialized dopamine transporter (DAT). In fact, a major mechanism for deactivation of dopaminergic signaling is presynaptic reuptake of dopamine through the DAT (Figure 1A), a member of the solute carrier family 6 (SLC6) group of Na^+/Cl^--dependent, transmembrane transport proteins that also includes transporters for serotonin, norepinephrine, GABA, and glycine, among others (5, 6).

A seminal breakthrough in our understanding of the mechanistic basis for membrane transport via these SLC6 proteins came from the determination of the crystal structure of a bacterial member of this family. The high-resolution structure (1.65 Å) of the dimeric leucine carrier $LeuT_{Aa}$ from the bacterium *Aquifex aeolicus* revealed 12 transmembrane domains (TMs) in each protomer, with both N and C termini bathed in the cytoplasm (7). This study also revealed a pseudo–two-fold axis of symmetry within the membrane, with TM1–TM5 closely corresponding to TM6–TM10, though with an inverted topology. The leucine substrate binds to non-helical regions within TM1 and TM6 (7). Since it was reported in 2005, this structure has served as a valuable template for a large number of structure-function studies of transporters in the SLC6 family, including the DAT (6).

DAT mutations are associated with autosomal recessive infantile parkinsonism-dystonia

Though the SLC6 protein family is very large and extensive, both phylogenetically and within species (5), the DAT has been among the most intensively studied members because of its functional relationships to disease, therapeutic agents, and drugs of abuse (8–13). In this issue of the *JCI*, Kurian et al. (14) have provided the first report of loss-of-function mutations in the SLC6A member 3 (*SLC6A3*; also known as *DAT1)* gene that directly links DAT dysfunction to a form of human autosomal recessive infantile parkinsonism-dystonia. This rare movement disorder presents in infancy with parkinsonism (slowness of movement,

muscle rigidity, rest tremor) and dystonia (sustained, abnormal muscle contractions often resulting in twisting movements). The authors describe three patients from two consanguineous families that harbor missense mutations (Leu368Gln and Pro395Leu) in the same general region, from the top of TM7 to the extracellular loop ending at TM8. On immunoblots, DAT migrates as both ~55- and ~85-kDa forms, and both mutations caused marked deficiency of the larger, mature form. The smaller form has been previously shown to have impaired transport activity relative to the larger form (10). Indeed, in heterologous cell studies, Kurian et al. (14) show that both DAT mutations resulted in markedly impaired dopamine uptake, supporting a loss-of-function mechanism underlying disease pathogenesis, and one mutation also decreased the binding affinity of dopamine to DAT (Figure 1B). The region of DAT harboring these mutations has been shown to be sensitive conformationally to a number of different agents, including Na^+, cocaine, and dopamine (12). Since the mutations occur at residues highly conserved among other SLC6 transporters, a more general functional role is also possible.

Upon initial consideration, a disease pathogenesis emphasizing DAT loss-of-function might seem surprising. Infantile parkinsonism-dystonia is typically caused by inborn errors of metabolism affecting the dopamine biosynthetic pathway, and thus represents a disorder of dopamine deficiency. Known disease mechanisms include inhibiting synthesis of the cofactor tetrahydrobiopterin or of dopamine biosynthetic enzymes themselves (e.g., tyrosine hydroxylase) (Figure 1A). In such cases, there is a severe reduction in dopamine metabolites such as homovanillic acid in the cerebrospinal fluid (15). In the Kurian et al. study (14), however, these metabolites were markedly elevated, prefiguring an excess of dopamine in the synaptic cleft. Thus, one could conclude that increased presence of these metabolites in the synaptic cleft reflects decreased dopamine reuptake and, as a result, depleted stores of intracellular dopamine to be packaged into synaptic vesicles for release. Excess dopamine in the synaptic cleft might also activate presynaptic D2 autoreceptors, thus inhibiting dopamine synthesis, or trigger desensitization or downregulation of postsynaptic dopamine receptors (Figure 1B). These notions are supported by studies of mutant mice lacking DAT and other monoamine transporters (9). One published report has described a similar clinical syndrome of infantile parkinsonism-dystonia, but in those three cases no DAT mutations were identified (16). Interestingly, one of the families in the Kurian et al. study (14) also exhibited the ocular flutter (involuntary bursts of oscillations of the eye around a point of fixation) and saccade initiation failure (inability to make rapid, abrupt eye movements) that characterized the parkinsonism-dystonia patients described in the study of Assmann et al. (16). Further identification of the pathogenic mechanisms underlying these latter cases is eagerly awaited.

Autozygosity mapping for identification of uncommon recessive disorder genes

Another aspect of the Kurian et al. study (14) worth emphasizing is how the causative gene was identified. Both families had known consanguinity, and the authors used an approach known as autozygosity (or homozygosity) mapping to identify the disease gene locus in one of them. Though this concept was first proposed over two decades ago (17), the availability of high-density SNP arrays has made this approach much more powerful, with the ability to identify disease loci quite rapidly in even very small inbred populations, where extended regions of homozygosity that segregate for disease signs and symptoms likely harbor the disease locus (18). One important caveat for this approach is that the clinical phenotype is often defined based on a very small number of subjects, and thus affected individuals from additional families are particularly helpful to clarify the major clinical features.

Transport to the future

This study (14) and those that will inevitably follow may require us to add "transportopathy" to the well-established "channelopathy" as a fundamental mechanism of disease in the neurological lexicon. No doubt, the *SLC6A3/DAT1* gene will now be scrutinized for additional mutations in movement disorders presenting with parkinsonism or dystonia, particularly those with early onset. These DAT missense changes will drive investigations of *SLC6A3/DAT1* knockin mice harboring the corresponding pathogenic mutations to understand their effects in a physiological context. Since the mutations identified in the Kurian et al. study (14) occur at residues broadly conserved in the SCL6 superfamily, studies of equivalent sites in other SLC6 transporters may also elucidate the fundamental biology of this protein family. We clearly need to learn more about how these transporters work — their interactions, regulation by posttranslational modifications, intracellular trafficking, etc. — before we can fully appreciate how they contribute to disease pathogenesis.

Lastly, it is important to emphasize that rare disorders such as this inherited infantile parkinsonism-dystonia have the potential to illuminate critical functions of the DAT, with important implications for far more common neuropsychiatric disorders. Fortunately, the tools available to link DAT biology to its function in the nervous system are quite extensive and powerful. In particular, imaging modalities for DAT using single positron emission computed tomography (SPECT) and PET with a host of available ligands will provide a crucial link among clinical characterizations, genetic investigations, and basic science studies (19, 20). For DAT, effects of alterations in protein structure on the response to pharmacological agents are also providing insights. In a study stimulated by the identification of two children with attention deficit hyperactivity disorder harboring a rare DAT Ala559Val coding variant, amphetamine was shown to attenuate, rather than enhance, dopamine efflux in this mutant DAT, emphasizing the critical role that response to pharmacologic agents can continue to play in dissecting the complex structure-function relationships of the DAT (13). Each new disease mutation will present an additional opportunity to help us unravel DAT function.

Acknowledgments

This work is supported by the Intramural Research Program of the National Institute of Neurological Disorders and Stroke, NIH.

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Can childhood viral infection protect from type 1 diabetes?

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While many candidate type 1 diabetes (T1D) susceptibility genes have been identified, evidence suggests that environmental stimuli, such as viral infections, may also be involved in T1D pathogenesis. However, how viral infections may prevent or trigger the diabetogenic process remains unclear. In this issue of the *JCI*, Filippi et al. show that infection of NOD mice with Coxsackie virus B3 or lymphocytic choriomeningitis virus, neither of which directly destroys insulin-secreting pancreatic β cells, triggers the activation of two distinct immunoregulatory mechanisms, involving both the innate and adaptive immune system, that protect against the development of T1D in these animals (see the related article beginning on page 1515).

Immune-mediated destruction of insulin-secreting pancreatic β cells results in type 1 diabetes (T1D). Genetic factors, including genes that regulate facets of the immune response, influence the susceptibility of an individual to T1D and other autoimmune diseases. Yet the concordance rate for T1D among monozygotic twins is only 40% (reviewed in ref. 1). Therefore, environmental or epigenetic factors must exist that trigger or mediate resistance to the development of overt T1D in genetically predisposed individuals. Among the environmental factors that might influence T1D expression, childhood infectious diseases have received considerable attention. Why? Clonotypic T cell receptors that recognize certain microbial antigens cross-react with self antigens. Some viruses can infect and damage pancreatic β cells. Hence, T cell clones activated by microbes can, on occasion, cross-react with molecularly related self antigens (a process known as *molecular mimicry*) and precipitate overt autoimmune disease. However, epidemiologic data do not support a link between childhood infection and autoimmune disease (reviewed in ref. 1). Indeed, developed countries, which have a low incidence of childhood infection, have a high rate of autoimmune disease, while the reverse is true for less-developed counties, which have a high rate of childhood infection (1). As persons from a less-developed region of a country with a low rate of autoimmune disease and high rates of childhood infection migrate to a more developed region of the country with lower rates of childhood infection, the incidence of autoimmune diseases among the migrants and their offspring reciprocally increases (1). Furthermore, it has been previously reported in rodent models of T1D, such as the NOD mouse, that exposure to infectious agents can provide powerful protection from T1D in these animals (reviewed in 1). However, how infections protect against autoimmune disease is unknown. In their current study in this issue of the *JCI*, Filippi et al. (2) demonstrate that infection of prediabetic NOD mice with Coxsackie virus B3 (CVB3) or lymphocytic choriomeningitis virus (LCMV) — pancreatotropic viruses that are known to cause systemic infection but do not lyse pancreatic β cells — reduces the incidence and delays the onset of T1D in these animals. The authors demonstrate that viral infection provides protection from T1D by triggering distinct immunoregulatory mechanisms, involving both the innate and adaptive immune systems, that prevent the expansion of diabetogenic T cells.

The inception and termination of T cell–dependant antiviral immune and autoimmune responses are orchestrated through

Conflict of interest: The author is a consultant for Quest Diagnostics Inc.

Nonstandard abbreviations used: CVB3, Coxsackie virus B3; IGRP, islet-specific glucose-6-phosphate catalytic-related protein; LCMV, lymphocytic choriomeningitis virus; PD-1, programmed cell death–1; PD-L1, PD-1 ligand 1; T1D, type 1 diabetes.

Citation for this article: *J. Clin. Invest.* **119**:1458–1461 (2009). doi:10.1172/JCI39565.