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REVIEWS

Reversal of Dopamine Flux in Health and Disease

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Abstract

The dopamine transporter (DAT) is responsible for regulating the concentration of dopamine (DA) in the synaptic space via a substrate re-uptake mechanism. This re-uptake mechanism can occur both as a facilitated exchange process, commonly described as the alternating access model (which is dependent on the concentration of Na^+, Cl^-, and DA), as well as a channel-like diffusion process where DA transport does not appear to be linked to Na^+ or Cl^- concentrations. The DAT is also capable of reverse transport of DA (DAT-RT) or moving DA from the cell cytosol into the extracellular space. This DAT-RT has been observed to occur under specific conditions: 1) upon the introduction of amphetamines (AMPH), 2) upon the activation of specific second messenger, DAT-influencing proteins, and 3) upon conditions of DAT sequence mutations, or coding variants of the DAT. Similar to DA re-uptake, this DAT-RT can occur both as a facilitated exchange process and a channel-like diffusion process. It is possible that the process of DA efflux, via a DAT-RT mechanism, plays a major, yet poorly understood, role in the DA homeostasis in the central nervous system. By further understanding the process of DAT-RT, it may be possible to shed new light on DA homeostasis and the diseases associated with alterations in this DA homeostasis.

Keywords

Addiction
Amphetamine
Dopamine
Transport
Mutation

Background

The neurotransmitter, dopamine (DA), plays an important role in the central nervous system by exerting influence over functions like voluntary movement, motivation, and reward[1]. DA's function as a neurotransmitter is regulated, in part, by the dopamine transporter (DAT) [2]. The DAT is a plasmalemmal phosphoprotein capable of DA re-uptake from the synaptic space, thereby limiting the post-synaptic exposure to DA which, in turn, regulates the intensity and duration of the dopaminergic response[2-4]. The DAT is also a vector for DA release, or DAT mediated reverse transport (DAT-RT), wherein the DAT is capable of slowly releasing large quantities of DA into the synaptic space via a vesicle-independent mechanism[2, 4, 5]. The DAT function, particularly DAT-RT function, can be altered by genetic mutations, activation of second messenger systems, and under the influence of exogenous pharmacological agents, such as psychostimulants like cocaine and amphetamines (AMPH)[6-8]. Therefore, alterations in DAT function by any of the aforementioned mechanisms will result in altered DA homeostasis.

Diseases implicated in altered DA homeostasis include attention deficit hyperactivity disorder (ADHD), affective disorders, schizophrenia, and drug abuse[6]. The National Institute of Mental Heath estimates that ADHD is exhibited by 3-5% of the American population. Adderal, a racemic mixture of S(+) and R(-) AMPH enantiomers, is the most prescribed treatment for juvenile ADHD in the United States. Furthermore, the number of American children exposed to an AMPH congener rose from 0.6 per 100 in 1987 to 2.4 per 100 in 1996[9]. The total annual economic cost of drug abuse in the United States rose from US$102 billion in 1992 to US$143 billion in 1998 and is currently estimated to be well above US$200 billion[10, 11]. Affective disorders and schizophrenia were attributed with contributing over US$300 billion to the global burden of disease in 2008 alone[11]. Given these data and innumerable other ways in which DA oriented diseases have affected individual and global health, it is important that the regulation of DA homeostasis be further understood.

This review will focus on the phenomenon of reverse transport of DA through the DAT and its role in DA homeostasis. This process of delivering large amounts of DA over extended periods may play a major, yet underappreciated, regulatory role in DA homeostasis. In this review, I will outline the mechanism for DA re-uptake via the DAT, followed by an overview of DAT-RT, detailing three documented factors that induce DAT-RT: (1) exposure to psy-

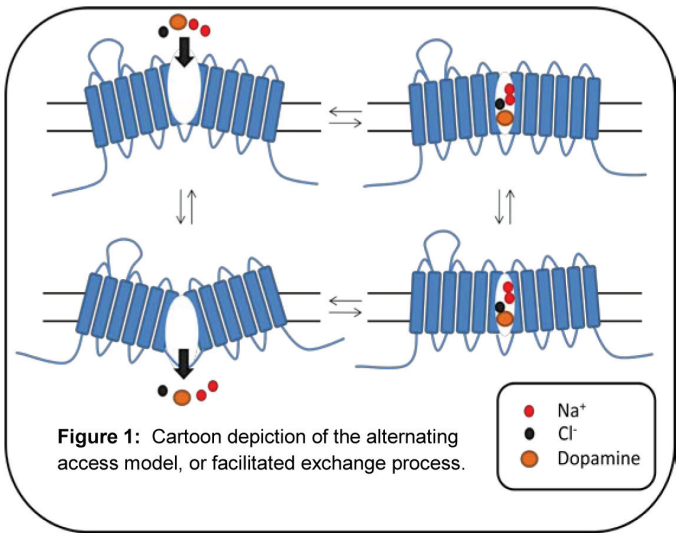

Figure 1: Cartoon depiction of the alternating access model, or facilitated exchange process.

chostimulants, particularly AMPH, (2) activation of second messenger signaling pathways, and (3) coding variants of the DAT.

Mechanism of Re-uptake

The DAT is a member of the solute carrier 6 (SLC6) gene family of Na^+/Cl^- symporters, which includes other monoamine transporters such as the serotonin transporter and the norepinephrine transporter. A characteristic of the transporters in this gene family is the use of the ion concentration gradient as the driving force for transporter-mediated re-uptake of their respective substrate[12]. In the case of the DAT, the uptake of DA is coupled with the translocation of two Na^+ ions and one Cl^- ion, resulting in a net movement of two positive charges per DA molecule (DA is positively charged at physiological pH)[13]. This results in the generation of an inward current in conditions of DA re-uptake. This model of moving two positive charges per transporter cycle stems from the classical alternating-access model of transporter function, which essentially assumes that the DAT function is analogous to a revolving door[14,15]. The model assumes that DAT is capable of an "outward-facing" conformation in which DA, Na^+, and Cl^- are required to bind to the transporter in a fixed ratio and induce a conformational change. This conformational change results in an "inward-facing" transporter and the dissociation of the cargo, thereby, completing the transport of DA from the synaptic space into the cytosol[14] (Figure 1).

Since the creation of the alternating-access model, researchers have been able to describe the types of conductance that occur in conjunction with substrate re-uptake through the DAT. They discovered a coupled "transport-associated" current, which obeys the properties of the alternating-access model and, notably, has a constitutive leakage conductance due to a previously unknown channel-like activity of the DAT where the transporter experiences a rapid, inward flux of ions and/or DA molecules without the requirement of Na^+ or Cl^-[16, 17]. The observation of the channel-like, constitutive leak conductance demonstrates that the DA re-uptake through the DAT behaves in a way that cannot be fully explained with classical alternating-access model of transporter function. This insight into the DAT function raises interesting questions about the molecular regulatory events that are responsible for this channel-like activity of the DAT, as well as questions about the directionality of the channel.

Reverse Transport of Dopamine via the Dopamine Transporter

There is an inherent asymmetry of ion concentration, substrate concentration, membrane potential, and DAT protein structure when using the neuronal cell membrane as the axis of symmetry. This asymmetry of obligate components for DA re-uptake would seem to suggest that the DAT is only capable of DA re-uptake. However there are very clear and documented instances of the reversal of the DAT function where the net flow of DA, mediated by the DAT, travels from the cell cytosol to the extracellular space[2, 4, 5, 7, 8, 17]. Therefore, these asymmetric conditions point to an asymmetry in the DAT function, with some molecular elements important for re-uptake and entirely different ones important for DAT-RT, depending on local and transient conditions. I will explore three conditions in which DAT-RT is known to occur: (1) exposure to psychostimulants, focusing on AMPH, (2) activation of second messenger signaling pathways, and (3) DAT coding variants.

Reverse Transport - Amphetamines

Pharmacological substances have long been used to study the process of DAT-RT, not only to understand the molecular events that surround the abuse of these substances, but also to study the molecular mechanisms that are required for reverse transport. Of these pharmacological substances, AMPH is the gold standard.

AMPH is a psychostimulant that exerts its physiological effect by primarily influencing the DAT[4, 18]. AMPH's molecular structure is very similar to DA and, as a result, is a substrate for the DAT. AMPH competitively inhibits DA re-uptake and ultimately promotes DAT-RT of

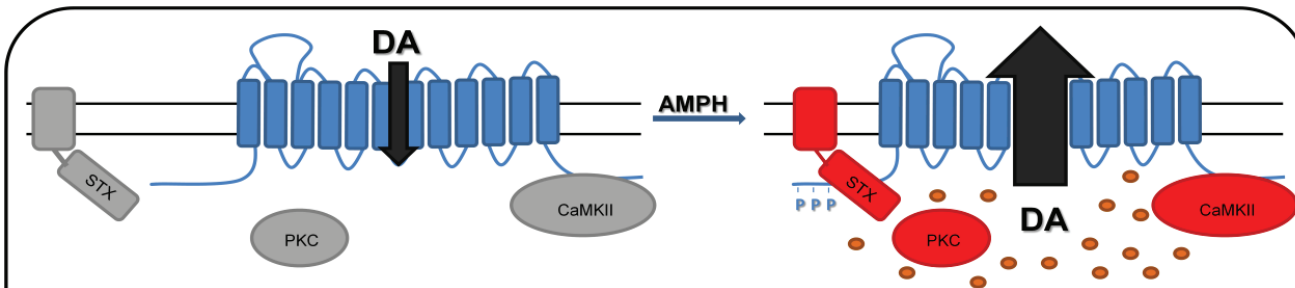

Figure 2: Cartoon depiction of molecular events surrounding the administration of AMPH. In basal conditions, the DAT favors re-uptake of DA. Administration of AMPH activates second messenger proteins, resulting in phosphorylation of N-terminus, promoting DAT-RT of DA molecules released from vesicles.

DA[18, 19]. Following the introduction of AMPH in the extracellular space, AMPH competes with other DAT substrates and interacts with the "outward-facing" conformation of the DAT. Thereafter, AMPH is transported into the intracellular space and interacts with the vesicular monoamine transporter. Being a weak base, AMPH alters the pH gradient between the cytoplasm and vesicles, resulting in the release of DA into the intracellular space. The newly released DA accesses the "inward-facing" conformation of the DAT and, along with the increased Na^+ concentration, results in DAT-RT of DA via a facilitated exchange process[20-22]. Other studies have revealed that this DAT-RT of DA can also occur in rapid bursts of DA efflux through the channel-like mode of the DAT which is independent of the facilitated exchange mechanism[17]. This process allowed researchers to conclude that the previously observed channel-like mechanism of DA re-uptake[16] is bi-directional and occurs in instances of DA re-uptake and DA efflux.

The scope of AMPH's influence on the DAT is not limited to direct interactions with the DAT protein itself; AMPH also appears to influence the local and transient environment surrounding the DAT which ultimately influences the properties of this transporter. Administration of extracellular AMPH increases the frequency of channel-like DA release via the DAT by approximately 8-fold[23]. Furthermore, exposure to AMPH shifts the DAT from a "reluctant" to a "willing" state-- an asymmetric state that favors AMPH-induced DA efflux via the DAT without disturbing normal DA re-uptake[24]. These modulations in DAT efflux are thought to be executed by the activities of DAT-interacting proteins (proteins that have become catalytically active in response to the administration of AMPH). The AMPH-dependent and –independent second messengers and associated proteins will be more thoroughly discussed in the next section.

Reverse Transport - Signaling Pathways

The model of AMPH-induced DA efflux cannot be accurately conveyed without considering its regulation by second messenger systems. As mentioned above, the administration of AMPH is thought to influence DAT-RT by altering the properties of DAT-associated proteins. For example, the first 22 amino acids of the intracellular-facing, N-terminal region of the DAT contain crucial serine residues that, when phosphorylated, alter the properties of DAT-RT. When truncated or replaced with non-phosphorylatable alanine residues, the AMPH-mediated DA efflux was reduced by 80%[24]. These N-terminal residues are phosphorylated by protein kinase C (PKC)[25, 26]. AMPH can increase the activity of PKC in vivo, and inhibition of PKC prevents AMPH-induced DAT-RT[25, 27]. Also, in the rat striatum, it was observed that there is a physical interaction between the DAT and $PKC\beta_{II}$[26].

Another major kinase implicated in the regulation of DAT-RT is CaMKII. CaMKII is also capable of phosphorylating the DAT N-terminal serine residues, and researchers have demonstrated that inactivation or inhibition of CaMKIIα reduces AMPH-induced DAT-RT[28]. A physical interaction between CaMKII and the DAT C-terminus is observed, and disruption of this interaction results in a diminished AMPH-induced DAT-RT[28]. A potential mediator of CaMKII's influence on the DAT-RT is the protein syntaxin 1 (STX). STX is a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) which is required for the docking and release of synaptic vesicles[29]. However, there is a growing body of evidence to suggest that STX associates with and regulates a variety of ion channels and neurotransmitter transporters, including the DAT[30, 31].

These DAT-STX interactions are demonstrated to increase in response to AMPH and are important for the regulation of AMPH-induced DAT-RT[30, 31]. The physical association between DAT and STX is also shown to influence the channel-like, AMPH-induced DA efflux via the DAT, and in C. Elegans a STX homologue suppresses the channel-like properties of the DAT-1[32, 33]. Moreover, researchers have established that the activity of the C-terminal associated CaMKII is responsible for mediating the DAT-STX interaction, and this interaction can be inhibited through the use of CaMKII inhibitors. Figure 2 depicts a representation of some of the molecular events that surround DAT-RT.

Reverse Transport – DAT Coding Variants

In the previous examples of DAT-RT, the efflux of DA via the DAT was stimulated by exogenous compounds (AMPH) and/or modulated by key second messenger proteins. However, researchers have recently described a DAT coding variant that is observed to produce an anomalous DAT-RT under physiologically normal conditions and without the administration of AMPH.

The A559V mutant of the DAT was identified in two brothers diagnosed with ADHD[6]. By cloning the DAT coding variant and expressing it in stable cell lines, the researchers were able to observe that the A559V mutant exhibited an anomalous DAT-RT that occurred under basal conditions[34]. The A559V DAT variant was also more sensitive to intracellular Na^+ concentrations and cell depolarization than wild-type DAT, as seen by increased magnitude of DAT-RT under these conditions[34]. These findings demonstrate the following: 1) a single amino-acid mutation in the DAT can produce significant functional differences in terms of DAT-RT properties and 2) the amino acid sequence of the DAT is important in regulating the dynamics of the DAT-RT.

Conclusions

As demonstrated by the studies presented in this review, the DAT is capable of both re-uptake of DA from the extracellular space and the release of DA via a non-vesicular, DAT-RT mechanism. The mechanisms of the DA re-uptake and DAT-RT are asymmetric; conditions that govern the properties of one mode of transport may not govern the other mode of transport. Despite this, both DA re-uptake and DAT-RT are capable of transporting DA via a facilitated exchange process and a channel-like process.

The significance of the DAT-RT mechanism is particularly remarkable considering that the amount of DA delivered via this flux can be larger than a vesicle-mediated, quantal release of DA delivered during activity dependent exocytosis[35]. In physiological terms, this indicates that the phenomenon of DAT-RT is capable of providing enough neurotransmitter to contribute to the micro-environment surrounding the neuron. This fact coupled with the observation that the DAT is localized not only in the synapse, but also extra-synaptically, on neuronal cell bodies, axons, as well as dendrites, and it becomes clear that dopaminergic neurons possess the capacity to significantly alter DA homeostasis through a potential DAT-RT mechanism[36, 37]. It is also clear that the DAT constantly maintains the capacity for DAT-RT as demonstrated by administration of AMPH, activation of key proteins, or expression of DAT coding variants that activate the DAT's endogenous potential for DAT-RT. Given these three scenarios for triggering DAT-RT, it is reasonable to begin asking the following questions: Does the DAT-RT occur in the CNS, under physiological conditions, without exposure to psychostimulants or genetic mutations? Given that the DAT-RT machinery exists and is exploited by the mechanisms outlined in this review, are there endogenous mechanisms for activating the second messenger proteins that result in DAT-RT? Is this potential DA release enough to act as a viable mechanism of neurotransmission? If any of these questions were even partly true, it would not be difficult to imagine the influence that DAT-RT would have on DA homeostasis in both health and disease.

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