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Review

Drosophila melanogaster as a genetic model system to study neurotransmitter transporters

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ABSTRACT

The model genetic organism *Drosophila melanogaster*, commonly known as the fruit fly, uses many of the same neurotransmitters as mammals and very similar mechanisms of neurotransmitter storage, release and recycling. This system offers a variety of powerful molecular-genetic methods for the study of transporters, many of which would be difficult in mammalian models. We review here progress made using *Drosophila* to understand the function and regulation of neurotransmitter transporters and discuss future directions for its use.

1. Introduction

Several features of the model genetic system *Drosophila melanogaster* make it attractive for the study of neurotransmitter transporters. These include a powerful molecular-genetic toolset, a short lifespan, low cost and the availability of an essentially limitless supply of "test subjects". Here we provide an overview of current studies on neurotransmitter transporters in *Drosophila*

Abbreviations: 5HT, 5-hydroxytryptamine (serotonin); ACh, acetylcholine; ASD, autism spectrum disorder; ChT, choline transporter; ChAT, choline acetyl transferase; DA, dopamine; Ddc, dopa decarboxylase; EAAT, excitatory amino acid transporter; GABA, gamma amino butyric acid; GAT, GABA transporter; KC, Kenyon cell; LDCV, large dense core vesicle; Mas, *Manduca sexta* (tobacco hornworm); MAO, Monoamine Oxidase; MB, mushroom body; MDMA, 3,4-methylenedioxy-N-methylamphetamine (Ecstasy); OA, octopamine; NMJ, neuromuscular junction; SV, synaptic vesicle; TNT, tetanus toxin; *T. ni* or *Trn*, *Trichoplusia ni* (cabbage looper caterpillar/moth); TbH, tyramine b hydroxylase; Tdc, tyrosine decarboxylase; TH, tyrosine hydroxylase; VGAT, vesicular GABA transporter; VGLUT, vesicular glutamate transporter; VMAT, vesicular monoamine transporter; UAS, (yeast) upstream activating sequence.

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and discuss potential uses for this system in the future. By including additional background, we hope to provide a general introduction to the field for both Drosophilists and non-Drosophilists alike. Conversely, for "fly people" with other primary interests, we also provide an overview of neurotransmitter transporters in general and some of the outstanding questions that remain unanswered.

2. Neurotransmitter transporters

Neurotransmitter transporters are responsible for the movement across biological membranes of classical neurotransmitters – the biogenic amines and acetylcholine – and the amino acid neurotransmitters – GABA, glutamate and glycine. Plasma membrane and vesicular neurotransmitter transporters represent two distinct activities (Fig. 1). Plasma membrane neurotransmitter transporters are responsible for the termination of synaptic transmission and recycling neurotransmitters after they are released (Blakely and Edwards, 2012) (Fig. 1). Vesicular neurotransmitter transporters localize to the membranes of secretory vesicles and are responsible for transport and storage of neurotransmitters into the vesicle lumen (Blakely and Edwards, 2012) (Fig. 1).

Vesicular transporters are required for the storage of neurotransmitters in synaptic vesicles (SVs) as well as large dense core vesicles (LDCVs), which also store and release peptide neurotransmitters (Fei et al., 2008). However, peptides are not transported into LDCVs via vesicular transporters, but rather packaged into the lumen of the vesicle as it is being formed (Dikeakos and Reudelhuber, 2007). Peptides also do not undergo transport at the plasma membrane. Similarly, "novel" neurotransmitters such as nitrous oxide do not require specific transport proteins since they are synthesized on demand and pass relatively freely through lipid membrane barriers (Boehning and Snyder, 2003). It remains unclear whether lipid-based signaling molecules such as anandamide require specific transporters for movement across biological membranes (Fowler, 2013).

All known plasma membrane and vesicular transporters are members of the Solute Carrier (SLC) family of proteins and share several characteristic features (Blakely and Edwards, 2012). All contain multiple transmembrane domains and are responsible for moving relatively small (~75–200 Da) hydrophilic molecules across lipid membranes. As "active" transporters, all use energy to drive the movement of neurotransmitter against a concentration gradient, and as "secondary" active transporters, the energy is supplied by an ion gradient rather than by direct hydrolysis of ATP. By contrast, the activities of "facilitated" transporters allow solute movement down a concentration gradient and do not require additional energy (e.g. (Richter and Hargreaves, 2013)), while primary active transporters directly hydrolyze ATP (e.g. (Dermauw and Van Leeuwen, 2014).

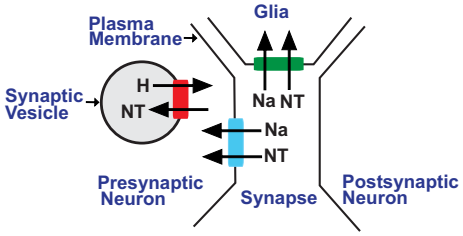

Fig. 1. Vesicular and plasma membrane neurotransmitter transporters. Vesicular and plasma membrane neurotransmitter transporters differ in several respects, including their localization and bioenergetics. In general, vesicular transporters localize to secretory vesicles, including synaptic vesicles in the presynaptic neurons that synthesize and release neurotransmitter. Depending on their subtype (see text) plasma membrane transporters may localize to either presynaptic neurons or surrounding glia. The movement of neurotransmitter via vesicular and plasma membrane transporters are coupled to proton and sodium gradients respectively. Vesicular transporters move protons (H) and neurotransmitter (NT) in opposite directions (antiport), using the high concentration of lumenal protons to drive transport. At the plasma membrane, sodium (Na) and neurotransmitters move in the same direction (symport).

At the plasma membrane, neurotransmitter transporters exploit the steep sodium gradient experienced by most eukaryotic cells. The sodium gradient drives the movement of the transporter and is coupled to neurotransmitter flux in a stoichiometrically defined manner, and in some cases with additional ions facilitating transport (Grewer et al., 2014; Kanai et al., 2014; Pramod et al., 2013; Rudnick et al., 2014). Since both sodium and the neurotransmitter move in the same direction at the plasma membrane, this is by definition a "symport" mechanism. Vesicular transporters use a distinct but related "antiport" mechanism in which a proton gradient is used to drive the movement of neurotransmitter in the opposite direction and into the lumen of the secretory vesicle (Parsons, 2000) (Fig. 1).

The manner in which the transporters couple the movement of ions and neurotransmitter is not yet fully understood, but biochemical experiments and more recent crystallographic and modeling studies have begun to unravel the underlying mechanisms (Penmatsa and Gouaux, 2014; Zhao et al., 2011). Plasma membrane transporters may also function in an uncoupled or loosely coupled mode characterized by relatively large ionic currents (Fairman et al., 1995; Galli et al., 1997; Schicker et al., 2013). The potential function of these currents in synaptic physiology is not yet clear. Efflux represents an additional functional mode in which neurotransmitter moves out of rather than into the cell (Fleckenstein et al., 2007; Sulzer, 2011). The mechanism by which this occurs is a very active area of investigation, and includes work in *Drosophila* described in more detail below (Pizzo et al., 2014, 2013).

Vesicular transporters generally localize to secretory vesicles in the presynaptic neurons that synthesize and release neurotransmitter (Blakely and Edwards, 2012). Plasma membrane transporters for biogenic amines also localize to presynaptic neurons (Blakely and Edwards, 2012). By contrast plasma membrane glutamate and GABA transporters may be expressed in either neurons or glia (Fig. 1) depending on their subtype (Zhou and Danbolt, 2013). We discuss the localization of specific *Drosophila* transporters below.

A circumscribed set of phylogenetic families encodes the plasma membrane and vesicular transporters (see Tables 1 and 2). Glutamate or excitatory amino acid transporters (EAATs) are members of SLC1; this family also contains neutral amino acid transporters (Grewer et al., 2014; Kanai et al., 2014; Sheldon and Robinson, 2007; Yang et al., 2009). All other known plasma membrane transporters, including those for GABA and the biogenic amines, are members of SLC6 (Pramod et al., 2013; Rudnick et al., 2014). SLC6 also includes a number of amino acid transporters, some of which are specific for insects, as well as a few remaining "orphans", whose substrate is not known (Boudko, 2012; Boudko et al., 2005; Caveney et al., 2006). Acetylcholine is hydrolyzed before reuptake and there is no plasma membrane acetylcholine transporter. Rather, following ACh hydrolysis, choline is transported into the presynaptic neuron by a high affinity choline transporter, a member of SLC5 (Ferguson et al., 2004; Ribeiro et al., 2006).

Vesicular transporter subfamilies include SCL32, of which VGAT is the lone member (Schioth et al., 2014). SLC18 includes vesicular transporters for the biogenic amines and acetylcholine (Lawal and Krantz, 2013) and SLC17 includes the vesicular glutamate transporters as well as other transporters with divergent functions such as organic anion uptake at the plasma membrane (Reimer, 2013). Another member of SLC17 was recently identified in mammals as a vesicular ATP or nucleotide transporter (VNUT) (Sawada et al., 2008); a possible VNUT ortholog is present in the fly genome but has not yet been characterized (see Table 1).

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