MQP-BIO-DSA-0603

DOPAMINE TRANSPORTER INTERNALIZES BY MEANS OF A DYNAMIN-INDEPENDENT ENDOCYTIC PATHWAY

A Major Qualifying Project Report

Submitted to the Faculty of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

in

Biology and Biotechnology

by

Patrick Kearney

April 26, 2012

APPROVED:

Haley Melikian, PhD
Psychiatry
UMass Medical School
MAJOR ADVISOR

David Adams, PhD
Biology and Biotechnology
WPI Project Advisor

ABSTRACT

The Dopamine Transporter (DAT) is a trans-membrane protein that binds dopamine and transports it into the cell, removing it from the synapse and terminating the signal to the post-synaptic cell. The endocytic pathway by which DAT internalizes is yet unknown. Dynamin is a GTPase that is known to be involved in the budding of vesicles during some endocytic pathways. The present study was performed to determine if DAT internalizes via a dynamin-dependent endocytic pathway. Data shows that DAT internalization is not dynamin-dependent and implicates that dynamin may be involved in recycling DAT to the cell surface.

TABLE OF CONTENTS

Signature Page ……. 1

Abstract …… 2

Table of Contents ……..…… 3

Acknowledgements …….. 4

Background ……... 5

Project Purpose ……. 11

Methods …… 12

Results …….. 14

Discussion …… 19

Bibliography …… 21

ACKNOWLEDGEMENTS

I would like to thank Dr. Haley Melikian for welcoming me into her laboratory and for her insight, guidance and patience throughout the year. I would also like to thank Luke Gabriel, Zack Stevens, and Sijia Wu for their assistance with my project. Finally, I would like to thank Dr. David Adams for his advice and guidance throughout the MQP and my time at WPI. The experiments for this project were carried out in Dr. Melikian's lab in the Department of Psychiatry at the University of Massachusetts Medical School. All cell lines, materials and reagents were provided by Dr. Melikian's lab.

BACKGROUND

The Dopamine Transporter

The Dopamine Transporter (DAT) is a trans-membrane protein that binds dopamine and transports it into the cell, removing it from the synaptic cleft and terminating the dopaminergic signal to the post-synaptic cell. Dopamine is an important neurotransmitter necessary in the central nervous system for correct motor function and rewarding behaviors. As such, the dopamine transporter has been implicated in a number of neurological disorders including attention deficit hyperactivity disorder, schizophrenia, and Parkinson's disease. DAT has also been linked to drug addiction because it is the target of many psychostimulants such as cocaine and amphetamines, which competitively inhibit the transporter's ability to remove DA from the synapse and cause an increase in the duration and magnitude of the synaptic signal. Fully understanding the activity and trafficking of DAT is vital for the potential treatment of these neurological disorders and addictions.

DAT is a member of the SLC6 transporter gene family that is characterized by the transporters' dependency on sodium and chloride ions for paired transport of dopamine into the cell. Other members of the SLC6 family include serotonin, norepinephrine and GABA transporters. All members of this gene family are highly homologous, having twelve transmembrane domains and a conserved endocytic signal (Figure 1).

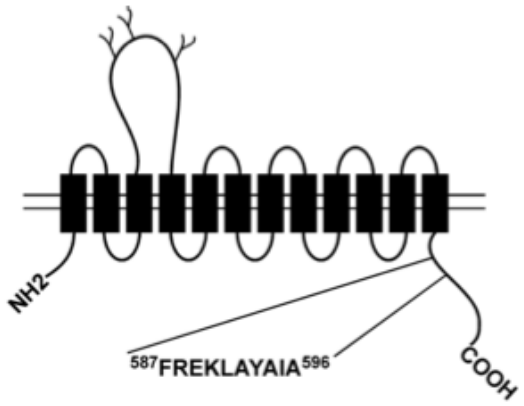

Figure 1: Structure of the Dopamine Transporter (DAT) DAT is an SLC6 transporter with 12 transmembrane domains and the endocytic signal "FREKLAYAIA" (Melikian, 1999).

Dopamine Transporter Internalization and Recycling

Protein trafficking via internalization and recycling plays a vital role in neuronal function. In the synapse, neurotransmitters are released by exocytosis and removed again by transporters. These transporters and the synaptic signal are regulated through transporter internalization and recycling. Dopamine reuptake by DAT terminates dopaminergic neurotransmission and is mediated by DA transporters. A careful balance must be maintained between the release and reuptake of dopamine to ensure proper dopaminergic neurotransmission.

Several studies have demonstrated that phorbal ester activation of protein kinase C (PKC) results in a decreased capacity of DAT to reuptake DA into the cell due to a decrease in DAT on the cell surface (Melikian & Buckley, 1999). The redistributing of DAT from the cell surface to endosomal compartments is what causes DA transport down-regulation following PKC activation (Loder & Melikian, 2003). This down-regulation is achieved by simultaneously increasing basal DAT endocytosis and slowing DAT delivery to the cell-surface (Holton et al, 2005). PKC relies on the endocytic signal that is conserved in the SLC6 family members at residues 587-596 "FREKLAYAIA" (Boudanova et al, 2008). Although the endocytic signal is known, the mechanism by

which DAT is endocytosed still remains unclear. A study using bafilomycin A_1 to facilitate recycling blockade has shown however that the DAT endocytic mechanism is distinct from the clathrin- and dynamin- dependent mechanism utilized by the transferrin receptor (Loder & Melikian, 2003).

The GTPase Dynamin

Dynamin is a large GTPase protein with a molecular weight of ~100kDa. As a GTPase, dynamin's main catalytic activity is the hydrolysis of GTP to GDP. The Dynamin I isoform is found primarily in neuronal cells and is the main isoform of interest. In its purified form, the protein is observed to spontaneously oligermerize in the form of rings and spirals.

Dynamin has been shown to be essential for vesicle budding in clathrin-dependent as well as other endocytic pathways, specifically at a late stage during the transition from a fully formed pit on the cell surface to pinched-off vesicle. This was first demonstrated by observations of a temperature sensitive mutation in the *Drosophila shibire* gene, which is a homologue to the human dynamin-I gene. In this study, flies observed at the nonpermissive temperature revealed a loss of synaptic vesicles and an accumulation of both coated and uncoated invaginations at the synaptic membrane (Damke et al, 1994). Furthermore, studies of human dynamin using a mutant that is defective in GTP binding and hydrolysis demonstrated that coated vesicles at the surface of cells expressing the mutant failed to constrict or bud from the membrane, indicating that dynamin is specifically required for coated vesicle endocytosis, in particular its GTP binding and hydrolysis activities (Damke et al, 1994).

In the mechanochemical models of activity, dynamin assembles as a collar on the neck of a budding vesicular pit, and the cooperative conformational change that accompanies GTP-hydrolysis leads to neck constriction and scission of the vesicle from the plasma membrane (Figure 2) (Macia et al, 2006). Additionally, it has been determined that when dynamin catalyzes GTP hydrolysis, it undergoes a conformation change in which it expands in length and contracts in width, resulting in the fission of the vesicle (Hill et al, 2009; Takei et al, 2005).

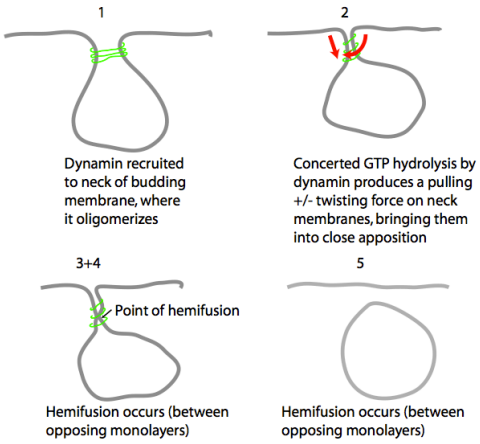

Figure 2: Mechanochemical Model of Dynamin-Dependent Endocytosis
Illustration of dynamin's role in membrane vesicle budding (Doherty & McMahon, 2009).

In addition to clathrin-mediated endocytosis, dynamin has been linked to transport from the trans-Golgi network, free vesicle formation by fission of caveolae from the plasma membrane and tubule fission from early endosomal compartments (Kirchhausen et al, 2006; Mesaki et al, 2011)

.

Dynamin Inhibition

In recent years, several small molecule inhibitors of dynamin activity have been discovered and synthesized. Such small molecule inhibitors are useful because they allow

researchers to freeze biological processes, permitting them to observe transient phenomena, such as membrane trafficking, that were nearly impossible to view when using prior methods of temperature sensitive mutants or permanent and nonspecific inhibition of GTP binding (Kirchhausen et al, 2008). Small molecule inhibitors are also useful because they are highly cell permeable and their effects can be reversed through washout (Hill et al, 2009; Kirchhausen et al, 2006).

Two important small molecule inhibitors of dynamin that were used in this study are Dynasore and Dynole 34-2. Dynasore is a noncompetitive inhibitor of dynamin I, II, and mitochondrial dynamin (Macia et al, 2006). Studies using Dynasore have shown it to be a potent inhibitor of known dynamin-dependent endocytic pathways by blocking coated vesicle formation within the first 1-2 minutes of exposure (Macia et al, 2006). The inhibitor Dynole 34-2 was discovered and synthesized more recently than Dynasore and is the most potent member of an indole-based inhibitor family known as dynoles (Hill et al, 2009). Dynole 34-2 inhibition of dynamin is also noncompetitive and studies suggest that the molecule must bind to an allosteric site that may become available upon binding of GTP to the enzyme's active site (Hill et al, 2009). The creators of Dynole 34-2 were also able to conclude that it was a superior dynamin inhibitor to Dynasore because it was 15-fold more active against dynamin I than Dynasore and it is more membrane permeable than Dynasore due to the lipophilic nature of the whole dynole family (Hill et al, 2009).

There have been previous studies using small molecule inhibitors to test the role of dynamin in synaptic vesicle endocytosis. Some of these studies claim that dynamin plays an undisputed role in synaptic vesicle recycling. One particular study concluded that dynamin is essential for all forms of synaptic vesicle endocytosis and that inhibition

of dynamin by Dynasore had no alternative effect on exocytosis (Newton et al, 2006). However, this claim is not consistent to data obtained during the experiments for this project. Another study demonstrated that the inhibition of LDL cholesterol trafficking by Dynasore led to an abnormal accumulation of the internalized cholesterol in the endolysosomal network (Girard et al, 2011). An additional study has demonstrated that dynamin inhibition with Dynasore prevents tubule fission from early endosome compartments, sequestering 63.2% of internalized transferrin within the compartment and impairing recycling to the cell surface (Mesaki et al, 2011). This sequestering of cholesterol and transferrin is more consistent with the apparent sequestration of DAT observed in this project.

PROJECT PURPOSE

This project was completed to experimentally determine the potential role of the GTPase dynamin in DAT endocytosis. While previous studies had shown that DAT did not internalize via the same clathrin-mediated endocytic pathway as transferrin, the particular mechanism utilized by DAT and whether this mechanism relied on dynamin for vesicular budding remained unknown. Characterization of the effects of dynamin inhibition on DA uptake in cultured cell lines would help elucidate the endocytic pathway by which DAT internalizes. The hypothesis being tested states that DAT internalization occurs via a dynamin-dependent but clathrin-independent pathway.

METHODS

Cell Lines and Culture

The cell line utilized in this study was of PC12 cells stably expressing hDAT. PC12 cells are derived from pheochromocytoma of the rat adrenal medulla. The cells were cultured at 37°C 10%CO_2 in DMEM supplemented with 5% Horse Serum, 5% Bovine Calf Serum, 2mM Glutamine, 10^2 units/ml Penicillin/Streptomycin antibiotic and 0.2mg/ml G418 to ensure expression of DAT.

Plasma Membrane Transport Assays

DAT PC12 cells were maintained in cell culture as described above. Cells were plated at a density of $2.5x10^5$ cells per well in 24 well scintillation plates. Before plating, each well was washed with 0.5 mg/ml poly-D-lysine to provide a layer for the PC12 cells to adhere to and prevent loss of cells during later washes of the assay. After 15 minutes the wells were washed with PBS to remove excess poly-D-lysine. Cells were plated and allowed to incubate for 24 hours. Following incubation, the cells were washed three times with KRH buffer (120 nM NaCl, 4.7mM KCl, 1.2mM MgSO4, 1.2mM KH2PO4, 2.2mM CaCl2, and 10mM HEPES, pH 7.4). A 0.18% glucose in KRH solution was used in the preparation of drugs for the membrane transport assays. All assays had a total volume of 250µl. Dynamin inhibiting drugs, Dynasore or Dynole, were added to their respective wells and allowed to pre-incubate for an allotted time. Following the pre-incubation, the cells were subsequently incubated with any other drugs being tested. After all drug incubations, [^{3}H]DA in a 10µM pargyline and 10µM ascorbic acid supplemented KRH/glucose solution was added to each well. The specific

activity of the [^{3}H]DA cocktail was diluted 20x by adding nonradioactive DA to obtain the desired concentration. Endogenous NE transporter activity was blocked by adding 0.1μM DMI to each well. Non-specific counts were obtained by using GBR 12909 to block DAT activity. In some assays, PMA was used to observe rapid DAT down-regulation. After incubating with [^{3}H]DA for 10 minutes, the cells were washed three times with ice cold KRH buffer to halt transporter activity. Cells were then lysed in 250μl scintillation fluid while shaking for 15 minutes before the plate was counted in a scintillation counter.

Plasma membrane transport assays were also performed to observe [^{3}H] Alanine uptake. These assays followed nearly the same procedure as those for [^{3}H]DA with the exception that non-specific counts were obtained by removing Na+ from any solutions that the cells in the NS condition were treated with. This was accomplished by making a KRH solution with Choline in place of Sodium Chloride (120 nM Choline, 4.7mM KCl, 1.2mM MgSO4, 1.2mM KH2PO4, 2.2mM CaCl2, and 10mM HEPES, pH 7.4). Alanine uptake assays also lacked DMI because endogenous NE transport was irrelevant or pargyline and ascorbic acid in the [^{3}H]Ala cocktails.

RESULTS

The purpose of this study was to experimentally determine the role of the GTPase dynamin in DAT endocytosis. Previously, dynamin had been shown to be involved in the budding of vesicles during several endocytic mechanisms, including clathrin-mediated endocytosis. However, additional studies have determined that DAT does not internalize via the same clathrin-mediated endocytic pathway as transferrin, and the particular mechanism utilized by DAT and whether this mechanism relies on dynamin for vesicular budding remains unknown. The original hypothesis being tested was that DAT internalization utilizes a dynamin-dependent endocytic pathway.

Studies of DA Uptake with Dynamin Inhibition

In order to determine whether dynamin is involved in DAT trafficking, dopamine uptake assays were performed on PC12 cells that stably expressed DAT. DA uptake assays were performed ±dynamin inhibitor ± the phorbal ester, PMA. Incubation with PMA results in DAT down-regulation through rapid internalization of the transporter. If Dynamin were involved in DAT endocytosis, incubation of DAT PC12 cells with a Dynamin inhibitor, either Dynasore or Dynole, would prevent or reduce this down-regulation. Incubation with PMA alone exhibited a loss in DA uptake characteristic of phorbal ester down-regulation; a 75% loss of specific DA uptake with Dynole (Figure 3A) and 70% loss with Dynasore (Figure 3B). In the case of both dynamin inhibitors, there was also a loss of specific DA uptake when incubated with the dynamin inhibitor alone. There was a 65% loss seen with Dynole (Figure 3A) and a 41% loss with

Dynasore (Figure 3B). An even more drastic loss of specific uptake was observed when DAT PC12 cells were incubated first with a dynamin inhibitor and then again with PMA. There was a total loss of 82% specific DA uptake when using Dynole and PMA(Figure 3A) total of 80.4% loss of specific uptake when using Dynasore and PMA(Figure 3B).

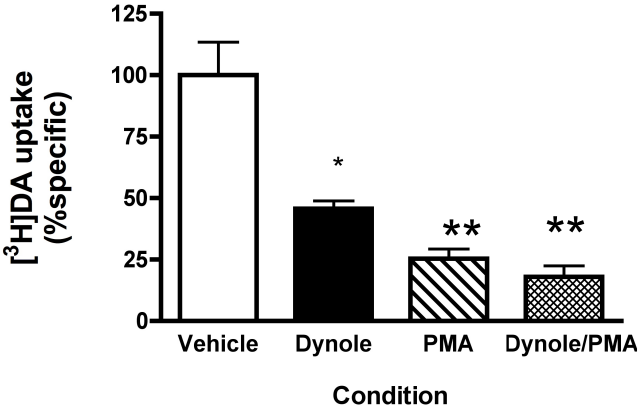

Figure 1A: Effect of Dynole on PMA-Mediated DAT Down-Regulation
[^{3}H]DA uptake ±Dynole ±PMA is measured as a percentage of vehicle DA uptake. *Significantly different from Vehicle, $p<0.01$ ** $p<0.001$, One-way ANOVA w/ Tukey's Multiple Comparison test, n=7.

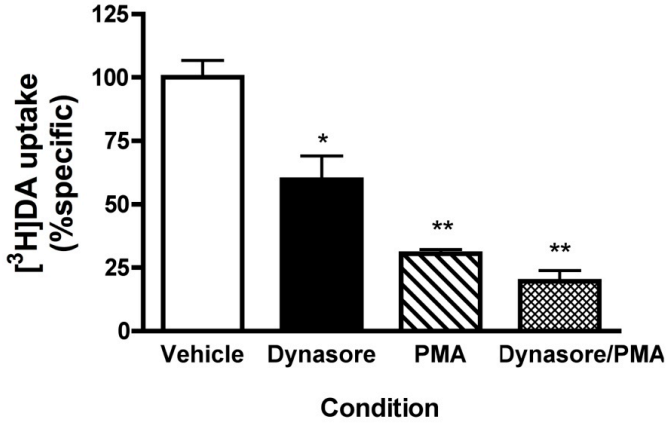

Figure 1B: Effect of Dynasore on PMA-Mediated DAT Down-Regulation
[^{3}H]DA uptake ±Dynasore ±PMA is measured as a percentage of vehicle DA uptake. *Significantly different from Vehicle, $p<0.01$ ** $p<0.001$, One-way ANOVA w/ Tukey's Multiple Comparison test, n=4.

The failure of dynamin inhibition to block or reduce PMA-mediated DAT down-regulation leads to the conclusion that DAT endocytosis must be dynamin-independent. However, because dynamin inhibition caused a reduction in DA uptake, dynamin must be involved at some other stage in the processing or recycling of internalized DAT.

DA Uptake Kinetics Following Dynamin Inhibition

After it was observed that dynamin inhibition caused a loss of specific DA uptake on its own, it was decided that the kinetics of DAT's interaction with DA in the presence of a dynamin inhibitor should be studied to determine how the addition of a dynamin inhibitor reduced DA transport. In the kinetics assays, uptake was recorded ±Dynasore with varying concentrations of [^{3}H]DA. These were performed to determine any changes caused by dynamin inhibition in either the maximum velocity of DA binding at saturation (V_{max}) or the concentration at which the velocity was half of V_{max} (K_m) of DA transport. Data from theses assays showed a drastic decrease in the V_{max} following Dynasore treatment as can be seen in a representative Michaelis-Menton curve in Figure 4A.

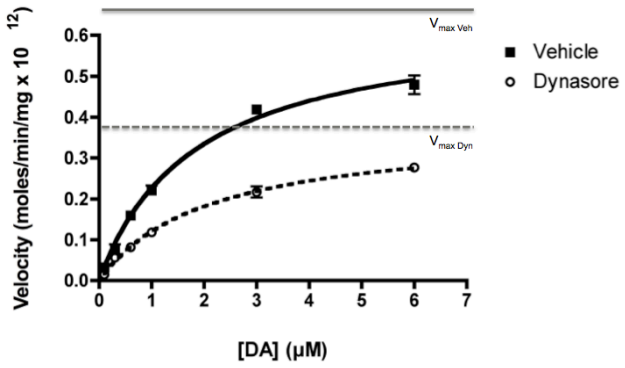

Figure 4A: Michaelis-Menton Kinetics of DA Uptake ±Dynasore Velocity of [^{3}H]DA uptake ±Dynole is measured at DA concentrations of 0.1, 0.3, 0.6, 1.0, 3.0 and 6.0µM. Graph is representative of at least 6 independent experiments.

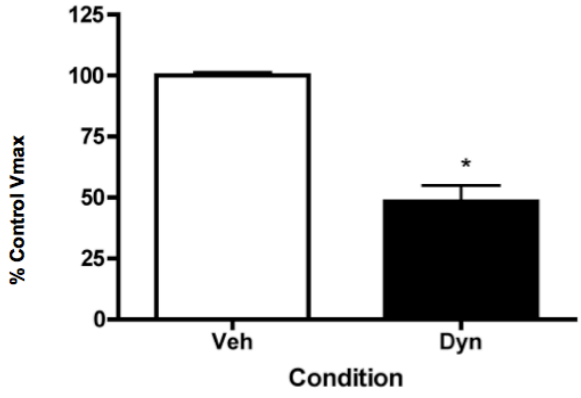

Figure 4B: Effect of Dynasore on the Vmax of DA Uptake Kinetics Vmax ±Dynasore is measured as a percentage of the vehicle Vmax. *Significantly different from Vehicle, $p<0.05$ Unpaired t test, n=6.

Over the course of six kinetics experiments, it was observed that there was a significant decrease in V_{max} following dynamin inhibition, but no significant change in K_m. On average, the V_{max} in the presence of Dynasore was only 48.3% of the vehicle V_{max} (Figure 4B).

[^{3}H] Alanine Uptake Following Dynamin Inhibition

To test if the loss in DA uptake and decrease in V_{max} that occurred following dynamin inhibition was the result of any disruption by the dynamin inhibiting drugs to either the cell membrane or the Na+ gradient that DAT relies on for transport, [^{3}H] Alanine uptake assays were performed ±Dynole. Alanine transport in PC12 cells is Na^+ dependent and an altering of the Na^+ gradient would be reflected in [^{3}H]Ala uptake. Concurrently, a disruption of the dynamics of the plasma membrane could also be reflected in [^{3}H] Alanine uptake.

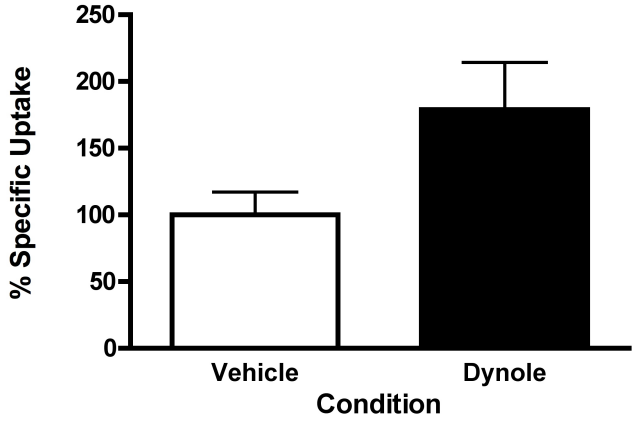

Figure 5: Effect of Dynole on Na+ Dependent Alanine Uptake
[^{3}H]Alanine uptake ±Dynole is measured as a percentage of vehicle Alanine uptake. There is no significant difference between conditions $p>0.05$.

The 3H Alanine studies showed that there was no significant loss in 3H Alanine uptake in the presence of Dynole and if anything, there was a trend that 3H Alanine uptake increased in its presence (Figure 5). Additionally, it suggested that the loss in DA

uptake following dynamin inhibition was not the result of any Na+ gradient change or a disruption of the cell membrane that would cause interrupted transporter functions.

Dynamin Inhibition Time Course

When it was determined that DAT internalization was dynamin-independent, further studies were performed to attempt to determine what point in DAT recycling was dynamin-dependent. To test this, time course assays were performed. In the time course, DAT PC12 cells were incubated with the dynamin-inhibitor Dynole for 5, 10, 15, 20, 30 and 45 minute intervals to determine how long of an incubation was needed for an effect.

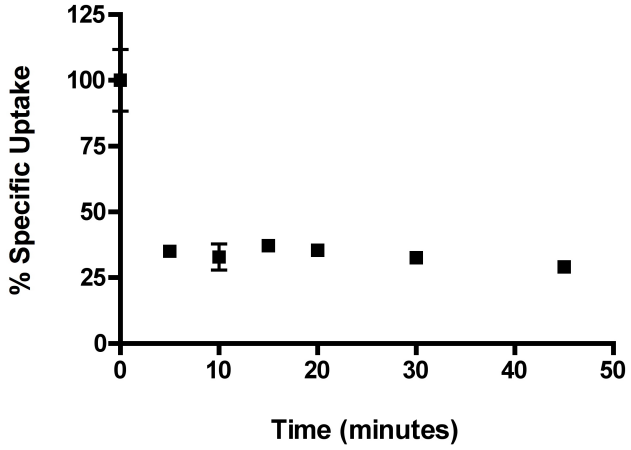

Figure 6: Effect of Dynole on DA Uptake
[^{3}H]DA uptake ±Dynole is measured as a percentage of vehicle DA uptake. Cells were incubated with Dynole for 5, 10, 15, 20, 30 and 45 minutes.
n=3.

The assays revealed that there was a drastic loss in specific DA uptake after only 5 minutes incubation with Dynole (Figure 6). Overall this was observed to be an average 65% loss of specific uptake and that Dynole reduced DA uptake regardless of the incubation period.

DISCUSSION

The overall conclusion that can be made from these experiments is that the dopamine transporter is internalized via a dynamin-independent endocytic pathway. The failure of dynamin inhibition to block or reduce PMA-mediated DAT down-regulation leads to this conclusion.

Furthermore, because there was an observed loss in DA uptake when cells were treated solely with a dynamin inhibitor and an additional loss in DA uptake when cells were treated with PMA following dynamin inhibition, the study suggests that dynamin must be involved at some subsequent stage in DAT trafficking. According to Michaelis-Menton kinetics, the observed reduction in the V_{max} of DA uptake following treatment with dynamin indicates that the transporter has either become inactivated or there has been a loss of total transporter from the system. Data from [3H] Alanine uptake assays can be used to infer that the loss in DA uptake from the dynamin inhibitor Dynole is not the result of a Na+ gradient change or a disruption of the plasma membrane that would cause interrupted transporter functions. Therefore, the decrease in V_{max} must be due to a subtraction of DAT from the cell surface and the inhibition of dynamin must sequester DAT in some compartment within the cell, suggesting that dynamin is involved at some point in the processing or recycling of internalized DAT.

Further studies on the internalization and recycling pathways utilized by DAT are needed to fully understand how exactly dynamin is involved in DAT trafficking. Cell surface biotinylation experiments could be used to quantitatively measure changes in DAT levels on the cell surface following dynamin inhibition. Immunocytochemistry

experiments are needed to observe where DAT is sequestered in the presence of a dynamin inhibitor and whether it co-localizes in any particular compartment, such as early endosomes, endosomal recycling compartments or lysosomes. Additionally, further experiments should be performed to determine the nature of the apparently dynamin- and clathrin-independent endocytic pathway utilized by DAT.

The dopamine transporter has been implicated in a number of neurological disorders including Bipolar Disorder, Attention Deficit Disorder, and drug addiction. Fully understanding the activity and trafficking of DAT is vital for the potential treatment of such disorders. The data from this MQP can help narrow down the possible endocytic pathways that could be employed in DAT endocytosis by eliminating any that are dynamin-dependent. Furthermore, the data raises new questions into how many other pathways there may be for the internalization of plasma membrane vesicles.

BIBLIOGRAPHY

Boudanova, E., Navaroli, D.M., Stevens, Z.H., & Melikian, H.E. (2008). " Dopamine transporter endocytic determinants: carboxy terminal residues critical for basal and PKC-stimulated internalization". *Mollec. & Cellular Neurosci*. 39: 211-217.

Boudanova, E., Navaroli, D.M., & Melikian, H.E. (2008). "Amphetamine-induced decreases in dopamine transporter surface expression are protein kinase C-independent". *Neuropharmacology*. 54: 605-612.

Buckley, K.M., Melikian, H.E., Provoda, C.J., & Waring, M.T. (2000). "Regulation of neuronal function by protein trafficking: a role for the endosomal pathway". *J. Physiol*. 525.1: 11-19.

Damke, H., Baba, T., Warnock, D.E., & Schmid, S.L. (1994). "Induction of mutant dynamin specifically blocks endocytic coated vesicle formation". *J. Cell Biology*. 127 (4): 915-934.

Daniels, G. M., & Amara, S.G. (1999). "Regulated trafficking of the human dopamine transporter". *J. Biol. Chem*. 274(50): 35791-35801.

Doherty, G.J., & McMahon, H.T. (2009). "Mechanisms of endocytosis". *Annu. Rev. Biochem*. 78: 31.1-31.46.

Girard, E., Paul J.L., Fournier, N., Beaune, P., Johannes, L., et al. (2011). "The dynamin chemical inhibitor Dynasore impares cholesterol trafficking and sterol-sensitive genes transcription in human HeLa cells and macrophages". *PLoS ONE*. 6(12): e29042. Doi:10:1371/journal.pone.0029042.

Hill, T.A., Gordon, C.P., McGeachie, A.B., et al. (2009). "Inhibition of dynamin mediated endocytosis by the dynoles - synthesis and functional activity of a family of indoles". *J. Med. Chem*. 52: 3762-3773.

Holton, K.L., Loder, M.K., & Melikian, H.E. (2005). "Nonclassical, distinct endocytic signals dictate constitutive and PKC-regulated neurotransmitter transporter internalization". *Nature Neurosci*. 8(7): 881-888.

Kirchhausen, T., Macia, E., & Pelish, H.E. (2008). "Use of dynasore, the small molecule inhibitor of dynamin, in the regulation of endocytosis". *Methods Enzymol*. 438: 77-93.

Kong, M.M.C., Hasbi, A., Mattocks, M., Fan, T., O'Dowd, B.F., & George, S.R. (2007). "Regulation of D1 dopamine receptor trafficking and signaling by caveolin-1". *Mol. Pharmacol*. 72:1157-1170.

Loder, M.K., & Melikian, H.E. (2003). "The dopamine transporter constitutively internalizes and recycles in a protein kinase C-regulated manner in stably transfected PC12 cell lines". *J. Biol. Chem.* 278(24): 221368-22174.

Macia, E., Ehrlich, M., Massol, R., Boucrot, E., Brunner, C., & Kirchhausen, T. (2006). "Dynasore, a cell-permeable inhibitor of dynamin". *Developmental Cell*. 10: 839-850.

Melikian, H.E., & Buckley, M.K. (1999). "Membrane trafficking regulates the activity of the human dopamine transporter". *J. Neurosci*. 19(18): 7699-7710.

Melikian, H.E., (2004). "Neurotransmitter transporter trafficking: endocytosis, recycling and regulation". *Pharmacol. & Therapeutics*. 104: 17-27.

Mesaki, K., Tanabe, K., Obayashi, M., Oe, N., & Takei, K. (2011). "Fission of tubular endosomes triggers endosomal acidification and movement". *PLoS ONE*. 6(5):e19764. Doi:10.1371/journal.pone.0019764.

Navaroli, D.M., Stevens, Z.H., Uzelac, Z., Gabriel, L., et al. (2011). "The plasma membrane-associated GTPase Rin interacts with the dopamine transporter and is required for protein kinase C-regulated dopamine transporter trafficking". *J. Neurosci*. 31(39): 13758-13770.

Newton A.J., Kirchhausen, T., & Murthy, V.N. (2006). "Inhibition of dynamin completely blocks compensatory synaptic vesicle endocytosis". *PNAS*. 103 (47): 17955-17960.

Saunders, C., Ferrer, J.V., Shi, L., Chen, J., et al. (2000). "Amphetamine-induced los of human dopamine transporter activity: an internalization-dependent and cocaine sensitive mechanism". *PNAS*. 97(12): 6850-6855.

Sorkina, T., Miranda, M., Dionne, K.R., Hoover, B.R., Zahniser, N.R., & Sorkin, A. (2006). "RNA interference screen reveals an essential role of Nedd4-2 in dopamine transporter ubiquitination and endocytosis". *J. Neurosci*. 26(31):8195-8205.

Takei, K., Yoshida, Y., & Yamada, H. (2005). "Regulatory mechanisms of dynamin-dependent endocytosis". *J. Biochem*. 137: 243-247.

Zahniser, N.R., & Sorkin, A. (2004). "Rapid regulation of the dopamine transporter: role in stimulant addiction?". *Neuropharmacology*. 47: 80-91.

Zahniser, N.R., & Sorkin, A. (2009). "Trafficking of dopamine transporters in psychostimulant actions". *Sem. Cell & Dev. Bio*. 20:411-417.