

AWARD NUMBER: **W81XWH-15-1-0557**

TITLE: Central and Peripheral Mechanisms of Antipsychotic Medication-Induced Metabolic Dysregulation

PRINCIPAL INVESTIGATOR: Gary J. Schwartz, Ph.D.

CONTRACTING ORGANIZATION: Albert Einstein College of Medicine
Bronx, NY 10463

REPORT DATE: October 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2018		2. REPORT TYPE Annual Report		3. DATES COVERED 30 Sep 2017 - 29 Sep 2018	
4. TITLE AND SUBTITLE Central and Peripheral Mechanisms of Antipsychotic Medication- Induced Metabolic Dysregulation				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-15-1-0557	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Gary J. Schwartz, Ph.D. E-Mail: gary.schwartz@einstein.yu.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Albert Einstein College of Medicine 1300 Morris Park Avenue Bronx, NY 10463				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Antipsychotic drugs (APDs) are widely used psychotropic medications, though they have significant metabolic side effects. While the mechanisms for these metabolic disturbances are poorly understood, the single known unifying property of all APDs is their blockade of the dopamine D ₂ (D2R) and D ₃ (D3R) receptors. We therefore hypothesize that D2R and/or D3R mediate the metabolic side effects of APDs both centrally in the hypothalamus and peripherally in pancreas, areas critical for metabolic regulation. In Year 1 of this award, we have completed the design of a D3R-flox mouse in order to selectively knock out expression of D3R in the hypothalamus and pancreatic beta cells. The resulting transgenic mice are being tested to confirm the successful production of the strain. In parallel, we have completed construction of novel inducible transgenic hypothalamic- and pancreatic beta cell-specific D2R knockout (KO) mice. Additionally, using pancreatic islets isolated from beta cell-selective D2R KO mice and complete D3R KO mice, we found diminished inhibition of stimulated insulin secretion in both strains relative to littermate controls, suggesting a role for both receptors in mediating insulin secretion.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	7	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	1
2. Keywords.....	1
3. Accomplishments.....	2
4. Impact.....	5
5. Changes/Problems.....	6
6. Products.....	6
7. Participants & Other Collaborating Organizations.....	7
8. Special Reporting Requirements.....	7
9. Appendices.....	7

1. INTRODUCTION

Antipsychotic drugs (APDs) are widely used psychotropic medications for numerous psychiatric illnesses including schizophrenia, posttraumatic stress disorder and depression. However, these medications also have significant metabolic side effects characterized by substantial weight gain, glucose intolerance, insulin resistance, hypertension and dyslipidemia as well as increased risks for type 2 diabetes and cardiovascular disease. Indeed, the prevalence of these APD-induced metabolic side effects in Veterans is more than twice that of the general population. However, the mechanisms for these metabolic disturbances are not well understood. Significantly, all APDs cause these side effects to differing degrees and ultimately result in life-shortening morbidity. A potentially important clue is that the single known unifying property of all APDs is their blockade of the dopamine D₂ (D2R) and D₃ (D3R) receptors, suggesting a role for these receptors in APD metabolic side effects. Consistent with this, D2R and D3R are expressed both centrally in the hypothalamus in regions mediating appetite and feeding behavior as well as peripherally in insulin-releasing pancreatic beta cells, key regulators of metabolism. We previously showed that activation of pancreatic beta cell D2R and D3R inhibited glucose-stimulated insulin secretion (GSIS) and that APD-induced receptor inhibition disrupted this regulatory mechanism. Thus, our central hypothesis is that D2R and/or D3R are critical regulators of metabolism and mediate the metabolic side effects of APDs both centrally in the hypothalamus and peripherally in pancreas. However, the relative contributions of peripheral and central D2R and D3R to APD-induced metabolic dysregulation are unknown. To disentangle these mechanisms, in partnership with PI Dr. Zachary Freyberg, we will aim to do the following: (1) to identify contributions of hypothalamic D2R and D3R action in APD-induced weight gain and metabolic dysregulation *in vivo*; (2) to identify the relationship of peripheral D2R and D3R to APD-induced weight gain and metabolic dysfunction *in vivo*; and (3) to identify APD-mediated effects on insulin and DA release in pancreatic beta cells using real-time imaging. Key to these aims is the generation of tissue-specific D2R and D3R knockout (KO) mice targeting either hypothalamus or pancreatic beta cells. Moreover, in focusing on the peripheral contributions of pancreatic D2R and D3R, we have also developed new and highly sensitive optical and biochemical assays to study D2R- and D3R-mediated effects on insulin and DA release in real-time. We have applied these new assays to an experimentally tractable model using the well-characterized rat beta cell-derived INS-1E cell line for our *in vitro* studies, in addition to our work in the D2R and D3R KO pancreatic islets. In the short term, our work will elucidate the anatomical and functional mechanisms of APD-induced metabolic side effects. In the longer term, we will use our findings to develop better-targeted APDs that can selectively reverse these drugs' metabolic side effects while preserving their clinical efficacy.

2. KEYWORDS

Keywords relevant to the work proposed here include:

1. Antipsychotic drug (APD)
2. Dopamine (DA)
3. Dopamine D₂ Receptor (D2R)
4. Dopamine D₃ Receptor (D3R)
5. Insulin
6. Glucose-stimulated insulin secretion (GSIS)
7. Diabetes
8. Metabolism

3. ACCOMPLISHMENTS

- **What were the major goals of the project?**

The major goals of the project as stated in the approved SOW are as follows:

- A. Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment
- B. Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or absence of APD treatment
- C. Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease
- D. Determine the precise contributions of D2R and D3R to glucose-stimulated insulin and dopamine release using pancreatic islets from pancreatic beta cell-selective D2R and D3R knockout mice as well as wildtype controls
- E. Determine effects of APDs on kinetics of real-time glucose-stimulated insulin and dopamine release in wildtype and beta cell-specific D2R or D3R knockout mouse pancreatic islets

- **What was accomplished under these goals?**

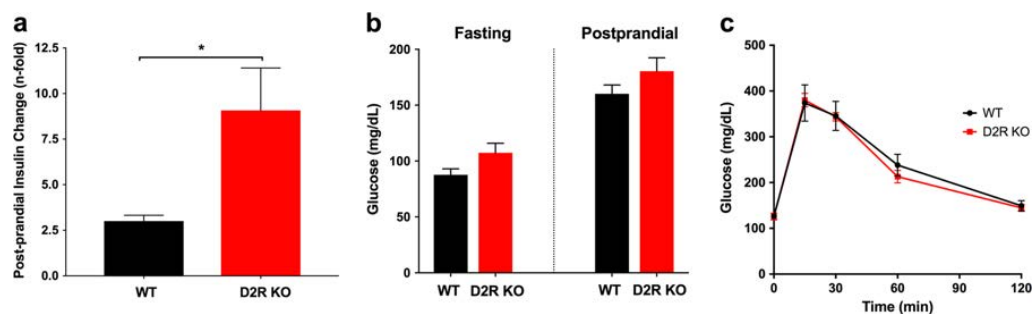
- **Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment**

To confirm a role for D2R/D3R in the modulation of systemic glucose tolerance we have been conducting measurement of weights and food consumption and systemic oral glucose tolerance test in wildtype littermate controls for the hypothalamus-specific D2R knockout mice. Mice were treated 2-6 weeks, 5 days per week, with intraperitoneal administration of the D2/D3 agonist bromocriptine, as well as a peripherally limited quaternary analogue of bromocriptine AB01, or vehicle. We have observed that the peripherally acting bromocriptine analog AB01 fails to alter glucose tolerance, while bromocriptine does. These data support a novel potential central neuronal action of dopamine in the beneficial control of glucose homeostasis in diet induced glucose intolerance. Our follow-up studies using our forebrain specific Nkx2.1-Cre D3Rflox mice (see below) will evaluate the degree to which forebrain D3 receptors are involved in this improvement.

We have also established and are now successfully breeding hypothalamus-specific D3R knockout mice (Nkx2.1-cre hemizygous, D3R-flox homozygous mice). These animals are currently being maintained on the high fat-high carbohydrate diet that we have used to promote the development of glucose intolerance. This is expected to take approximately 16 weeks. Once this dietary regimen is complete, we will perform basal glucose and insulin measurements as well as place the animals in metabolic cages to identify and characterize the role of forebrain-specific D3R in the development of glucose intolerance, food intake, obesity and energy expenditure. Based on our recently completed and published work in Rip1cre-Drd2-flox beta cell-selective KO animals, we do not anticipate any difficulties in performing these metabolic characterizations.

We also investigated D2R's role in maintaining insulin homeostasis in vivo using our β -cell-selective D2R KO mice. We measured the effects of D2R KO on changes in serum insulin levels in response to a meal challenge. Although there were no significant differences in fasting serum insulin

levels between D2R KO and the WT control mice preceding the meal challenge ($P > 0.05$), we observed significantly higher serum insulin levels in D2R KO mice following a meal challenge ($P = 0.038$). We explored the possibilities that these insulin increases were in response to concomitant elevations in blood glucose and/or increased insulin resistance in the D2R KO mice relative to the WT control animals. Comparisons of fasting or postprandial glucose levels between the two genotypes did not reveal any significant differences ($P > 0.05$). Furthermore, we found no significant differences in insulin sensitivity either at basal or post-glucose infusion timepoints between D2R KO and WT mice as measured by intraperitoneal glucose tolerance testing (ipGTT) ($P > 0.05$) or via calculation of the HOMA-IR ($P > 0.05$; WT: 4.3 ± 0.8 , D2R KO: 8.9 ± 2.4). These data suggest that the constituents of the food pellets provided in the oral challenge were sufficient to generate DA precursors that are absent when glucose is administered i.p. Importantly, our results also suggest that the differences in postprandial insulin levels may therefore be a consequence of direct changes in insulin secretion rather than in response to insulin resistance or elevated blood glucose levels in the D2R KO mice. Overall, these data suggest that D2R modulation of insulin release is especially sensitive to acute food intake. Moreover, blunted D2R signaling in β -cells may cause decreased DA-mediated GSIS inhibition and lead to the postprandial hyperinsulinemic state observed.



CAPTION: Glucose-stimulated DA secretion is reduced in D2R and D3R KO pancreatic islets. A) Postprandial elevation in serum insulin levels was threefold higher in homozygous pancreatic β -cell-selective D2R KO mice ($n = 12$) compared with WT littermate controls ($n = 9$; $P = 0.038$). Postprandial serum insulin values were normalized to subjects' respective pre-meal fasting serum insulin levels. Assays were conducted in triplicate on $n \geq 3$ independent experimental days. b There were no significant differences in either pre-meal fasting or postprandial glucose levels between homozygous pancreatic β -cell-selective D2R KO mice ($n = 12$) compared with WT littermate controls ($n = 9$; $P > 0.05$). c Intraperitoneal glucose tolerance test (ipGTT, 2 g/kg). There were no significant differences in glucose tolerance between homozygous pancreatic β -cell-selective D2R KO mice ($n = 6$) compared with WT littermate controls ($n = 10$; $P > 0.05$). All bars and points represent the mean $\pm$ SEM. * $P < 0.05$

- **Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or absence of APD treatment**

We are in the process of characterizing the quantity and duration of tamoxifen necessary to induce successful deletion of D2R in our inducible pancreatic beta cell-specific D2R knockout mice. Our current studies have not yet produced reliable significant knockdown, using an intraperitoneal route of administration, 100 mg/kg, every other day (3 times a week) for 1 week, where the tamoxifen is

dissolved in Prepare 90% corn oil and 10% ethanol mix. We are evaluating alternative vehicles such as dimethyl sulfoxide as the tamoxifen emulsion seems to precipitate and is not well distributed once injected. Once we have confirmed deletion of D2R in pancreatic islets isolated from beta cells, we will begin characterizing the metabolic status of these animals from week 3 of life onwards following completion of weaning. Specifically, we will conduct weekly measurement of weights and food consumption in beta cell-specific D2R (and wildtype littermate controls) treated with either with first-generation APD haloperidol or second-generation APD olanzapine (via i.p. administration). We will also measure serum fasting glucose and insulin levels in hypothalamus-specific D2R knockout mice and wildtype littermate control mice in the presence or absence of APD treatment; serum will be collected at weeks 13 and 26 of APD treatment. Once this is complete, we will begin a similar process for pancreatic beta cell-specific D3R knockout mice.

- **Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease**

We have now finalized the dietary conditions necessary to induce insulin resistance, and we are preparing to use this diet to compare effects of domperidone on the rate of development of insulin resistance both in wildtype as well as in beta cell-specific D2R or D3R knockout mice. Besides insulin resistance, we will look at other markers of metabolic disease including adiposity, fatty liver and pancreatic beta cell mass.

- **What opportunities for training and professional development has the project provided?**

Nothing to Report.

- **How were the results disseminated to communities of interest?**

Work resulting from this award were presented at a national meeting the Behavior, Biology and Chemistry meeting in San Antonio, TX in March 2018. At this meeting, the data was presented both as an abstract as well as through a poster presentation, demonstrating a weight-independent improvement in glucose tolerance following chronic administration of the D2R/ D3R agonist bromocriptine. Presently, we are preparing two manuscripts based on our characterization of the mechanisms by which dopamine and dopamine D₂ and D₃ receptors mediate glucose-stimulated insulin secretion. We hope that the publication of these manuscripts will facilitate the dissemination of the experimental data derived from the aims of this project.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

The presentation of our preliminary results at scientific meetings including at a national conference, the Behavior, Biology and Chemistry meeting in San Antonio, TX in March 2018, were instrumental in advancing the concept that APDs may act on peripheral dopaminergic targets. In presenting this work during talks and poster presentations, our findings were broadly disseminated to a broad scientific audience whose expertise spans multiple disciplines including neuroscience, endocrinology, cell biology and clinical medicine.

- **What was the impact on other disciplines?**

In the longer term, the knowledge resulting from our work may directly lead to development of better APDs free of metabolic side effects. This could significantly reduce serious morbidity and mortality from medication-associated type II diabetes and cardiovascular disease. Moreover, better understanding the mechanisms by which dopamine and dopamine receptors mediate insulin release may also significantly contribute to our fundamental understanding of obesity and lead to novel treatments. Since APD-induced metabolic disturbances also increase risks of developing type II diabetes and Alzheimer's disease, further elucidating the mechanisms of APD-induced weight gain may also lead to fundamental insights into the mechanisms for development of these disorders.

- **What was the impact on technology transfer?**

Nothing to Report.

- **What was the impact on society beyond science and technology?**

Nothing to Report.

5. **CHANGES/PROBLEMS**

Nothing to Report.

6. **PRODUCTS**

- **Publications, conference papers, and presentations**

Journal publications

Farino ZJ, et al. New roles for dopamine D2 and D3 receptors in pancreatic beta cell insulin secretion. Mol Psychiatry. 2019 Jan 9. doi: 10.1038/s41380-018-0344-6. [Epub ahead of print]

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations

Abstract for Behavior, Biology and Chemistry meeting in San Antonio, TX in March 2018; "Novel Tools to Investigate the Role of Dopamine D2/D3 Receptors in Antipsychotic Drug-Induced Metabolic Disease"

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Nothing to Report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

• **What individuals have worked on the project?**

• Name:	Gary Schwartz
• Project Role:	Principal Investigator
• Researcher Identifier (e.g. ORCID ID):	ORCID ID: 0000-0003-0446-5553
• Nearest person month worked:	3
• Contribution to Project:	Dr. Schwartz has designed performed and analyzed all experimental data in the areas of metabolic and behavioral assessments of dopamine action at pancreatic and central neural sites.
• Funding Support:	National Institutes of Health/ R01

• **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report.

• **What other organizations were involved as partners?**

Nothing to Report.

8. **SPECIAL REPORTING REQUIREMENTS**

• **Collaborative Awards**

We have worked closely with the Partnering PI of this award, Dr. Zachary Freyberg.

9. **APPENDICES**

Below is the abstract presented as a poster at a national meeting (Behavior, Biology and Chemistry meeting in San Antonio, TX in March 2018) (see the **Products** section).

A. Abstract and poster presented at the Behavior, Biology and Chemistry meeting in San Antonio, TX in March 2018

Novel Tools to Investigate the Role of Dopamine D2/D3 Receptors in Antipsychotic Drug-Induced Metabolic Disease

Bonifazi, Alessandro,¹ Ellenberger, Michael P.,¹ Schwartz, Gary J.,² Freyberg, Zachary,³ Newman, Amy H.¹

¹*Medicinal Chemistry Section, Molecular Targets and Medications Discovery Branch, NIDA-IRP, NIH, Baltimore, MD 21224.* ²*Departments of Medicine and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.* ³*University of Pittsburgh, Department of Psychiatry, Pittsburgh, PA 15213.*

Antipsychotic drugs (APDs) cause significant metabolic side effects and increased risks for type II diabetes with consequent high rates of treatment discontinuation. The ubiquitous trait amongst effective clinical APDs is their antagonism at dopamine D2R and D3R playing a role in

the mediation of APD-induced metabolic side effects. Dopamine (DA) signaling through D2R and D3R in the central nervous system (CNS) mediates appetite and feeding behavior. Because both D2R and D3R are also expressed peripherally in insulin secreting pancreatic beta cells, DA signaling outside the CNS may be involved in systemic metabolic regulation. Activation of these receptors in beta cells mediates a negative feedback where DA co-released with insulin inhibits further insulin secretion. To avoid exacerbating psychosis by countering APDs' actions in the CNS, we have designed peripherally-limited D2R or D3R agonists via quaternization at the basic nitrogen. Most of the quaternary ammonium salts showed only a moderate loss of affinity at the D2R, retaining agonist profiles when compared with their parent molecules. We examined these quaternary salts and their parent drugs in beta cell islet assays to determine their effects on glucose-stimulated insulin release. Bromocriptine methiodide, the most promising quaternary analogue, was compared to bromocriptine and evaluated for metabolic stability in mouse microsomes as well as for blood/brain plasma ratios. Bromocriptine methiodide is currently being evaluated in *in vivo* metabolic analyses in mice including indirect calorimetry, food intake and body weight in the presence or absence of APD treatment. In total, these studies will further illuminate mechanisms underlying APD-induced metabolic syndrome and may lead to improved APD design.