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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 D2 (D2R) and D3 (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. We had completed the design of a novel inducible D3R-flox mouse in order to selectively knock out expression of D3R in the hypothalamus and pancreatic beta cells, but had lost them due to Covid. We are reconstituting them now. We did not identify major metabolic deficits in central neuronal Nkx 2.1 D3R, D2R, or D3/R D2R knockouts relative to their respective controls. We are currently evaluating metabolic and glucose homeostasis phenotypes following Olanzapine and Bromocriptine treatment in HFD mice.

15. SUBJECT TERMS
None listed.

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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**

We continue to be significantly delayed due to COVID related restrictions on animal availability and the ability to perform experiments in the laboratory. We have been successful rebreeding hypothalamus (Nkx2.1) -specific D2R and D3R knockout 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. One of the cohorts of males and females have developed slightly but significantly increased adiposity and glucose intolerance and they are being tested in metabolic cages to identify changes in energy expenditure and food intake that may contribute to these body mass and glucose phenotypes. Basal glucose and insulin levels in these animals are elevated, and we have placed the animals in metabolic cages to identify and characterize the role of forebrain-specific D2R and D3R in the development of glucose intolerance, food intake, obesity and energy expenditure. We have not yet found significant differences between these hypothalamic D2R or D3 knockout animals and their respective genetic background strain controls. We hypothesize that this may in part be due to the relative lack of specificity of the D2/D3 knockdown, which includes non-hypothalamic neurons. We have accordingly pursued more hypothalamic specific Cre- mouse lines unique to hypothalamic neuronal populations implicated in the control of blood glucose and energy balance, such as agouti-related protein (AGRP). We have been delayed by COVID in acquiring the Agrp-Cre line but have now bred these animals and are maintaining them on HFD, to be followed by tests of glucose and insulin tolerance, food intake, body composition and energy expenditure. We do not anticipate any difficulties in performing these metabolic characterizations.

- **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 have recently been submitted in abstract form to the American Diabetes Association Annual Meeting, June 2022, New Orleans, LA, “Novel tools to dissect the metabolic roles of Central and Peripheral Dopamine D2 receptors” , in addition to our recently published work in Molecular Psychiatry, documenting for the first time the direct involvement of both D2 and D3 dopamine receptors in the control of insulin secretory evens in the beta cell.

New roles for dopamine D2 and D3 receptors in pancreatic beta cell insulin secretion.

Farino ZJ, Morgenstern TJ, Maffei A, Quick M, De Solis AJ, Wiriyasermkul P, Freyberg RJ, Aslanoglou D, Sorisio D, Inbar BP, Free RB, Donthamsetti P, Mosharov EV, Kellendonk C, Schwartz GJ, Sibley DR, Schmauss C, Zeltser LM, Moore H, Harris PE, Javitch JA, Freyberg Z.

Mol Psychiatry. 2020 Sep;25(9):2070-2085. doi: 10.1038/s41380-018-0344-6.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?** The identification of our novel findings and this signaling pathway has spurred interest by other major diabetes and obesity research teams in evaluating both the peripheral and central effects of D2 and D3 stimulation in diabetes. Recent work of Mori et al. has demonstrated that one potential mode of olanzapine action at the level of the pancreatic islet may be to protein misfolding doi: 10.7554/eLife.60970. Olanzapine reduced maturation of proinsulin, and thereby inhibited secretion of insulin; and specifically shifted the primary localization of proinsulin from insulin granules to the endoplasmic reticulum. This was due to olanzapine's impairment of proper disulfide bond formation in proinsulin, although direct targets of olanzapine remain undetermined. We anticipate that harvested pancreatic tissue from our studies can be used to determine if such misfolding is also characteristic of the pancreas from our olanzapinie treated mice.

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
Nothing to report.

Books or other non-periodical, one-time publications
Nothing to report.

Other publications, conference papers, and presentations

- Abstract for the American Diabetes Association Annual Meeting, June 2022, New Orleans, LA, "Novel tools to dissect the metabolic roles of Central and Peripheral Dopamine D2 receptors"

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 with the Partnering PI of this award, Dr. Zachary Freyberg.

9. APPENDICES

None.