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PRINCIPAL INVESTIGATOR: Nira Ben-Jonathan

CONTRACTING ORGANIZATION: University of Cincinnati
Cincinnati, OH 45221

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Nira Ben-Jonathan

E-Mail: Nira.Ben-Jonathan@uc.edu

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14. ABSTRACT

Atypical antipsychotics (AAP) are prescribed to millions of patients with neuropsychiatric disorders. Although AAP can ameliorate mental dysfunctions, they have serious metabolic side-effects such as weight gain, the metabolic syndrome, and increased risk of diabetes and cardiovascular disease. The current dogma is that the metabolic side effects of AAP are attributed to their action on neuronal circuits the brain. However, we discovered expression of functional dopamine and serotonin receptors in human and rodent adipocytes and found that these receptors are targeted by AAP. *In vivo* studies with rats and *in vitro* studies with human adipocytes revealed multiple direct effects of AAP on adipose tissue. These include increased food intake, fat accumulation, enlargement of adipocytes, alterations in key metabolic genes, changes in the secretion of leptin and adiponectin, suppression of basal and isoproterenol-stimulated lipolysis, and increased preadipocyte proliferation. We conclude that AAP-induced metabolic dysregulation is caused, in part, by their direct action on adipose tissue, presumably via local dopamine and serotonin receptor subtypes.

15. SUBJECT TERMS
Antipsychotics, adipocytes, metabolic syndrome, in vivo, in vitro, dopamine, serotonin, receptors

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Introduction

Atypical antipsychotics (AAP) are used chronically to treat millions of pediatric, adult, and geriatric patients with schizophrenia, bipolar disorder, major depression, post-traumatic stress disorder and autism [1, 2]. While most drugs alleviate neurobehavioral symptoms, many cause serious metabolic side-effects such as weight gain, and the metabolic syndrome [3]. The metabolic syndrome is defined as a cluster of disorders that include obesity, insulin resistance, glucose intolerance, hypertension and dyslipidemia, and is associated with increased morbidity, and high risk of mortality due to cardiovascular disease.

The precise targets of AAPs are unclear, but they bind primarily to dopamine and serotonin receptors [4, 5]. The current dogma is that AAPs bind to these receptors within the brain. Yet, our laboratory discovered that the same receptors are also expressed in adipose tissue [6], where they can be directly activated by the AAP.

Among the most widely prescribed AAP, olanzapine (Zyprexia) and clozapine (Clozaril) carry the greatest risk of the metabolic disturbances, quetiapine (Seroquel) and risperidone (Risperdal) have an intermediate risk, while ziprasidone (Geodon) and aripiprazole (Abilify) confer lower risks [7]. **Table 1** shows an example of three AAP and their relative effects on weight gain, glucose homeostasis and dyslipidemia. For our studies, we selected Olanzapine and Zipresidone which represent high and low risk of the metabolic syndrome. We also compiled data from various publications on the binding affinities of these AAP to dopaminergic (DAR) and serotonergic (5HTR) receptor subtypes. **Table 2** shows relatively high binding affinities of most drugs to D1R and D2R, and variable binding affinities to some serotonergic receptors, indicating that they act via multiple mechanisms of action that cannot be ascribed to a single receptor subtype.

Table 1: Metabolic disturbances associated with selected AAP

	Weight Gain	Glucose Abnormalities	Dyslipidemia	Metabolic Syndrome
Olanzapine	High	High	High	High
Risperidone	Medium	Medium-Low	Low	Medium
Ziprasidone	Low	Low	Low	Low

Table 2: Binding affinities (Ki=nM) of AAP to DAR and 5HTR subtypes

	D1R	D2R	5HT1a	5HT2a	5HT2c	5HT6	5HT7
Olanzapine	31-58	5-20	600-2000	1.5-8	11-14	6	105
Risperidone	61-75	1-8	190-490	0.2-0.6	26-33	2240	6.6
Ziprasidone	30	3-8	1.9-3.4	0.1-3	1.3-13	61	6

Hypothesis/Objectives

Hypothesis: Adipose tissue, via its endogenous DAR and/or 5-HTR, is a major target of AAP. We proposed that AAP-induced suppression of leptin release from adipocytes leads to increased food intake and weight gain, while the suppression of adiponectin exacerbates the metabolic syndrome. The objectives were to establish adipose tissue as a critical target of AAP and elucidate the mechanisms by which they alter adipose tissue functions. A rat model was used to determine the *in vivo* effects of the drugs and examine the putative role of leptin as the mediator of drug-induced increased appetite and weight gain. Human adipocytes were used to document the *in vitro* effects of AAP on preadipocyte proliferation, gene expression, and lipid accumulation.

Specific Aims:

Aim 1: To document the effects of the drugs in a rat model and examine whether drug-induced leptin suppression is a major drive for increased appetite and weight gain.

Aim 2: To determine if weight-inducing AAP increase cell proliferation, stimulate adipogenesis, enhance lipid accumulation and/or alter expression and release of selected adipokines in human adipocytes.

Methods

Rat Model: Adult female Sprague-Dawley rats under normal diet were given cookie dough mixed with Olanzapine or Ziprasidone (4 mg/kg), or vehicle, twice a day for 7 days. Body weight and food intake were measured each day. Body composition by non-invasive NMR was analyzed on days 1,3, and 7. On days 3 and 7, rats were euthanized and subcutaneous (sc) and periovarian (vis) fat was harvested and analyzed by custom-designed PCR arrays for selected adipose-related genes. Incubated rat sc explants or adipocytes were used for analyzing the effects of AAP on multiple parameters.

Human adipocytes: Human subcutaneous adipose tissue was obtained with informed consent from patients undergoing elective abdominoplasty procedures. Preadipocytes and mature adipocytes were isolated by

collagenase digestion followed by differential centrifugation, induced differentiation, and treatment as indicated.

RNA extraction and purification: RNA was isolated using a trizol protocol to remove excess triglycerides and produce crude RNA. Purified DNA-free RNA was isolated from crude RNA using a RNAspin mini kit.

RT-qPCR: After reverse transcription, cDNA was analyzed by two methods: 1) custom-designed RNA arrays with 21 metabolic-related genes. β-2 microglobulin (B2M) and hypoxanthine phosphoribosyl transferase (HPRT) were used as reference genes.

Lipolysis: Adipocytes were incubated with various treatments for 72h and then were incubated with (2h) and without (4h) the β-adrenergic agonist isoproterenol in Krebs-Ringers buffer containing 2% BSA. Glycerol release was measured by colorimetry.

ELISA: Paired commercial capture and detection antibodies were used to analyze leptin or adiponectin by respective sandwich ELISAs using fluorometric detection.

Body

A. Studies with rats

A1: Effects of AAP on body weight, food intake and body fat in rats

The goal was to compare the effects of treating rats with Olanzapine (Olan) and Ziprasidone (Zip), which represent high and low risk of metabolic disturbances, on food intake, body weight and body composition. Rats were given cookie dough mixed with vehicle, Olan or Zip at 4 mg/kg twice/day for 7 days. **Fig 1** shows rapid increases in food intake and body weight, with Olan exhibiting stronger effects than Zip. The weight gain was attributed to fat mass expansion, as confirm by *in vivo* NMR. Although the Olan-induced food intake leveled off after 2-3 days, fat mass continued to rise, suggesting direct drug effects on fat accumulation.

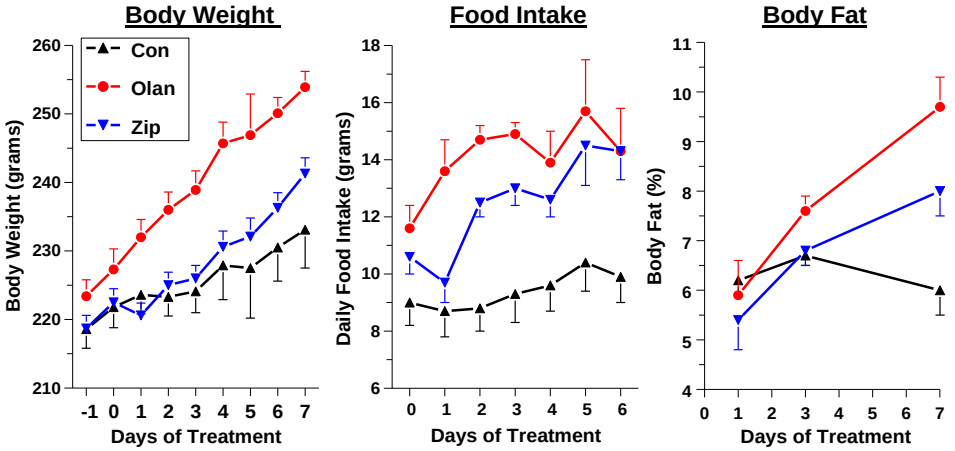

Fig 1: Differential increases in body weight, food intake and body fat induced by olanzapine and ziprasidone. Female rats were given 4 mg/kg of drug or vehicle orally twice/day for 7 days. Each value is a mean±SEM of 8 rats.

A2: AAP directly Increase the size of rat adipocytes

Sc adipose explants from rats (N=8) were incubated in DMEM/F12 and 5% FBS with vehicle control, Olan (10 and 100 nM) or Zip (10 and 100 nM) for 7 days. Explants were fixed in paraformaldehyde, paraffin-embedded and 8µm sections mounted on slides were stained with H&E and photographed. Using the Adiposoft software, the surface area of adipocytes was measured in six fields in each section in a blinded manner. As shown in **Fig 2**, Olan and Zip at 100 nM increased adipocyte size by 50% and 20%, respectively. The low dose of Olan was ineffective while Zip at a low dose caused a small, but significant, reduction in adipocyte size. These data indicate that the observed *in vivo* effect of the drugs on body weight and body fat in rats were due, in part, to their direct ability to cause enlargement of the adipocytes.

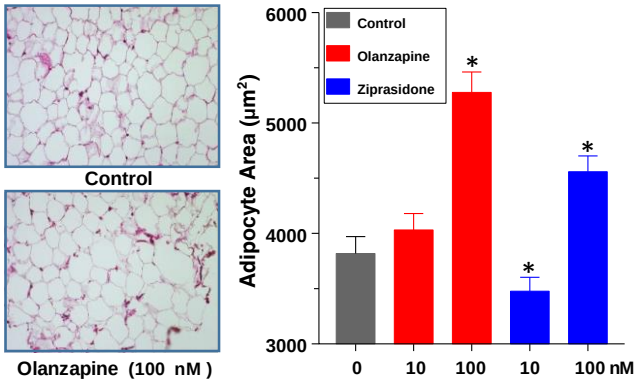

Fig 2: Direct effects of Olan and Zip on rat sc adipocyte size. Explants were incubated with vehicle, Olan or Zip for 7 days. Adipocyte size was determined using H&E-stained sections (N=8).

A3: Changes in gene expression in sc and vis fat following in vivo treatment of rats with AAP

Our next objective was to determine whether *in vivo* treatment with Olan and Zip alter selected metabolic-related genes. To this end, we used custom-designed PCR array with a carefully selected set of genes, grouped by function into: 1) metabolic components, 2) adipokines/cytokines, 3) transcription factors, and 4) various

receptors. We examined changes in these genes in both sc and vis fat in response to treatment with the two AAP for 3 and 7 days. **Fig 3** shows a complex outcome, with many of the genes suppressed in sc, but not in vis, fat after 3 days. Without exception, olanzapine showed stronger effects than ziprasidone. Several key lipolytic

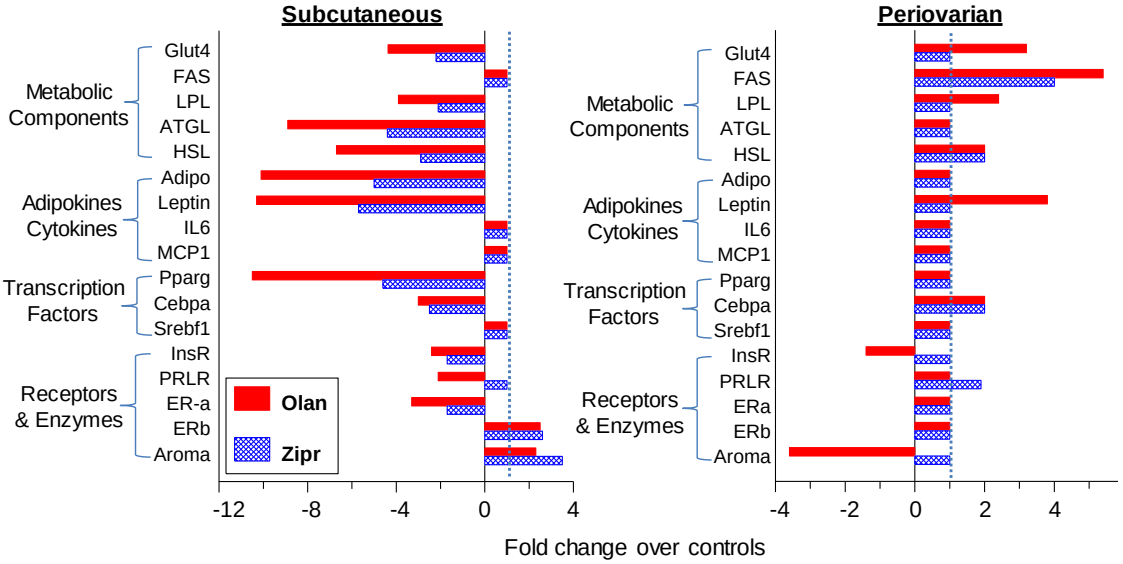

Fig 3: AAP-induced changes in expression of 17 metabolic-related genes in sc and periovarian (vis) adipose tissue. Female rats were orally-treated for 3 days with olanzapine (Olan), ziprasidone (Zipr) or vehicle. Data are expressed as fold positive or negative changes over controls, after correction for 2 house-keeping genes. Blue dotted line = no change.

enzymes (LPL, ATGL and HSL) as well as transcription factors (PPARγ and c/EBPα) that regulate adipogenesis, were suppressed in sc fat, while FAS, a major enzyme that regulates lipogenesis, as well as Glut4, an insulin-regulated glucose transporter, were increased in periovarian fat. Adiponectin and leptin were suppressed in sc fat after 3 days of treatment, while IL-6 and MCP-1 increased after 7 days (data not shown). Insulin receptor expression was moderately suppressed in both fat depots, suggesting induction of insulin resistance. Expression of Srebf1, which regulates lipid homeostasis, significantly increased after 7 days by both drugs (not shown), suggesting a delayed fat accumulation and a potential induction of liver steatosis [8].

Adiponectin, an insulin-sensitizing adipokine [9], was markedly suppressed in sc fat but did not change in vis fat. Unexpectedly, leptin was markedly suppressed by both drugs in sc fat, but increased in response to Olan in vis fat. Since the relative contributions of sc and vis adipose depots to the circulating levels of adipokines is unknown, their direct analysis in serum following drug treatment should provide a true assessment of their impact on targets such as brain, liver or cardiovascular system. Notably, estrogen receptor alpha (ERα) was reduced, but ERβ was moderately increased in sc fat, while aromatase, which converts androgen precursors to estrogens was suppressed. Future studies should examine the role of gonadal steroids and their receptors in metabolic homeostasis in response to AAP treatment.

A4: Effects of AAP on prolactin release in rats

To examine for the dopaminergic component of each drug, blood collected on days 3 and 7 of treatment was analyzed for prolactin, a pituitary hormone which is under tonic inhibition by dopamine [10]. **Fig 4** shows 80-100 fold increases in serum prolactin levels in response to Olan, with lesser, but significant, increases in response to Zip, confirming the critical role of D2R in mediating some actions of the AAP on the brain.

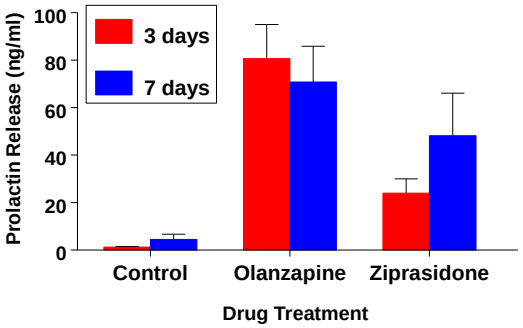

Fig 4: Both Olan and Zip increase serum PRL levels. Female rats were given 4 mg/kg of drug or vehicle orally twice/day for 7 days. Tail blood, collected on days 3 and 7, was analyzed or prolactin by a bioassay. Each value is a mean±SEM of 8 rats.

A5: Lack of effects of either leptin or leptin receptor super-antagonist

Leptin, whose release is proportional to fat mass, regulates food intake and energy expenditure [11]. Leptin administration reduces appetite while its chronic deficiency results in extreme obesity. Being the major satiety hormone, we assumed that drugs that increase appetite do so by suppressing leptin release. The availability of pegylated leptin and leptin receptor super-antagonists from our collaborator, Dr. Gertler [12], provided a unique opportunity for examining the effects of acute hypoleptinemia on food intake and weight gain in rats. Pegylation increases the serum half-life of both compounds from 1 hr to 14 hrs. Our main objective was to use the leptin antagonist to induce severe central leptin deficiency, enabling us to examine the role of leptin as a possible mediator of olanzapine-induced increased appetite and weight gain, presumably by blocking the transport of endogenous leptin into the brain.

Two approaches were initially used to examine the putative role of leptin in mediating AAP actions. We first examined if the leptin receptor antagonist mimics the olanzapine-induced rise in food intake and body weight gain. Rats were injected ip once a day with 2.5 mg/kg of the pegylated antagonist or vehicle control, followed by analysis of food intake and body weight for 7 days. Disappointedly, there were no differences in food intake or body weight in the antagonist-treated rats (data not shown). We then examined if peguylated leptin abrogates the effects of Olan. Rats were treated with both, daily ip injection of pegylated leptin at 0.5 mg/kg, and twice a day with Olan at 4 mg/kg, followed by the same analyses as above. Whereas Olan caused increases in all parameters, as was shown in **Fig 1**, pre-treatment with leptin did not alter the effects of Olan (data not shown).

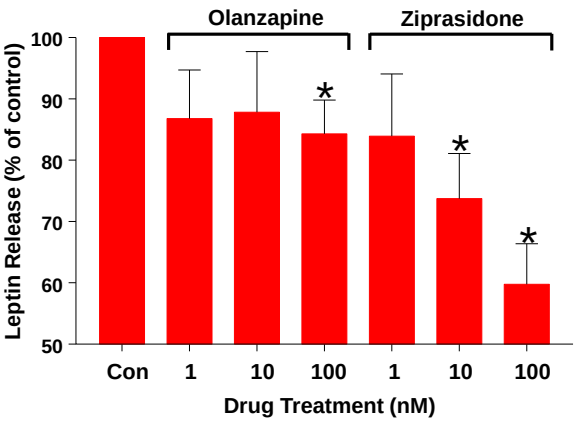

Fig 5: Suppression of leptin release from mature adipocytes. Cells were incubated with Olanzapine or Ziprasidone for 72 hrs. Conditioned media were analyzed for leptin by ELISA. Each value is a mean±SEM; N=6.

Indeed, little or no effect of the AAP on leptin release was also observed using isolated vis mature rat adipocytes. As evident in **Fig 5**, Olan at a high dose of 100 nM caused a small, 15% suppression of leptin release from mature adipocytes, while Zip 100 nM caused 40% inhibition. Given the disappointing *in vivo* and *in vitro* results, we decided to discount our original hypothesis that leptin is a major mediator of AAP actions.

B. Studies with primary human adipocytes

B1: Effects of AAP and dopamine on adiponectin and leptin release from mature human adipocytes

Using isolated mature human adipocytes, we examined the effects of dopamine sulfate (DAS), Olan and Zip on leptin and adiponectin release. As shown in **Fig 6**, a 3 day incubation with 1 nM olan caused >65% inhibition of adiponectin release, while 1 nM Zip had no effects. Notably, 10 nM Olan was less effective than the 1 nM dose, suggesting activation of opposing receptors at higher doses; 10 nM Zip cause 50% inhibition of adiponectin, while DAS was without effects. As was seen using rat adipocytes (**Fig 5**), Zip was more effective than Olan in suppressing leptin release, but without an obvious dose-dependent actions. This experiment suggests that future studies should identify which receptors mediate the actions of Olanzapine vs ziprasidone.

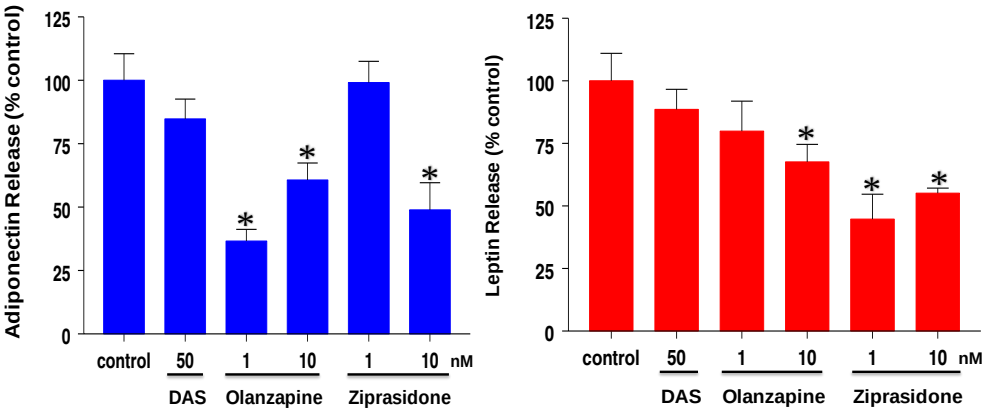

Fig 6: Effects of dopamine sulfate (DAS), Olan, or Zip on adiponectin (*left panel*) and leptin (*right panel*) release from sc mature primary human adipocytes incubated for 72 hrs. Media were analyzed for the two adipokines by respective sandwich ELISAs.

B2: Suppression of basal lipolysis by the AAP

We next examined the direct effects of AAP on lipolysis, using either isolated mature adipocytes or primary preadipocytes which were induced to differentiate in culture over a 10 day period by incubation with a differentiation cocktail [13]. Cells were incubated with the drugs for 72 hrs, and after media replacement, conditioned media were collected for 4 hrs and analyzed for glycerol by a colorimetric assay. As evident in **Fig 7**, Olanzapine caused dose-dependent inhibition of lipolysis in differentiated preadipocytes, having a similar effect in mature adipocytes except at the higher dose. Ziprasidone was less effective than Olanzapine. These data indicate that the suppression of basal lipolysis by AAP likely contributes to fat accumulation and enlargement of the adipocytes. The non-linear dose-dependence effect suggests activation of various receptors at different doses, an issue that should be examined in future studies by knocking out selected dopaminergic and serotonergic receptors.

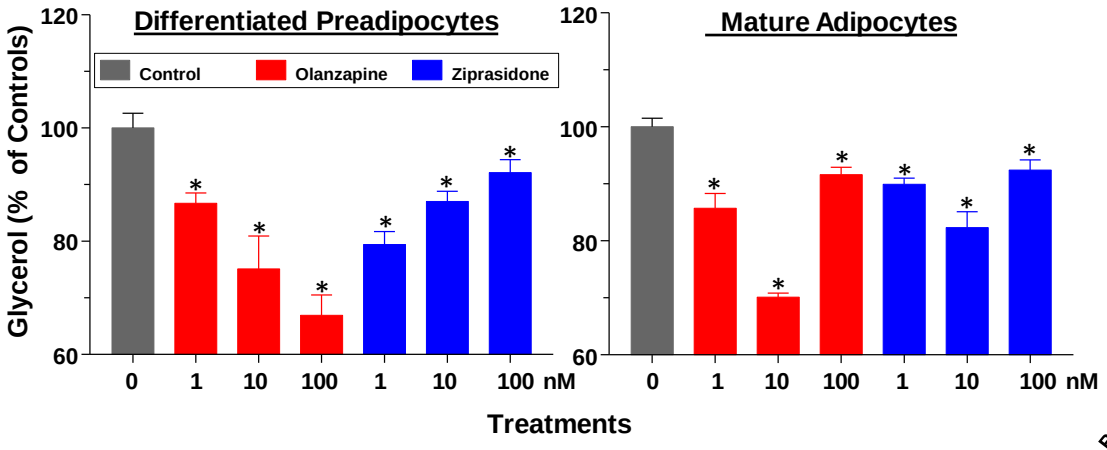

Fig 7: Suppression of basal lipolysis in differentiated human preadipocytes (*left panel*) or isolated mature adipocytes (*right panel*). Cells were incubated with Olan or Zip for 72 hrs. Conditioned media collected after 4 hr were analyzed for glycerol release by colorimetry.

B3: Expression of serotonergic receptors in human adipocytes

Given the increasing evidence for a critical role played by serotonergic receptors as mediators of AAP actions [4], we next examined expression of selected 5HTR in both primary human adipocytes and our LS15 human adipocyte cell line (see below) before and after differentiation (**Fig 8**). As determined by RT-PCR, 5HTR1a was expressed at variable levels by all cells examined, 5HTR7 was expressed in primary preadipocytes and differentiated LS14 adipocytes, while mature adipocytes express only 5HTR2c. Primary preadipocytes also express 5HTR2a and 5HTR6. Since classical RT-PCR is only semi-quantitative, more detailed analysis should be done in the future using quantitative real-time PCR.

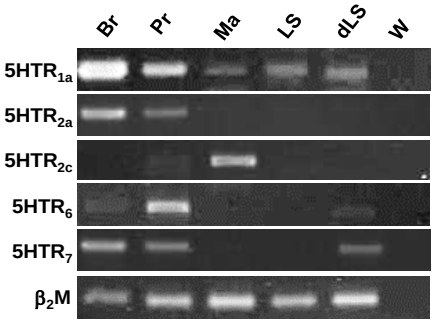

Fig 8: Expression of serotonergic receptors in human adipocytes, analyzed by RT-PCR. Br: brain, Pr: primary preadipocytes, Ma: mature adipocytes, LS: proliferating LS14, dLS: differentiated LS14, W: no template.

B4: Suppression of lipolysis by DAR and 5HTR agonists

Pooled preadipocytes from several patients were induced to differentiate for 10 days. The cells were then incubated for 72 hrs with DAR and 5HTR agonists (all at 10 nM doses), washed, and conditioned media were collected for 5 hrs for basal lipolysis. This was followed by cell incubation with 1μM isoproterenol (Iso), a β-adrenergic receptor agonist, and media collection after 2 hrs. Glycerol release was analyzed by a colorimetric assay. As shown in **Fig 9**, both Fenoldopam, a peripheral D1R agonist, and Cabergoline, a D2R agonist, significantly suppressed basal lipolysis but had no, or little, effect on iso-stimulated lipolysis. None of the tested 5HTR agonists affected basal lipolysis, while Tan, a HT1A agonist, caused a significant suppression of iso-stimulated lipolysis. These data indicate that the suppression of lipolysis by AAP likely contribute to fat accumulation and enlargement of the adipocytes. This involves activation of various receptors at different doses, an issue that should be examined in future studies by conducting knockout studies of selected dopaminergic and serotonergic receptors.

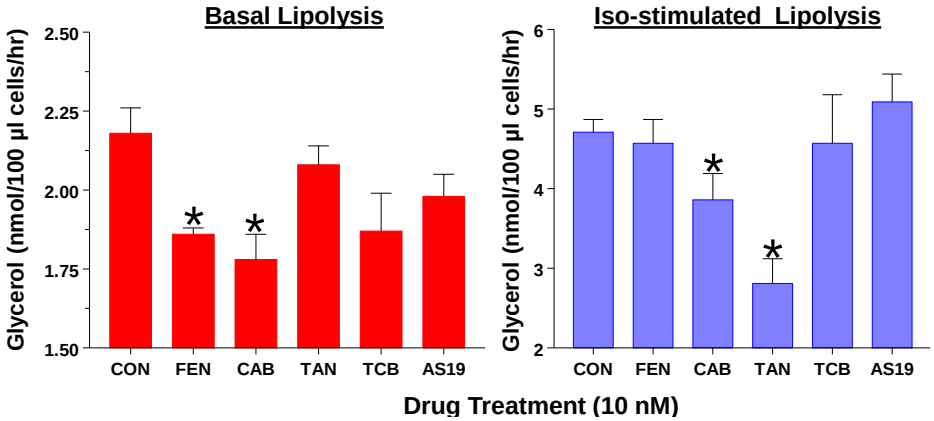

Fig 9: Differential suppression of basal (*left panel*) and isoproterenol (iso)-stimulated (*right panel*) lipolysis from differentiated primary adipocytes. Fen: Fenoldopam (D1R agonist); Cab: Cabergoline (D2R agonist); Tan: Tandospirone (HT_{1A} agonist); TCB: TCB2 (HT_{2A} agonist); AS19: AS19 (HT_7 agonist); Each value is a mean±SEM of N=6.

C. Studies with LS14 human adipocytes

C1: Characteristics of LS14 cells

We previously cloned a unique human adipocyte cell line, named LS14, from a patient with a freshly removed liposarcoma [14]. These spontaneously immortalized cells have been maintained in culture for many generations, and were provided to many investigators in the USA and abroad. Extensive characterization

revealed that they resemble vis adipocytes and express all the receptors, i.e., DAR and several 5-HTR, as well as adipose-specific markers, as do primary vis adipocytes (**Fig 10**).

C3: AAP increased the proliferation of LS14 cells

Given their immortality, LS14 cells can be used as a cellular model for studying both: proliferating preadipocytes and fully differentiated mature adipocytes (**Fig 11**).

Using LS14 cells as preadipocytes, we examined the effects of AAP on cell proliferation. Cells were incubated with the drugs for 72 hrs, and cell proliferation was then determined by the Resazurin assay. **Fig 12** shows that Zip increased cell proliferation at all doses tested while Olan was effective as 10 and 100 nM but not at 1 nM. These data indicate that in addition to expanding fat mass by cell enlargement, AAP can increase the pool of preadipocytes that eventually differentiate into lipid filled mature adipocytes.

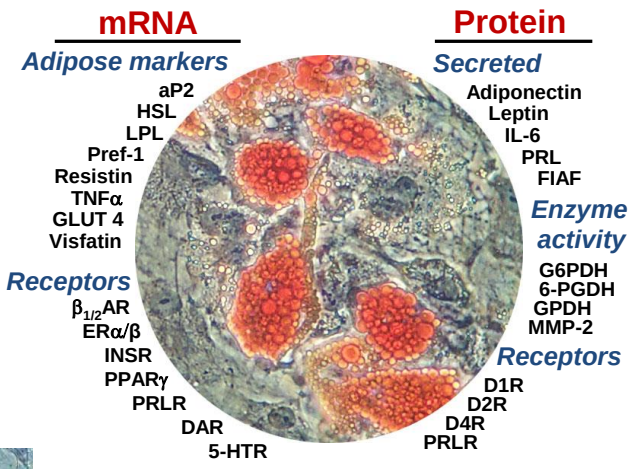

Fig 10. Characterization of LS14 adipocytes.

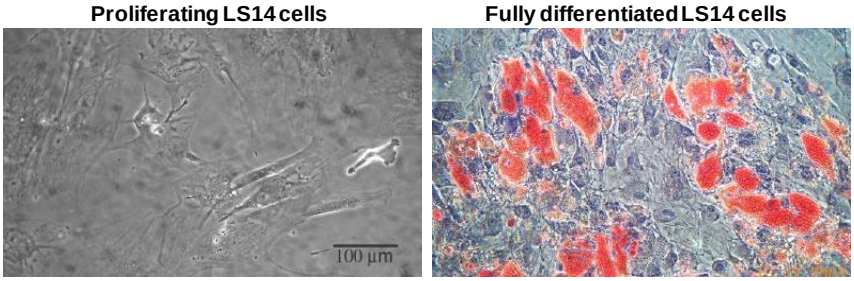

Fig 11: *Left panel:* photographs of proliferating LS14 preadipocytes. *Right panel:* 10 days after induction of differentiation, showing marked changes in cell shape and lipid accumulation, determined by staining with Oil-Red-O.

Key Research Accomplishments

- AAP administration in rats results in rapid increases in body weight, food intake and adiposity, as reflected by increased adipose tissue mass.
- Multiple metabolic-related genes are altered in both sc and vis fat in response to *in vivo* administration of AAP.
- Olanzapine, known to cause more severe metabolic disturbances in humans, was also potent in causing metabolic alterations in rats.
- Studies with isolated human adipocytes demonstrate direct effects of the AAP on both adipokine release and lipolysis.
- AAP exert direct actions on fat accretion by increasing preadipocyte proliferation as well as by augmenting the size of mature adipocyte.
- Enhanced fat accumulation caused by AAP is also due to the suppression of basal lipolysis.

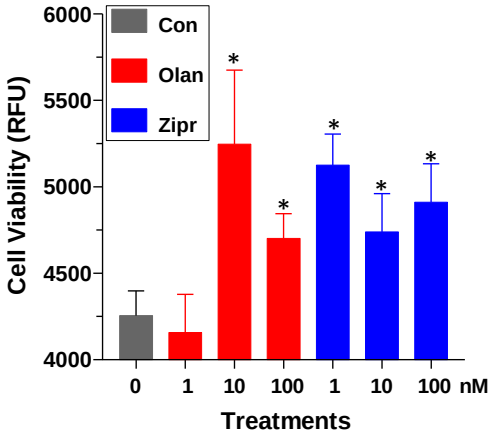

Fig 12: AAP increase the proliferation of human preadipocytes. LS14 cells were incubated with Olan or Zipr for 72 hrs. Cell viability was determined by the Resazurin assay. RFU: relative fluorescence units.

Reportable Outcome

Presentations in Scientific Meetings:

- Ben-Jonathan: Antipsychotics induced obesity: **Direct actions on the Adipocytes**, Invited Speaker, the 2014 Obesity Summit, London, UK, April 2014.
- Eric R Hugo, Randall R Sakai, Eric J Phillips, Sejal R Fox, Vidjaya LV Premkumar, Nira Ben-Jonathan: **Direct Effects of Weight-Inducing Antipsychotics on Adipose Tissue from Humans and Rats,** Annual Meeting of the Endocrine Society, Chicago, Illinois, June 2014.
- Ben-Jonathan and Hugo: **Direct actions of antipsychotics: A cause for metabolic dysregulation.** The 7th International Congress of psychopharmacology, Antalya, Turkey, August, 2014.

Manuscripts:

- Eric R. Hugo, Dana C. Borcherding, Neil M. Richtand, and Nira Ben-Jonathan. Dopamine and Serotonin

Receptors in Human Adipocytes: Do they Mediate Adverse Metabolic Effects of Antipsychotics? Molecular Medicine (submitted; Appendix 1)

- Hugo, Sakai, Phillips, Fox, Premkumar, and Ben-Jonathan: Direct effects of weight-inducing antipsychotics on adipose tissue from humans and rats (Manuscript in preparation).

Overall Conclusions

Collectively, these studies support our major hypothesis that AAP-induced metabolic dysfunctions in patients that are treated with AAP are due, in part, to their direct action on the adipocytes. As predicted by its actions in patients, Olanzapine is more potent than Ziprasidone in affecting lipolysis and adipokine release from human adipocytes. The mechanism of action of AAP is highly complex as it is involved activation of a variety of dopaminergic and serotonergic receptors, release of several adipokines, and reciprocal interactions with many factors that are produced by other target organs, including the brain, liver and the GI tract. Deciphering which of these receptors in adipose tissue is particularly critical would be extremely difficult, necessitating the application of gene silencing of each receptor alone as well as in combination.

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Dopamine and Serotonin Receptors in Human Adipocytes: Do they Mediate Adverse Metabolic Effects of Antipsychotics?

Eric R. Hugo, Dana C. Borcherding, Neil M. Richtand, and Nira Ben-Jonathan

Departments of Cancer and Cell Biology (ERH, DCB, NBJ), and Psychiatry and Behavioral Neuroscience (NMR)

University of Cincinnati, Cincinnati Ohio 45267, USA

Corresponding Author: Ben-Jonathan, N. (Nira.Ben-Jonathan@uc.edu)

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Abstract

Atypical antipsychotics (AAP) are used to treat millions of patients with schizophrenia, bipolar disorder, depression, posttraumatic stress disorder, and autism. Although effective in ameliorating many psychiatric symptoms, AAP also induce substantial weight gain and other metabolic disturbances. The overarching consensus is that they do so by acting on brain dopaminergic (DAR) and serotonergic (5-HTR) receptors. We challenge this dogma by reviewing evidence that adipose tissue, via endogenous DAR and 5-HTR, is a major target of AAP. This model underscores peripheral actions of AAP which complement, or override, their central actions. This knowledge should serve as a guide for the rational selection of existing drugs, and as a benchmark for developing a new generation of drugs devoid of adverse metabolic side-effects.

The issue of metabolic disturbances by atypical antipsychotics

AAP are composed of a group of medications with high affinity binding to dopamine (DAR) and serotonin (5-HTR) receptors, that are used to treat a steadily growing list of psychiatric disorders. In 2004, four scientific societies: American Diabetes Association, American Psychiatric Association, American Association of Clinical Endocrinologists, and The Obesity Society, convened a consensus development conference on AAP evaluation [1]. The panel concluded that although AAP have lower neurological side-effects than conventional antipsychotics, their association with dramatic weight gain, diabetes and an atherogenic lipid profile has become a major public health concern with great economic impact. The panel emphasized the need for elucidating the mechanism by which AAP exert their adverse metabolic effects.

Presently, a clear understanding of the receptors and/or anatomical sites that mediate the metabolic side effects of AAP has not emerged. The general consensus is that AAP bind differentially to DAR and 5-HTR subtypes, but also to some adrenergic, histaminergic and cholinergic receptors [2-4], albeit with much uncertainty as to the most critical receptor(s) targeted by each drug. There is also no agreement with respect to their sites of action within the brain, implicating mesolimbic, mesocortical, and nigrostriatal dopaminergic pathways, all of which have multiple interactions with other neurotransmitters and neuropeptides.

While not disputing the therapeutic effects of AAP on the brain, our main premise is that adipose tissue, via endogenous DAR and 5-HTR subtypes, mediates many metabolic actions of AAP (Figure 1). We propose that AAP-induced suppression of leptin stimulates food intake and weight gain, while inhibition of adiponectin and stimulation of pro-inflammatory cytokines exacerbate the metabolic syndrome. AAP may also increase adiposity by augmenting the differentiation of preadipocytes into mature adipocytes (adipogenesis), and/or by enhancing their lipid accumulation. To lay the foundation for this premise, we review metabolic dysregulation by AAP and discuss adipose functions known to be affected by AAP such as adipogenesis, lipogenesis/lipolysis and production of key adipokines. We then present data on the expression and functions of DAR and 5-HTR subtypes in adipocytes, evaluate clinical data and animal studies on AAP actions on adipose functions, and discuss the implications of this knowledge for the future development of improved antipsychotics.

Antipsychotic medications

Conventional and atypical antipsychotics

The dopamine (DA) theory of schizophrenia was based on the observations that drugs that effectively increase DA in certain brain areas caused schizophrenic-like symptoms, while blockade of DA actions

ameliorated such symptoms [5]. DA is a catecholamine which binds to five receptors, classified by structure, pharmacology and function into D_2R-like (D_2R, D_3R and D_4R) and D1R-like (D_1R and D_5R) receptors [6,7]. Serotonin (5HT) is an indoleamine which binds to seven classes of 5-HTR and is implicated in the pathophysiology of depression, anxiety and schizophrenia, as well as in obsessive-compulsive and eating disorders [8]. Conventional antipsychotics, e.g., chloropromazine, haloperidol and perphenazine, act primarily as D2R antagonists and ameliorate positive symptoms of psychosis. However, their tolerability is limited by neurological side effects such as Parkinsonism, akathisia and tardive dyskinesia [9]. The search for drugs with lower extrapyramidal side effects led to the development of 'atypical' antipsychotics, or AAP, which maintain high affinity binding to D_2R, but also have high affinity binding to serotonin $5HT_{2A}$R [10]. Box 1 lists the various mental disorders, i.e., schizophrenia, bipolar disorder, major depression, posttraumatic stress disorder, and autism [11,12], which are currently treated with AAP.

Metabolic side effects of AAP

The initial optimism with regard to the tolerability of AAP has been tempered by serious metabolic disturbances [13-15]. Excessive weight gain and changes in serum lipids, adipokines and inflammatory cytokines in response to AAP are well documented in both adults and pediatric patients. A case in point is a recent report on the effects of AAP in 272 youth, aged 4 to 19 years, diagnosed with schizophrenia, bipolar disorder and autism [16]. Although most drugs improved behavioral problems, they came at the expense of substantial weight gain. Within 10-12 weeks of treatment, the mean weight gain was 8.5 kg for olanzapine (Zyprexa), 6.1 kg for quetiapine (Seroquel), 5.3 kg for risperidone (Risperdal), and 4.4 kg for aripiprazole (Abilify), as compared to 0.2 kg in untreated controls. Hyperlipidemia and insulin resistance were also seen. Children are especially vulnerable to early weight gain, as it increases the risks for developing the metabolic syndrome in adulthood [17]. As depicted in Table 1, among the various AAP in current practice, olanzapine and clozapine carry the greatest risk of weight gain, diabetes and dyslipidemia, quetiapine and risperidone carry an intermediate risk, while ziprasidone and aripiprazole confer the lowest risk [1,18].

Key features of adipose tissue and adipocytes

Adipose tissue composition and distribution

The most abundant cell type within adipose tissue is the large, lipid filled adipocyte, with a small number of proliferation-competent preadipocytes. The stroma also contains endothelial cells, pericytes, mast cells, fibroblasts, nerve endings, and immune cells. Two types of adipocytes are recognized: brown and

white. Brown adipocytes have a major role in thermogenesis and are most abundant in neonates. White adipocytes are segregated into discreet anatomical depots, i.e., visceral and subcutaneous, with a clear sexual dimorphism in their distribution [19]. The depots differ in the expression of several receptors, responsiveness to circulating and local regulators, and lipolytic activity [20,21]. Excess abdominal fat is the major contributor to obesity-related metabolic disorders (Box 2).

Adipogenesis

Obesity results from increased adipocyte size (hypertrophy) and number (hyperplasia). Fat cell number is primarily determined by adipogenesis, a terminal differentiation process which takes 10-14 days and results in the conversion of preadipocytes into mature adipocytes. Preadipocytes are fibroblast-like unipotent cells that are 'committed' to the adipocyte lineage [22]. Adipogenesis proceeds in several intertwined steps which begin with an initial growth arrest followed by clonal expansion and the initiation of differentiation. The early stages of adipogenesis are characterized by coordinated waves of expression of several transcription factors, extracellular matrix and cytoskeletal proteins, whereas the later stages are denoted by increased expression of lipogenic enzymes and adipokines [23].

Lipogenesis and lipolysis

Lipogenesis encompasses the processes of fatty acid synthesis and production of triglycerides by esterification of fatty acids with glycerol. Lipolysis is the process by which lipids are broken down step-wise into free fatty acids (FFA) and glycerol. Catecholamines, acting via β-adrenergic receptors (β-AR) are the most potent lipolytic hormones, while insulin is a potent anti-lipolytic hormone [24]. Notably, norepinephrine (NE), but not selective β1- or β2-AR agonists, was still capable of stimulating lipolysis in triple (β1/β2/β3) AR-knockout mice [25]. As there is no identified fourth β-AR, the authors proposed that the lipolytic activity of NE in the β-less adipocytes was due to an unknown Gs-protein-coupled receptor with low affinity for NE. Alternative explanation is the possibility that NE at high concentrations binds DAR [6].

Selected adipokines

Leptin is a key adipokine which regulates food intake and energy expenditure. In humans, leptin administration reduces appetite while its chronic deficiency causes extreme obesity; leptin production is proportional to adipose tissue mass [26,27]. Adiponectin is a potent adipokine which protects against the metabolic syndrome [28,29]. It has insulin-sensitizing, anti-inflammatory and anti-atherogenic properties, with weight reduction accompanied by increased serum adiponectin levels and improved

insulin sensitivity. IL-6 is a pro-inflammatory cytokine which contributes to the low level of inflammation associated with obesity. Elevated serum IL-6 increases the production of c-reactive protein and can lead to coronary heart disease and atherosclerosis [30]. Within adipose tissue, IL-6 is mainly produced by macrophages and preadipocytes and to a lesser extent by mature adipocytes [31].

The dopaminergic system in adipocytes

Sources of dopamine to the adipocytes

DA synthesis is regulated by the rate limiting step tyrosine hydroxylase (TH). Whereas most studies have focused on DA production and release within the brain, the rather large concentrations of DA, primarily as DA-sulfate (DA-S), in the human circulation have been largely ignored. As illustrated in Figure 2, peripheral DA originates from the gastrointestinal (GI) tract, spleen, paraganglia, and adrenal medulla [32,33]. Ingestion of a meal in fasting individuals causes a 50-fold rise in circulating DA-S levels. Under basal conditions, serum DA-S at ≈5 ng/ml exceeds the combined levels of free DA (0.3 ng/ml), NE (0.2 ng/ml) or epinephrine (0.05 ng/ml). Adipose tissue can also receive DA input from adjacent mesenteric neurons and paraganglia, as well as from lymphocytes and macrophages which accumulate in fat and can produce DA [34,35].

Although DA-S comprises >95% of serum DA, it escapes detection by most analytical methods. Sulfoconjugation is done in the liver, GI tract and platelets by SULT1A3 sulfotransferase. In humans, a single amino acid substitution confers the enzyme with higher affinity for DA than NE or epinephrine [36]. Unlike the irreversible DA inactivation by deamination, O-methylation or glucoronidation, sulfo-conjugation is reversible, and DA-S can be converted to DA by arylsulfatase A (ARSA), a releasable lysosomal enzyme [37,38]. ARSA is expressed in human adipose tissue and its activity increases after adipogenesis [39]. Although DA-S does not activate DAR, when incubated with human adipocytes, its activity is indistinguishable from that of DA, suggesting that adipocytes can de-conjugate DA-S to DA [39]. Given that DA-S has an half-life of 4.5 hrs, as compared to a few minutes for free DA [40], DA-S may serve as a stable reservoir of bioactive DA to adipose tissue (see Figure 2).

Expression of dopamine receptor subtypes

DAR are made of a single polypeptide chain, ranging in size from 387 to 475 residues. Each receptor is composed of seven transmembrane spanning helices that form a hydrophobic ligand-binding pocket. The D1R-like genes have no introns whereas the D_2R, D_3R and D_4R have 6, 5, and 3 introns, respectively [6]. The presence of introns enables the generation of receptor variants by alternative splicing. Some variants have distinct anatomical, physiological, and pharmacological properties [41].

The ability of DAR to couple to several G-proteins is at the heart of their action. The D2R-like are coupled to Gαi and Gαo proteins and inhibit adenylate cyclase (AC), whereas the D1R-like are coupled to Gαs, Gαolf or Gαq proteins, and stimulate AC and PKA, but can also activate phospholipases (e.g., PLC) as well as the MAPK and PI3K/Akt pathways [7,42]. With the exception of D3R, all DAR are expressed in human adipose tissue, primary adipocytes and two human adipocyte cell lines, LS14 and SW872, at both the RNA and protein levels [39]. An older study described D1R-like in brown rat adipocytes [43], but there are no comparable data on brown human adipocytes.

DA signals through several pathways in human adipocytes. For instance, at low nM doses, DA suppresses cAMP in differentiated LS14 adipocytes, but increases cGMP at higher doses. DA also rapidly activates the MAPK system but has little effect on Akt signaling [39]. The binding affinity of DA to DAR ranges from 50 nM for D_3R to >2 μM for D_1R [6], underscoring the variable response to DA by cells that express more than one receptor subtype. Indeed, DA exhibits a non-monotonic, U-shaped activity curve on several molecular targets in human adipocytes [39]. This suggests that activation of inhibitory DAR at low DA concentrations is counteracted by activation of stimulatory DAR at higher concentrations, resembling the opposite regulation of lipolysis by ∝ and β adrenergic receptors [24]. Less ambiguity should results from the use of selective DAR agonists and antagonists in combination with targeted DAR knockdown.

Putative functions

The expression of D2R increases, while that of D1R decreases, during the first few days of human preadipocyte differentiation [39], raising the possibility that they participate in some manner in adipogenesis. A search using the Genomatic Matinspector program (http://www.genomatix.de/online) identified a putative PPARγ binding site in the promoters of D_1R and D_2R, while the D_1R promoter also has a C/EBP binding site. Given that these transcription factors play critical roles in the initiation and progression of adipogenesis [23], DAR may be among their regulated genes. The use of selective DAR agonists and antagonists revealed that DA acts via D_2R to inhibit adipocyte PRL expression and release while the D_1R/D_5R appear to mediate the suppression of leptin release and the stimulation of adiponectin and IL-6 release [39]. Locally-produced PRL has multiple roles in adipose functions, including stimulating of adipogenesis, inhibition of lipolysis and variable effects on adipokine release [44]. Notably, short term treatment of obese women with bromocriptine, a D_2R agonist, resulted in increased serum FFA levels and suppressed leptin [45], indicating either a direct or an indirect effects of DA on lipolysis and leptin release. Results from our laboratory (Hugo et al, unpublished) show direct lipolytic effects of low nM doses of DA on cultured human adipocytes.

The serotonergic system in adipocytes

Sources of serotonin to the adipocytes

Serotonin is synthesized from 5-hydroxytryptophan by tryptophan hydroxylase (TPH), a rate limiting enzyme which exists in two isoforms: peripheral TPH1, and brain-specific TPH2 [46]. Over 95% of peripheral serotonin is synthesized by the enterochromaffin cells within the GI tract [47]. After its release, serum serotonin is rapidly taken up by platelets via the serotonin transporter (SERT), and is sequestered into dense granules by vesicular monoamine transporters [48]. Platelets activation can result in release of serotonin which then becomes available to various tissues. Adipocytes also have the capacity for *de-novo* synthesis of serotonin (see Figure 2). Both 3T3-L1 murine adipocytes [49] and visceral rat adipocytes [50] express TPH1, and are capable of storing and releasing serotonin.

Expression of serotonin receptor subtypes

The 5-HTR are grouped into seven sub-classes comprised of 14 distinct receptors. With the exception of 5-HT_3R which gate an ion channel, all others are G-protein-coupled receptors that activate second messengers such as AC, PLC and PKA and result in excitatory or inhibitory responses [51]. As illustrated in Table 1, the general consensus is that some AAP act by blocking 5-HT_{2A}R, stimulating 5-HT_{1A}R, and variably binding to 5-HT_{2C}R, 5-HT_6R or 5-HT_7R [52]. A cross-talk between serotonergic and dopaminergic receptors and their downstream signaling pathways [4] can alter antipsychotic effectiveness, metabolic disturbances, and neurological adverse effects of each drug.

Most 5-HTR subtypes are expressed in peripheral organs where they participate in the regulation of cardiovascular and respiratory functions, bowel motility, ejaculatory latency and bladder control [8]. Expression of the following 5-HTR: 1A, 1B, 1D, 1F, 2A, 2C, 5A, 5B, 6 and 7 have been detected in 3T3-L1 adipocytes, with expression of 2C receptors increasing, and 2_A receptors decreasing, during cell differentiation [49]. As evident at both the gene and protein levels, visceral rat adipocytes express 2A, 2B and 2C [50]. Studies in our laboratory (E. Hugo et al., unpublished) confirmed expression of 1A, 2A, 2B, and 7 serotonin receptor subtypes in primary human adipocytes and LS14 adipocytes.

Putative functions

Peripheral serotonin administration affects glucose and lipid metabolism [53,54], but only limited data are available on the direct effects of serotonin on the adipocytes. One study reported a rapid, biphasic inhibition of leptin expression and release from cultured primary rat adipocytes by serotonin [50]. Another study found that selective 5-HT_{2A}R and 5-HT_{2C}R antagonists inhibited 3T3-L1 cell differentiation, with similar effects achieved by overexpression of micro-RNA(miR)-448 which targets

5-HT_{2C}R [49]. A recent paper reported that treatment of 3T3-L1 adipocytes with serotonin inhibited insulin-stimulated glucose uptake via the transactivation of the EGF receptor and the ERK1/2-mTOR pathway, but did not identify the 5-HTR involved [55].

Effects of atypical antipsychotics on adipocyte functions

Clinical data

Treatment of patients with AAP, especially olanzapine and clozapine, is accompanied by rapid increases in appetite and caloric intake [56-58]. Appetite is controlled by a network of hypothalamic and extra-hypothalamic neurons that are modulated by hormones from the stomach (ghrelin), pancreas (insulin), and adipose tissue (leptin), and are also affected by circulating glucose and lipid levels [59]. Given that leptin is a major satiety hormone, it stands to reason that drugs which increase appetite are associated with suppressed leptin. Yet, most studies reported elevated serum leptin levels in response to weight-inducing AAP [60]. Close examination of these data reveals that leptin was determined after weeks or months of drug treatment, suggesting that the observed rise in serum leptin is secondary to increased fat tissue mass. Because appetite is regulated at much shorter time intervals, data on acute effects of AAP on serum leptin would be highly informative, but are scarce and inconclusive [57,61]. Clearly, a functional link between AAP, circulating leptin, hyperphagia, and weight gain warrants a close examination. As it is not feasible to rapidly suppress leptin independent of food intake, or reversibly block its actions in human subjects, animal models are needed.

Elevated FFA flux from adipose tissue amplifies many of the metabolic derangements that underlie obesity-associated insulin resistance. However, the reports on serum FFA levels following treatments with AAP are inconsistent, with some observing marked increases [62], while others finding no changes or a decline [63,64]. A large study with 567 schizophrenic patients treated with clozapine, olanzapine and risperidone, found lower serum adiponectin levels [65], while others found no change [60]. Treatment of patients with clozapine increased serum IL-6 within one week [66], whereas chronic treatment with risperidone was ineffective [67]. The discrepancies among the above studies undoubtedly reflect the use of different drugs, variable doses, different length of treatment, and a non-standardized time of blood sampling relative to food intake. Furthermore, clinical studies cannot resolve whether the observed drug effects are due to their direct or indirect actions on adipose tissue.

Animal studies

Both mice [68] and rats [69] have been used as acceptable, albeit imperfect, animal models for studying the metabolic side effects of AAP. A major advantage of using rats is their larger blood volume for sampling, and a more sizable adipose tissue. Treatment of female rats with olanzapine increased food

intake, body weight and fat deposits within one week, and elevated serum ghrelin levels while reducing circulating insulin within two weeks [69]. Acute changes in response to AAP were also reported, with oral administration of olanzapine stimulating food intake within 24 hrs and increasing weight gain within 2-3 days [70,71]. Importantly, within 5 hrs of ip injection of olanzapine, plasma leptin was reduced by 50%, the glucose-induced leptin rise was blunted, and ghrelin was suppressed. The authors concluded that acute hypoleptinemia is a major contributor to AAP-induced hyperphagia, but cautioned that more data are needed to substantiate this postulate.

Chronic treatment of rats with AAP induces several biochemical and cellular alterations in adipose tissue, including decreased expression of hormone sensitive lipase and enhanced expression of fatty acid synthase [72,73]. The combined effect of suppression of lipolysis and augmented lipogenesis resulted in fat accumulation and enlargement of the adipocytes. As drug usage *in vivo* cannot distinguish between central vs peripheral actions, data obtained with isolated adipocytes are critical. Indeed, AAP enhanced triglyceride storage by upregulating the lipogenic enzymes and reducing lipolysis in cultured rat and murine adipocytes [74,75]. The drugs also impaired insulin-stimulated glucose uptake. These reports indicate that AAP can directly stimulate lipid accumulation and alter the size of adipocytes, but did not address their mechanism of action. Studies in our laboratory (Hugo et al unpublished) found that several AAP directly suppressed leptin and adiponectin and stimulated IL-6 release as well as increased lipolysis from human adipocytes.

Concluding remarks

The dramatic worldwide epidemics of obesity and diabetes has attracted significant attention to the central role played by adipose tissue in metabolic homeostasis and its dysregulation. The last few decades have witnessed a remarkable pace of discovery of transcription factors that regulate adipogenesis, enzymes that regulate lipid assembly and breakdown, and adipose-secreted factors that regulate brain, cardiovascular and hepatic functions (Box 2). One of the objectives of this review was to underscore the emerging knowledge on the impact of dopaminergic and serotonergic receptors on adipocyte biology, while recognizing the need for more information on the identity of the receptor subtypes involved and their full spectrum of actions.

Antipsychotic medications have become a mainstay of treatment for a steadily growing list of mental disorders. Of particular concern is the increased exposure of pediatric patients at higher risks for metabolic disturbances. Unquestionably, many patients do benefit from AAP medications in ameliorating psychiatric symptoms, except that these benefits are often diminished by severe metabolic side effects that increase mortality risk from cardiovascular disease. The overarching consensus in this

field is that drug actions are confined to the brain. Although previous studies showed drug effects on typical pancreatic, liver and adipose tissue functions, most of these effects were ascribed to indirect drug actions on peripheral organs via brain-derived mechanisms. Based on animal experimentation and studies with isolated adipocytes, but not yet supported by extensive clinical data, we offer a working model which implicates direct actions of AAP on adipose tissue which complement, or override, their central actions. The model emphasizes three major components: AAP-induced changes in leptin which increase appetite, alterations in adipokines/cytokines which exacerbate the metabolic syndrome, and changes in adipocyte differentiation and/or lipid accumulation which augment adiposity.

The proposed model stipulates that acute suppression of leptin by AAP stimulates appetite, increases food intake and results in weight gain. However, following chronic treatment with AAP, the expanding fat mass leads to hyperleptinemia and the development of leptin resistance. Presumably, an initial rapid weight gain induced by acute suppressions of leptin by AAP eventually levels off because of the lower ability of elevated leptin to suppress appetite as a consequence of altered responsiveness of brain orexigenic/anti-orexigenic circuits to leptin. Granted, this concept is oversimplified because it does not incorporate other appetite regulating factors such as glucose, insulin, ghrelin and PYY, as well as AAP-induced activation of brain DAR/5-HTR which impinge on appetite regulating neurons [76,77]. Nonetheless, the focus on leptin serves as a reasonable starting point for assessing the role of adipose-derived adipokines as mediators of AAP-induced weight gain. Future clinical studies which compare acute vs chronic changes in leptin and weight gain in response to AAP treatment are critical for testing this concept.

Weight gain is the most obvious side effect of AAP, but more subtle effects should be taken into account. A given drug can alter the endocrine functions of adipose tissue, its lipid storage capacity or both. Changes in adipokine release can affect insulin resistance (i.e., adiponectin) or cardiovascular functions (i.e., pro-inflammatory cytokines). Notably, drug-induced changes in adipokine release can occur without a major weight gain. Again, clinical studies which take into account both drug doses and timing of blood sampling with respect to food intake on circulating cytokines should be undertaken.

Because clinical studies cannot effectively address the mechanism of drug actions, studies with animals that afford invasive procedures, as well as the use of genetically-modified animals, can fill the gaps. Yet, the control of metabolic homeostasis as well as drug responsiveness can differ between rodents and humans. A case in point is an obvious sexual dimorphism in AAP-induced weight gain in rats, i.e. females are more sensitive to AAP-induced adiposity than males, which is not apparent in humans. Additional pharmacological and biochemical studies with isolated human adipocytes or adipocyte cell lines would be required to establish which DAR and 5-HTR subtypes are targeted by each

drug. Again, the limitations of this approach in terms of their extrapolation to the behavior of an intact adipose tissue should be recognized.

There is a good opportunity for future intervention aimed at finding drugs with little or no metabolic side effects. Once the full spectrum of drug actions on the adipocytes is delineated, practical means for identifying drugs that do, or do not, inflict metabolic disturbances can be devised. We foresee that the creation of a 'metabolic signature' could serve as a benchmark for developing new drugs devoid of undesirable metabolic side effects. Given the millions of patients worldwide that are chronically treated with antipsychotics, many pharmaceutical companies are actively engaged in the development of new drugs in this class. Human adipocytes could be easily integrated into the screening paradigms of candidate new drugs for an early identification of potential adverse metabolic activities prior to costly animal experimentation and prolonged and expensive clinical trials.

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Conflicts

N. Richtand has the following disclosures: Speaker's Bureau: Bristol-Meyers Squibb, Schering-Plough Corporation, Novartis Pharmaceuticals, Sunovion Pharmaceuticals Inc. Consultant: Bristol-Meyers Squibb, Sunovion Pharmaceuticals Inc. Grant/Research Support: Ortho-McNeil Janssen Scientific Affairs, LLC, AstraZeneca Pharmaceuticals.

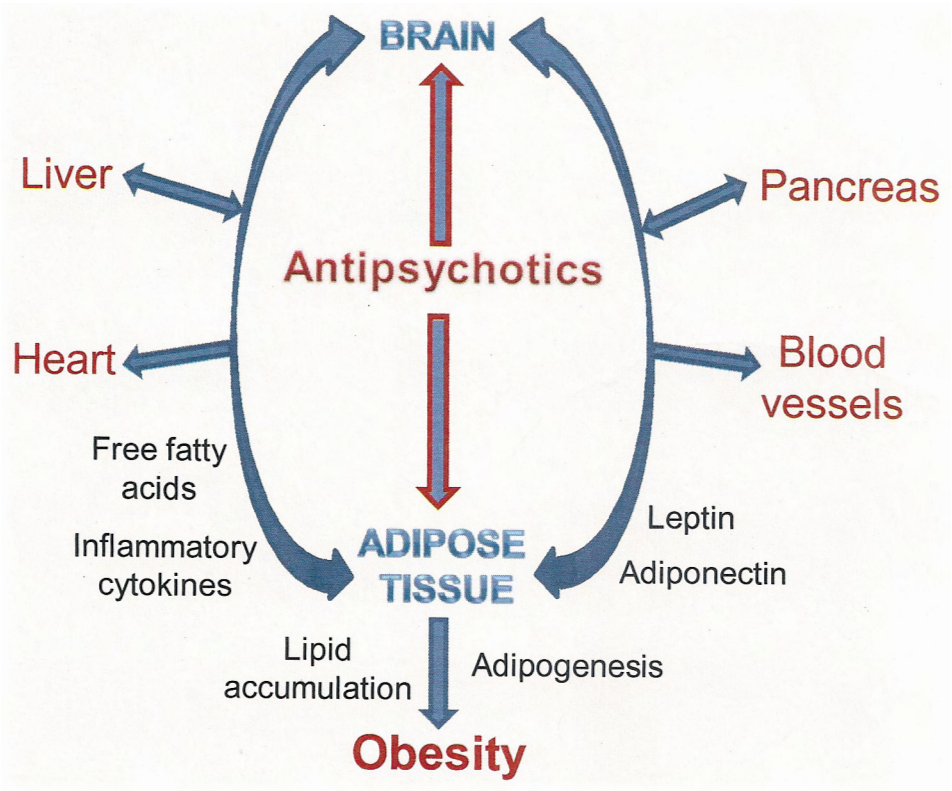
BRAIN
Liver
Pancreas
Antipsychotics
Heart
Blood vessels
Free fatty acids
Inflammatory cytokines
ADIPOSE TISSUE
Leptin
Adiponectin
Lipid accumulation
Adipogenesis
Obesity

Figure 1

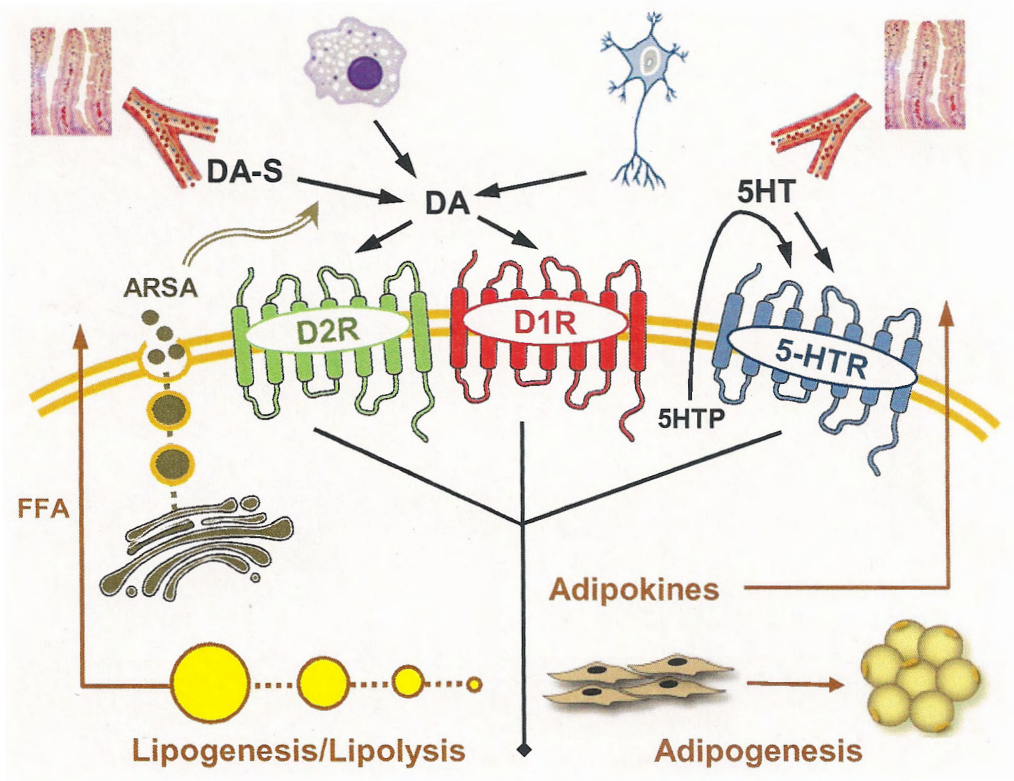
DA-S
DA
5HT
ARSA
D2R
D1R
5-HTR
5HTP
FFA
Adipokines
Lipogenesis/Lipolysis
Adipogenesis

Figure 2

Figure Legends

Figure 1: The role of adipose tissue in mediating metabolic disturbances by atypical antipsychotics (AAP). Acting via dopamine and serotonin receptors on the adipocytes, AAP accelerate adipogenesis and stimulate lipid accumulation, resulting in fat mass expansion. Acute suppression of leptin by AAP leads to increased appetite and weight gain, whereas a reduction in circulating adiponectin leads to insulin resistance. The metabolic syndrome, which involves target tissues such as liver, pancreas and the cardiovascular system, is further exacerbated by AAP-induced increases in circulating free fatty acids and inflammatory cytokines. Activation of neuronal circuits in the brain by AAP directly affect the regulation of food intake, and indirectly affect adipose tissue functions via the sympathetic nervous system.

Figure 2: Sources and actions of dopamine (DA) and serotonin (5HT) on the adipocyte. Sources of DA include the GI tract, peripheral nerves, paraganglia and immune cells. DA primarily circulates as a metabolically inactive sulfated form (DA-S), but can be deconjugated to bioactive DA by aryl sulfatase A (ARSA), a secretable lysosomal enzyme. DA can bind to either D_2R-like or to D_1R-like receptors and activate a variety of signaling pathways which culminate in alterations in lipogenesis, lipolysis, adipogenesis and adipokine release. Serotonin (5HT) input to the adipocytes comes primarily from the GI tract via circulating platelets. Adipocytes can also synthesize serotonin *de novo* from tryptophan (Trp) by peripheral tryptophan hydroxylase. Serotonin can bind to a number of receptor (5-HTR) subtypes and affect similar parameters as does DA. Whether the simultaneous activation of DAR and 5-HTR by any given drug results in augmentation, synergism or antagonism in each of the putative functions remains unclear.

Glossary

Adiponectin: a 30 kDa protein produced by adipocytes which circulates as high and low molecular weight isoforms, and its serum levels are inversely related to adiposity. Adiponectin reduces insulin resistance, inflammation, and atherosclerosis.

Adrenergic receptors: a class of G-protein-coupled receptors, divided into two main subtypes, α and β, which bind the catecholamines norepinephrine and epinephrine. These receptors are involved in many functions, including fight or flight response, lipolysis, smooth muscle contraction, heart rate, and vasoconstriction.

Arylsulfatase A (ARSA): a secretable lysosomal enzyme that can convert the inactive dopamine-sulfate back into bioactive dopamine by removing the sulfate group.

Extrapyramidal Side Effects: movement disorders such as akinesia (decreased voluntary movement), pseudoparkinsonism and akathisia (feeling of restlesness) often resulting from the use of dopamine antagonists (neuroleptic drugs).

Free fatty acids (FFA): carboxylic acids with a long aliphatic tail that is not attached to other molecules. Usually derived from triglycerides, they are a source of energy for peripheral tissues.

G-protein-coupled receptors (GPCR): a large superfamily of seven-transmembrane receptors that activate G-proteins when bound to a ligand.

Ghrelin: a 28 kDa protein hormone produced by the stomach which stimulates hunger. Ghrelin levels decrease in the fed state and increase with fasting.

Interleukin-6 (IL-6): a 22 kDa inflammatory cytokine produced by preadipocytes and macrophages in adipose tissue, which increases c-reactive protein production and heart disease. Plasma levels of IL-6 correlate with BMI and the size of adipocytes.

Leptin: a 16 kDa protein hormone produced primarily by adipocytes. Leptin functions as an energy sensor in the hypothalamus by inhibiting appetite and stimulating energy expenditure. Leptin knockout mice (ob/ob) are hyperphagic and obese. However, in humans leptin levels increase with obesity.

Lipogenesis: the process by which acetyl-CoA is converted into lipids, which allows for the efficient storage of energy.

Lipolysis: the process by which triglycerides are hydrolyzed to FFA and glycerol. The breakdown of lipids allows for their mobilization and use as energy for other tissues, and results in a reduction in lipid droplet size and overall adipose tissue mass.

Tryptophan hydroxylase (TPH): the rate-limiting enzyme for the synthesis of serotonin from tryptophan. TPH2 is the brain-specific isoform, while TPH1 is found in multiple peripheral tissues.

Box 1. Therapeutic use of antipsychotic medications: Past and present

Chlorpromazine, the first phenothiazine antipsychotic medication, was developed as an adjuvant to anesthetics [78]. The recognition in the early 1950s of its antipsychotic efficacy resulted in the liberation of thousands of institutionalized patients from insane asylums. Indeed, U.S. state mental hospitals housed 560,000 patients in 1955, but less than 80,000 patients by 1999. The discovery of clozapine in 1958 identified the improved neurological side effect profile of "atypical" antipsychotics (AAP), with a lower incidence of dystonia, parkinsonism, akathisia, and tardive dyskinesia compared to "typical" antipsychotics [79]. However, the association between clozapine and agranulocytosis has limited its therapeutic usage to treatment-resistant schizophrenia. In contrast, the lower neurological side effects of the subsequently developed AAP resulted in an exponential increase in their use, and they now account for 5% of U.S. drug expenditures [80]. FDA-approved indications for AAP include: schizophrenia, bipolar mania and mixed state, adjunctive treatment for major depression, irritability associated with autism, Tourette Syndrome, and bipolar depression. Yet, almost two-thirds of prescriptions for antipsychotic medications are for 'of label' non-FDA approved indications, including agitation in delirious patients, psychosis and agitation secondary to dementia, symptoms of post-traumatic stress disorder, personality disorder, attention deficit hyperactivity disorder, anxiety and insomnia [77].

Box 2. Obesity, the metabolic syndrome, and adipose tissue homeostasis.

- Obesity is one of the most challenging health problems of the 21st century. More than 60% of the US population is overweight (body mass index or BMI >25), with half of those individuals classified as obese (BMI>30).
- Obesity (increased adiposity) results from an imbalance between food intake and energy expenditure.
- The most common health complications associated with obesity are cardiovascular disease (hypertension, atherosclerosis, and coronary artery disease), the metabolic syndrome, and type II diabetes.
- As defined by the International Diabetes Federation, the metabolic syndrome is central (abdominal/visceral) obesity with any two of the following conditions: elevated serum triglycerides, reduced high density lipoprotein (HDL) cholesterol, hypertension, or impaired glucose tolerance.
- The metabolic syndrome is developed, in part, from disturbances of adipose tissue homeostasis, including increased release of inflammatory cytokines and free fatty acids, as well as decreased release of protective adipokines.
- Inflammatory cytokines consist of, but are not limited to, interleukin (IL)-1β, IL- 6, monocyte chemotactic protein-1 (MCP-1), and tumor necrosis factor α (TNFα). These cytokines are major contributors to cardiovascular impairments associated with the metabolic syndrome.
- Protective adipokines such as adiponectin increase insulin sensitivity. Their suppression during the metabolic syndrome contribute to insulin resistance and can lead to the development of diabetes.

Table 1. Antipsychotic medications: Receptor binding affinities and metabolic activities.

Drug[a]		receptors[b]			Metabolic effects		
generic	**trade®**	**DAR**	**5-HTR**	**other**	**weight**	**IR**	**Refs[c]**
1st generation							
High DAR affinity							
Haloperidol	Haldol	2>4>3>1>5	2A>1B>7	H1	=/+	=	[2,52,81-83]
Med. DAR affinity							
Perphenazine	Trilafon	2~3>4	2A~2B>2C~6~7>1F~1A>3>1B>1e>1D>5A	H1	=	=	[2,82,83]
Low DAR affinity							
Chlorpromazine	Thorazine	2~3>4>1>5	2A>2C~6~7>1e>1D	H1,A2	++++	↑	[81-83]
2nd generation							
Asenapine	Saphris	1~2~3~4	1A~1B~2A~2B~2C~7	H1,A1,A2	+	nr[d]	[84]
Clozapine	Clozaril	4>1>2>3	2A~2B>2C~6~7>1F~1A>3>1B>1e>1D>5A	H1,M1,A1	+++	↑	[2,52,81,82]
Iloperidone	Fanapt	2>3>4>1	2A>1B~1A>7>2C	A2	+	=	[2,81-83]
Olanzapine	Zyprexa	2>4>1	2A>2C>6>1F~7>1B>1D>1e>1A	H1,M1,A1	++++	↑	[2,52,81-83]
Paliperidone	Invega	3>2>1>4	2A~7>2C>2B>1B	H1,A1,A2		↑	[83]
Quetiapine	Seroquel	3>2>1	7>1A>2A>1B	H1,A1,A2	+++	=	[2,52,81-83]
Risperidone	Risperdal	2>4~5>3>1	2A>7>1D>1B~2B~2C>1B>1A~5A	H1,A1,A2	++	↑	[2,52,81-83]
Sertindole	Serdolect	2~3>4	2A>2C>6>7>1B>1D>1A>1F>1e	A1	+++	nr	[2,81,82]
Ziprasidone	Geodon	2>3>1~4>5	2A>1B~1D~7>2C>6>1A	H1,M1,A1	=/+	nr	[2,81,82]
3rd generation							
Aripiprazole	Abilify	2>3>1	2B>1A>7>2A~2C>1D	H1,A1	=/+	=	[81,83]

[a] Abbreviations: IR-insulin resistance; = no significant change; + relative increase from slight (+) to major (++++);↑ increased insulin resistance; H1 - H_1 histamine receptor, A1 - α_1-adrenergic receptor, A2 - α_2-adrenergic receptor, M1 - muscarinic acetylcholine receptor M_1.

[b] Ligand type: agonist - black; reverse agonist/antagonist - red; allosteric effect - blue.

[c] Additional data from pharmaceutical manufacturers.

[d] Not reported.

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