Schizophrenia Bulletin Advance Access published January 17, 2013

Schizophrenia Bulletin
doi:10.1093/schbul/sbs196

Second-Generation Antipsychotics Cause a Rapid Switch to Fat Oxidation That Is Required for Survival in C57BL/6J Mice

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Some second-generation antipsychotics (SGAs) increase insulin resistance and fat oxidation, but counter intuitively they do not activate lipolysis. This seems unsustainable for meeting energy demands. Here, we measured dose-dependent effects of SGAs on rates of oxygen consumption (VO_2), respiratory exchange ratio (RER), and physical activity in C57BL/6J mice. The role of H1-histamine receptors and consequences of blocking fat oxidation were also examined. Olanzapine, risperidone, and clozapine (2.5–10 mg/kg) elicited rapid drops in dark-cycle RER (~0.7) within minutes, whereas aripiprazole exerted only modest changes. Higher doses of olanzapine decreased VO_2, and this was associated with accumulation of glucose in plasma. Clozapine and risperidone also lowered VO_2, in contrast to aripiprazole, whereas all decreased physical activity. Astemizole and terfenadine had no significant effects on RER, VO_2, or physical activity. The VO_2 and RER effects appear independent of sedation/physical activity or H1-receptors. CPT-1 inhibitors can enhance muscle glucose utilization and prevent fat oxidation. However, after etomoxir (2 × 30 mg/kg), a low dose of olanzapine that did not significantly affect VO_2 by itself caused precipitous drops in VO_2 and body temperature, leading to death within hours or a moribund state requiring euthanasia. One 30 mg/kg dose of either etomoxir or 2-tetradecylglycidate followed by olanzapine, risperidone, or clozapine, but not aripiprazole, dramatically lowered VO_2 and body temperature. Thus, mice treated with some SGAs shift their fuel utilization to mostly fat but are unable to either switch back to glucose or meet their energy demands when either higher doses are used or when fat oxidation is blocked.

Key words: respiratory exchange ration/fat oxidation/glucose/body temperature/risperidone/olanzapine/clozapine/histamine/oxygen consumption

Introduction

Second-generation antipsychotics (SGAs) including olanzapine, clozapine, and risperidone are commonly associated with metabolic side effects including insulin resistance, glucose intolerance, overeating, increased adiposity, metabolic syndrome, and diabetes.[1] These side effects reduce quality of life and increase the risk of cardiovascular disease and mortality.[2] Additionally, the diagnoses for which SGAs are prescribed along with their overall utilization in adults and children have increased dramatically,[3–5] thus increasing the pool at risk.[6,7] It is currently unclear whether children, a population in which diabetes appears to be more difficult to treat,[8] are appropriately screened for these side effects.[9] Another issue is that the weight gain and metabolic side effects contribute to reduced compliance, increasing the risk of psychosis and the potential for reduced efficacy of subsequent SGA treatment.[10] Although some SGAs, like aripiprazole, have a lower incidence of adverse effects, they may not possess the same clinical efficacy as olanzapine, clozapine, and risperidone. So it is important to understand the mechanism of these effects in order to develop approaches or targets to reverse these trends.

Surprisingly, some of the glycemic effects of olanzapine and other SGAs manifest quickly. For example, elevated plasma glucose, glucose intolerance, or lower glucose infusion rates during a euglycemic clamp have been observed acutely in preclinical and clinical studies within minutes or days.[11–16] Another metabolic disturbance, decreased circulating free fatty acids (FFA), has also been described after both acute (1 h to 3 days) and chronic (2–3 weeks) treatment.[11–13,17,18]

The converse effects of SGAs on glycemia and FFA are of interest for several reasons. First, these effects appear to precede significant weight gain. Second, the pattern of these changes among the SGAs tested so far is

similar to the diabetes and weight-gain side-effect profile of these drugs. For example, FFA lowering was found after risperidone and olanzapine treatment but not after aripiprazole or haloperidol treatment in humans.[10,12,17]

In diabetes and other insulin-resistant states, peripheral tissues increasingly utilize fat rather than glucose as a fuel, leading to the accumulation of glucose in plasma. Similarly, in olanzapine-treated, ad libitum-fed rodents, a dramatic increase in FFA metabolic rate is observed (~2-fold), coinciding with a rapid fall in the dark-cycle respiratory exchange ratio (RER) to ~0.7, as well as glucose accumulation in plasma.[13] RER measures provide a non-invasive assay for the rapid and robust shift in fuel utilization from glucose to fat observed after SGA treatment. It is unclear why RER lowering has not been reported in humans treated with SGAs; however in humans, calorimetry is usually performed in the fasted state, a time when RER is already lowered. Thus, in diabetes and insulin-resistant states and after acute SGA treatment, glucose accumulates in the plasma and, at least in animals, there is increased lipid oxidation. However, there are notable differences between these 2 situations. In insulin resistance and diabetes, FFA are mobilized due to increased adipose tissue lipolysis.[19] FFA mobilization in this case is important to sustain metabolic rate and meet whole-body energy demands. In contrast, olanzapine does not increase glycerol rates of appearance in humans[17] despite causing glucose intolerance within days.[12] Furthermore, in both acute and chronic preclinical studies, basal and isoproterenol-stimulated lipolyses were actually impaired in vivo by olanzapine.[13] From an energy-balance standpoint, increasing reliance on fat as a fuel while not increasing lipolysis would seem to be a potentially unsustainable situation. We have investigated the potential for such unsustainability here by examining dose-related effects of SGAs on dark-cycle VO_2, RER, and physical activity. Recent studies have implicated H1-histamine blockade in some metabolic side effects of SGAs,[20] and so we also compared the effect of antihistamines and SGAs on VO_2, RER, and physical activity.

Noncompetitive or chronic blockade of the rate-controlling step in fat oxidation catalyzed by carnitine palmitoyltransferase-1 (CPT-1) has been shown to improve skeletal muscle glucose utilization.[17,21–23] Therefore, we also determined whether CPT-1 blockade would restore glucose utilization in SGA-treated mice, which is reflected in RER measurements. However, rather than reversing the SGA stimulation of lipid oxidation, after partial CPT-1 treatment (1 dose), olanzapine, risperidone, and clozapine, but not aripiprazole, dramatically lowered VO_2 even though the SGA doses used had no significant effect on VO_2 when used alone. A regimen of 2 doses of etoxomir (each 30 mg/kg) had no significant effect by itself but was toxic when combined with olanzapine, leading to rapid and lethal lowering of VO_2 and body temperature along with morbidity. Although these results were unexpected, they nevertheless provide insight into potential sites at which SGAs may bring about this switch from glucose to fat oxidation.

Materials and Methods

Materials

Olanzapine and the noncompetitive CPT-1 inhibitor, tetradecylglycidic acid (TDGA; CAS # 68170-97-8), were generous gifts of Neuland Laboratories Ltds and Janssen Research & Development, LLC, respectively. Clozapine (Alexis Biochemicals), risperidone (Amneal Pharmaceuticals), aripiprazole (Bristol-Myers Squibb), the noncompetitive CPT-1 inhibitor etoxomir (Sigma-Aldrich), and terfenadine and astemizole (Tocris Bioscience) were purchased from the indicated suppliers.

Animals

All procedures were conducted after review and approval by the Penn State Hershey Institutional Animal Care and Use Committee (IACUC). The Animal Resource Program is operated by the Department of Comparative Medicine and is accredited by AAALAC International. All animal living conditions are consistent with standards laid forth in the *Guide for the Care and Use of Laboratory Animals*, 8th edition, published by the National Research Council.

Male C57BL/6J mice were from The Jackson Laboratory or from our colony back-crossed to mice from this supplier about every third generation. All of the mice tested had been in our facility for at least 3 weeks. The average body weight was 31 ± 0.3 g, and the ages ranged from 5 to 6 months. Mice were maintained on a 12:12 light–dark cycle (lights on at 0700h) with ambient room temperature at 20.6 ± 1°C. Food (Harlan-Teklad Rodent Chow, no. 2018; Harlan-Teklad) and water were provided ad libitum unless otherwise indicated. Food intake before the acute experiments described was 4.8 ± 0.1 g.

Body composition was measured in conscious animals using a Bruker LF90 proton-NMR Minispec (Bruker Optics). The mice had the following pretreatment body composition (mean ± SE): lean body mass, 71.4 ± 0.7 %; fat mass, 10.7 ± 0.6 %; and body fluid, 8.0 ± 0.1 %, and there was no significant effect of the acute treatments on body composition. Shapiro–Wilk test was used to test the hypothesis that these mice might display a lack of normality with regard to body composition. However, the results of that testing indicated that the body composition follows a normal distribution (lean mass: $P = .16$; Fat mass: $P = .17$; Fluid mass: $P = .55$). Mice were routinely allocated to groups to minimize pretreatment differences in body weight.

Drug Preparation

All drugs were administered to mice via oral gavage that had been previously exposed to vehicle gavage treatments

for at least 3 days. Olanzapine was prepared using 0.1N HCl adjusted to pH 5 using NaOH. Other SGAs and etomoxir were suspended in 2% polyethylene glycol and 98% carboxymethylcellulose as previously described.[11,13] TDGA was prepared using 90 mg/ml of fatty acid–free bovine serum albumin in 0.9% NaCl.

SGAs were given at clinically relevant doses that induce varying levels of glycemia in rodents.[16,24] Furthermore, the doses of SGAs chosen conform to recent criteria using dopamine D2 receptor occupancy to simulate human drug dosing.[24–26] The dose of the noncompetitive CPT-1 inhibitors was based on previous literature and consultation with the supplier of TDGA.[27,28] Where indicated, 1 or 2 doses of etomoxir or TDGA (both 30 mg/kg) were given 3 and 15 h prior to the SGA to induce presumptive full or partial blockade of CPT-1.[28] Two doses are thought to provide a more complete inhibition of CPT-1. The doses of H1-antagonists in the current experiment were chosen based on their effective behavioral response in mice exposed to allergens.[29]

VO_2 and Locomotor Activity

Male C57BL/6J mice were acclimated to single cage housing for a week or more and then were accustomed to receiving daily gavages of 0.2 ml water for 4 to 7 days. They were then acclimated to Columbus Instruments (CI) metabolic chambers fitted with an air circulation fan for a minimum of 24 h and up to 96 h before studies. The CI system we used does not have automated food intake, water intake, and spillage detection. The water bottles used were the same as in the single housing, and the food was provided on the plastic decking (~3 pellets per day) so that it was easy for the animals to find and was changed daily. We have compared this CI system to another that does have automated food intake detection. In our experience, the required acclimation time for mice, evaluated by measuring body weight, is less when the food is provided on the decking compared with the automated system that we have access to. In our nonautomated CI system, the mice being acclimated to oral gavage have an elevated VO_2 that subsides over 6 h or so, thus, justifying the 24-h acclimation.

Researchers did not perform studies on the mice if they had lost weight by the dosing time, if 2100 h target RER values were not above 0.8, or if body weight–corrected VO_2 values were above 3500. In these cases, the animals were allowed to acclimate further until these objective endpoints were observed.

Vehicle-treated mice were tested concurrently with drug-treated mice for all replicates. VO_2, RER, and locomotor activity were measured as previously described.[13] Briefly, O_2 and CO_2 concentrations were measured from sealed chambers to calculate O_2 consumption (VO_2) and CO_2 expiration (VCO_2) (Oxymax; Columbus Instruments). Each chamber was measured for 1 min at 15-min intervals. Flow rate was set to 0.6 l.p.m. RER was calculated by dividing VCO_2/VO_2. Locomotor activity was measured simultaneously using infrared technology (OPT-M3; Columbus Instruments). The number of light-beam breaks were counted along the *x*-axis and *z*-axis and averaged over 3 or 6 h of treatment.

There is current controversy about how to normalize VO_2 or energy expenditure data when groups of animals with different body compositions are being compared.[30,31] Such data are usually collected over time and then averaged for some time period (eg, 24 h, dark cycle or light cycle, energy expenditure or VO_2). However, these approaches that employ ANCOVA do not seem to be appropriate for displaying and comparing repeated measures over time in the same animals or comparisons to vehicle-treated mice with the same body composition, genotype, and diet. Mice in this study were all lean with a narrow range of body weight and composition. In pilot studies, we found that body composition did not change over the time course we were studying in animals treated with olanzapine, data not shown. Even et al.[31] addressed a second situation applicable to our study where adult, lean animals of the same body composition were being compared. They indicate that when lean rodents are being studied, a normalization procedure is needed even if the animals are all of the same genotype, and they showed that body weight is a better normalizer compared with an exponent of body weight or fat-free mass for this purpose. This was validated in their study by plotting the body weight (x) against body weight–corrected energy expenditure (y). Although Even et al.[31] indicated that using body weight for a denominator was valid for lean adult rats and mice, only rat data was shown. To help validate body weight normalization of our VO_2 data, we used their approach by plotting the body weight of vehicle-treated mice against body weight–corrected dark-cycle VO_2 values. We included mice at the lighter and heavier end of the spectrum. The data were fitted to a linear equation $F(X) = MX + B$ and analyzed to determine the correlation coefficient using GraphPad Prism 6.0 software. The results of this analysis are described and support the use of body weight for our comparisons.

Blood Glucose and Body Temperature

Glucose was measured from tail blood in duplicate using an Ascencia Contour blood glucose meter (Bayer Health Care LCC). Rectal body temperature was measured using a NIST-certified electronic thermometer (Fluke Corporation) with a mouse thermocouple probe from Harvard Apparatus.

Statistical Analysis

Data are expressed as the mean ± SEM. Multiple comparisons were performed using one-way ANOVA

with significance at $P < .05$ using GraphPad Prism computer software (GraphPad Software). To determine when VO_2 and RER were changing after SGA treatment, data were analyzed using repeated measures ANOVA (GraphPad Prism) with time and treatment as main effects. Experimental treatment values were compared with that of controls using a Dunnett's post test and the vehicle group as the indicated control. Because in many instances drug treatments decreased both VO_2 and activity, an ANCOVA was performed using XLSTAT software (Addinsoft). We assessed the relationship between body weight–corrected VO_2 and activity using the total number of beam breaks along the *x*-axis (XTOT) summed over 3 h, and we compared this to the 3-h average of VO_2 during this same time. We chose to use data from 3 h after treatment because this is when repeated measures ANOVA detected differences in VO_2 between most doses of SGAs and vehicle. We also compared 3-h XTOT with the 3-h average of VO_2 after treatment subtracted from the 3-h average of VO_2 before treatment (VO_2 difference) to account for potential differences in VO_2 prior to treatment. Differences among groups were analyzed using Bonferroni's multiple comparison post test where appropriate.

Results

Olanzapine Has Converse, Dose-Dependent Effects on Plasma Glucose and RER

Recent studies from our group and others have shown that olanzapine acutely raises plasma glucose in Sprague-Dawley rats and FVB/N mice within 1–2 h.[13,16,32] An acute glycemic effect of olanzapine is also observed in euglycemic clamp studies performed on rats.[11,13–15,33] In this study, in male C57BL/6J mice, olanzapine dose dependently increased plasma glucose 1 h after oral administration (figure 1). Glucose was elevated 43% at the 5 mg/kg dose and 61% at 10 mg/kg. We did not evaluate higher doses; however, in acute rat studies by Assie et al., the acute glycemic effects had not been saturated by 40 mg/kg.[16]

Olanzapine lowers plasma FFA, increases FFA metabolic rate, and dramatically lowers dark-cycle RER. These findings support the idea that olanzapine increases fat oxidation.[10–13,17] Here, we also monitored changes in dark-cycle RER as a noninvasive approach to monitor changes in major fuel selection. At 5 mg/kg, olanzapine caused a sustained lowering of RER to ~0.7, which began to recover around 8 h later. A dose of 2.5 mg/kg was somewhat less efficacious, lowering RER early on and exhibiting a faster recovery (figure 2A). Repeated measures ANOVA indicated that compared with vehicle treatment, 2.5 mg/kg olanzapine caused a significant decrease in RER between 2.25 and 8.5 h compared with vehicle ($P < .0001$ to $P < .05$) with effects lasting longer at the higher dose of at 5 mg/kg (2.25–13 h; $P < .0001$). Thus, hyperglycemia after olanzapine (figure 1) is associated with a dose-dependent transition to fat oxidation as measured by this drop in the RER (figure 2A).

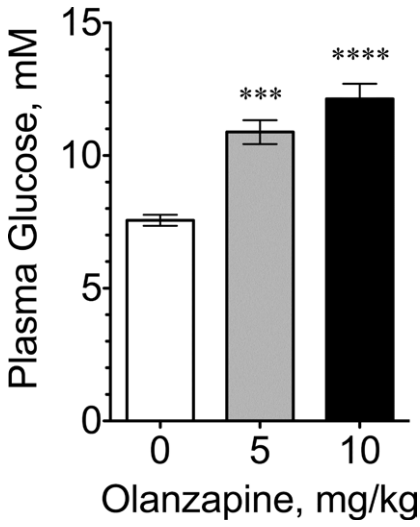

Fig. 1. Olanzapine acutely raises plasma glucose in male C57BL/6J mice. Food was removed 3 h prior to oral gavage of olanzapine or vehicle at 2100 h. Blood glucose was measured 1 h later. Results are mean ± SE (n = 5/bar). Asterisks indicate statistically different from vehicle at ***$P < .001$ or **** $P < .0001$.

Effects of Risperidone, Clozapine, and Aripiprazole on RER

In their study, Assie et al.[16] found that clozapine and risperidone also efficaciously raised glucose, whereas comparatively, aripiprazole had only marginal affects that were not dose related. Here, we examined the dose-dependent effects of risperidone, clozapine, and aripiprazole on RER (figures 2B–D). At 2.5 and 5 mg/kg, risperidone had RER lowering effects (figure 2B), decreasing the dark-cycle RER to ~0.7 within 1 h after gavage (Repeated measures ANOVA: vehicle and 2.5 mg/kg risperidone were significantly different between 2.5 and 13 h [$P < .0001$]; vehicle and 5 mg/kg RIS were different between 2.75 and 13 h [$P < .0001$]). Comparable to the lower dose of olanzapine (figure 2A), 5 and 10 mg/kg clozapine had a similar RER nadir (~0.7) and similar rates of recovery (figure 2C; Repeated measures ANOVA: vehicle and 5 mg/kg clozapine were different between 2 and 8 h [$P < .0001$ to $P < .05$] and 10 mg/kg clozapine was different from vehicle treatment between 2 and 8.25 h [$P < .0001$ to $P < .05$]). Compared with these other SGAs, aripiprazole caused smaller effects on RER as we previously reported.[13] The RER after a 1 mg/kg aripirazole dose was not significantly different from vehicle at any time point ($P > .05$). However, at 5 and 10 mg/kg, aripiprazole lowered RER, albeit with less efficacy compared with the other SGAs (figure 2D; Repeated measures ANOVA: vehicle and 5 mg/kg aripiprazole were different between

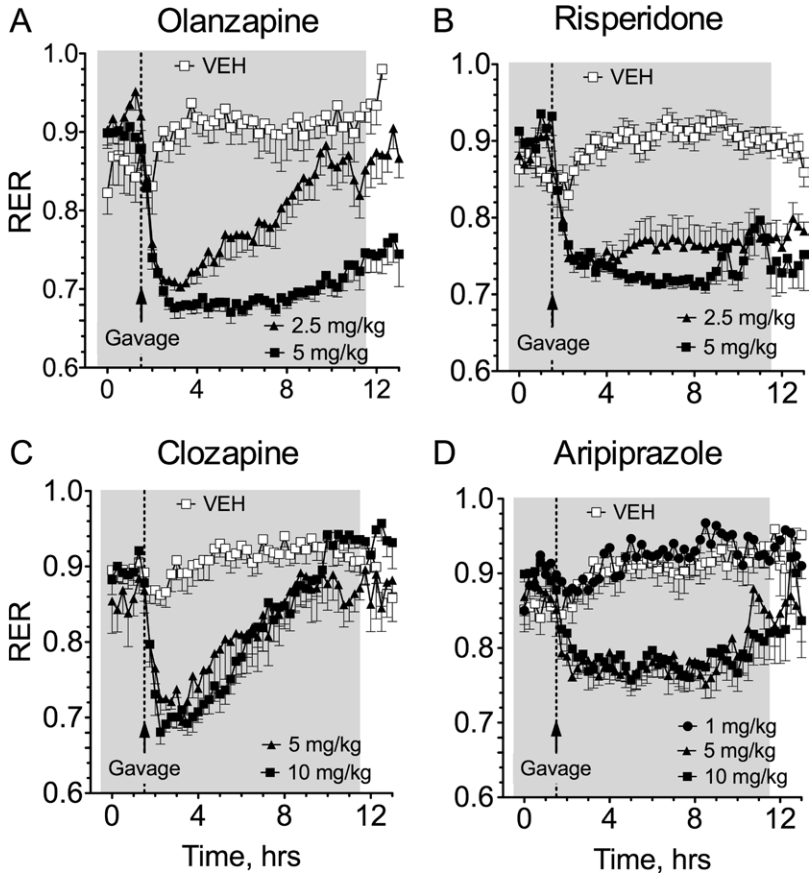

Fig. 2. Effects of second-generation antipsychotics on respiratory exchange ratio in mice. Male C57BL/6J mice were acclimated to single cage housing for a week or more and became accustomed to receiving daily gavages of 0.2 ml water. They were then acclimated to Columbus Instruments metabolic chambers fitted with an air circulation fan for a minimum of 24 h as described in Materials and Methods. respiratory exchange ratio was then recorded before and after vehicle (-□-) or indicated doses of (A) olanzapine (OLZ), (B) risperidone (RIS), (C) clozapine (CLZ), or (D) aripiprazole (ARI) at $t = 2100$ h (indicated by the arrow). Results of repeated measures ANOVA are described in the text. Gray background indicates dark cycle; white background indicates light cycle times. Mice had access to food and water ad libitum before and after gavage. Mean and SE are shown, $n = 5$–11/group (grp).

2.25 and 10.5 h [$P < .0001$ to $P < .01$], whereas 10 mg/kg aripiprazole was different from vehicle between 2.5 and 13 h [$P < .0001$ to $P < .01$]).

Some SGAs Lower Whole-Body Respiration at Higher Doses

Next, we examined the time-course effects of different doses of SGAs on VO_2. As described in the methods, we used an approach from Even et al.[31] to validate the use of body weight as a normalizer for the VO_2 values. Using the approach from their article, we plotted the average dark-cycle body weight–corrected VO_2 of vehicle-treated mice against their body weight (figure 3). We included mice at the lighter and heavier end of the spectrum such as those used in this study. We found that the relationship between the body weight and the per weight VO_2 for our mice appears similar across the range of weights we used (the fitted line had a very low r^2 and has a very slight negative slope with a ratio of M to B less than 0.001), which is consistent with the rat data obtained by Even et al.[31] Notably, the average body weight of all of the animals used was ~31 g. For these reasons, we decided to use body weight as a normalizer for our VO_2 measurements and analyzed the time courses for differences using ANOVA repeated measures as with the RER data.

The dose-dependent effects of olanzapine on body weight–corrected VO_2 is shown in figure 4A. The lower dose of olanzapine (2.5 mg/kg) that dramatically lowered RER had no significant effect on VO_2 (compare figure 2A with figure 4A). In contrast, at 5 mg/kg, olanzapine, which also lowered RER, caused a roughly 50% decrease in the VO_2 (figure 4A; repeated measures ANOVA indicated treatment differences from vehicle after 5 mg/kg olanzapine between 2.25 and 6.25 h [$P < .0001$ to $P < .05$]; it should be noted that at the higher dose, the ratio of VCO_2/VO_2 (RER) is based on a much lower VCO_2 and VO_2.)

The dose-dependent actions of risperidone, clozapine, and aripiprazole on VO_2 were also examined (figures 4B–D). Although 2.5 and 5 mg/kg doses of risperidone had similar efficacy of lowering RER (figure 2B), they exhibited dose-dependent effects on VO_2 (figure 4B),

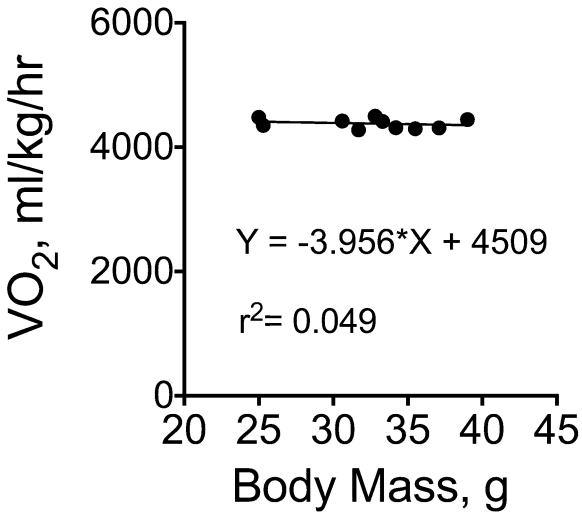

Fig. 3. Comparison of body weight to body weight–corrected dark-cycle rates of oxygen consumption in lean vehicle-treated C57BL6/J mice used in our study. Average dark-cycle body weight–corrected rates of oxygen consumption was calculated for vehicle-treated mice and plotted against their different body weights as described in the Materials and Methods. The data were fitted to a line to calculate the correlation coefficient, slope, and intercept.

decreasing the VO_2 by ~33% and 52%, respectively, within 1 h after gavage. Repeated measures ANOVA for 2.5 mg/kg risperidone was significant compared with vehicle-treated mice between 1.75 and 2 h ($P < .0001$ to $P < .05$), whereas 5 mg/kg risperidone was different from vehicle between 1.75 and 3.5 h ($P < .0001$ to $P < .05$). Although 5 and 10 mg/kg clozapine had a similar RER nadir, the higher dose led to a ~64% transient reduction in the VO_2 compared with a ~40% reduction at the lower dose (figure 4C; repeated measures ANOVA: 5 mg/kg clozapine compared with vehicle was significantly lower between 2 and 4.5 h [$P < .001$ to $P < .05$], and for 10 mg/kg clozapine, a difference was observed between 1.75 and 4.5 h [$P < .0001$ to $P < .05$]). None of the aripiprazole doses affected VO_2 at any time point (figure 4D).

H1-Antihistamines Do Not Affect RER, VO_2, or Physical Activity

Blocking of H1-histamine receptors has been implicated in some metabolic side effects of SGAs.[34–37] Therefore, we examined the effect of 2 H1-blocking exemplars, astemizole (Hismanal) and terfenadine (Seldane), using effective H1-blocking doses from previous literature. Neither

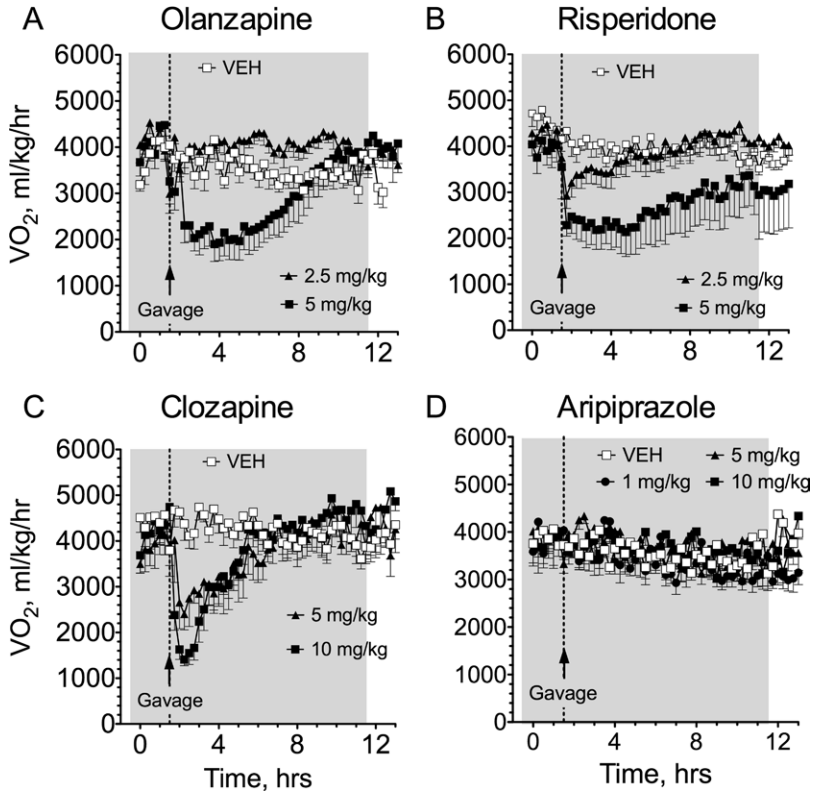

Fig. 4. Effects of second-generation antipsychotics on rates of oxygen consumption. Rates of oxygen consumption shown for the same mice from figure 2 before and after vehicle (-□-) or indicated doses (closed symbols) of (A) olanzapine (OLZ), (B) risperidone (RIS), (C) clozapine (CLZ), or (D) aripiprazole (ARI) at $t = 2100$ h (indicated by the arrow). Gray background indicates dark cycle; white background indicates light cycle. Mice had access to food and water ad libitum before and after gavage. Mean and SE are shown ($n = 5$–11/grp).

astemizole (3 mg/kg) nor terfenadine (10 mg/kg) had any significant effect on RER or VO_2 (figure 5). While there was a tendency for reduced physical activity after terfenadine administration, neither terfenadine nor astemizole elicited a statistically significant decrease in physical activity (table 1).

SGAs Including Aripiprazole Acutely Decrease Physical Activity

The calorimeter is fitted with 2 light sensor arrays to measure horizontal (*X*) and vertical (*Z*) light-beam breaks that were monitored in the above experiments. All of the SGAs tested decreased *X* movement 50% or more during the dark cycle compared with corresponding vehicle controls, with some also affecting rearing behavior (*Z*), which was more variable between the controls (table 1). Activity levels neither increased nor plummeted immediately following vehicle gavage, reflecting our acclimation procedure. Thus, any change in activity likely represents a drug treatment effect. Presumably, this decrease in physical activity represents sedation by the drugs. Aripiprazole, which did not affect VO_2 (figure 3D), is included in the drugs that caused this decrease in physical activity (table 1). This observation would seem to disassociate the sedative/physical activity and VO_2 effects of the SGAs, an interaction that is presented later in the analysis.

This apparent lack of association between physical activity and VO_2 was also found in another analysis using ANCOVA. In many instances, SGA treatment decreased VO_2 and physical activity. ANOVA repeated measures analyses suggested that some SGA doses had consistent effects on body weight–corrected VO_2 at least over 3 h after gavage. Therefore, we used ANCOVA analysis to examine the potential relationship between vehicle and drug effects on physical activity and VO_2 using a 3-h average of the physical activity and VO_2 after gavage (data not shown). We found no interaction between XTOT and VO_2 for olanzapine (P = .49), risperidone (P = .45), clozapine (P = .60), aripiprazole (P = .86), terfenadine (P = .70), or astemizole (P = .66). However, consistent with repeated measures ANOVA, there was a treatment effect for olanzapine (P = .001), risperidone (P = .03), and clozapine (P = .008). Furthermore, the coefficient of determination (R^2) for each regression line was less than 0.1 for most of the VO_2 and XTOT interactions tested including the vehicles, indicating that VO_2 is not a good predictor of total activity and vice versa. We extended the

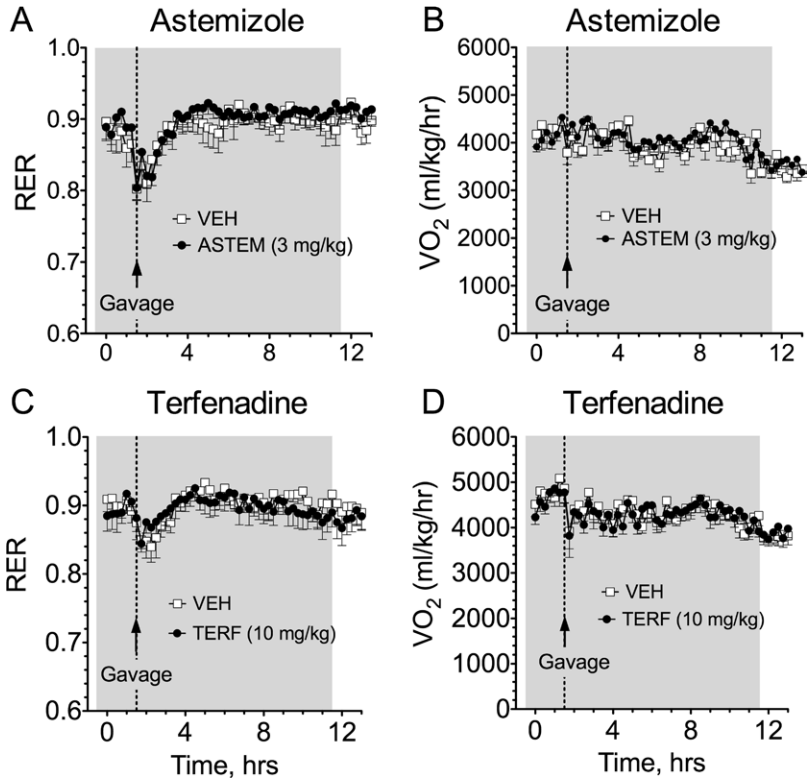

Fig. 5. Lack of effects of H1-antihistamines on respiratory exchange ratio or rates of oxygen consumption. Using the same protocol from figures 2 and 3, the effects of indicated single doses of either (A and B) astemizole (ASTEM) or (C and D) terfenadine (TERF) on (A, C) respiratory exchange ratio and (B, D) rates of oxygen consumption were measured. Repeated measures ANOVA did not detect any differences between vehicle (VEH) and antihistamines on respiratory exchange ratio or rates of oxygen consumption at any time point (P > .05). Oral gavages were performed at t = 2100 h, indicated by an arrow. Gray background indicates dark cycle; white background indicates light cycle. Mice had access to food and water ad libitum before and after gavage. Mean and SE are shown (n = 7–10/grp).

Table 1. Six-Hour Cumulative Physical Activity (Light Array Beam Breaks) After Treatment

Drug	Dose (mg/kg)	Activity on *X*-axis	Activity on *Z*-axis	*n*
Vehicle	0	5948 ± 1050	812 ± 176	8
Olanzapine	2.5	2817 ± 1011*	404 ± 111	6
	5	821 ± 232**	225 ± 74*	6
Vehicle	0	5603 ± 943	1968 ± 576	8
Risperidone	2.5	1938 ± 479**	324 ± 73**	10
	5	1777 ± 356***	263 ± 51*	5
Vehicle	0	5565 ± 938	2204 ± 1147	8
Clozapine	3	5566 ± 1284	1039 ± 292	7
	5	3917 ± 1467	720 ± 249	5
	10	2284 ± 600	400 ± 97	7
Vehicle	0	5617 ± 764	1048 ± 272	8
Aripiprazole	1	2280 ± 428*	626 ± 224	6
	5	2887 ± 444*	623 ± 131	8
	10	3647 ± 924*	867 ± 218	7
Vehicle	0	5463 ± 1386	1556 ± 567	8
Terfenadine	10	3428 ± 861	1021 ± 337	9
Astemizole	3	6109 ± 1258	1360 ± 405	8

Note: Activity was measured using infrared beam breaks during the dark phase of the light/dark cycle. Data are mean ± SE for 6-h cumulative activity after oral gavage at 2100 h.
Asterisks indicate number of beam breaks different from vehicle (*$P < .05$, **$P < .01$, ***$P < .001$).

analysis to include ANCOVA testing for the difference in VO_2 before and after treatment, and again, we detected no interaction between VO_2 difference and XTOT activity for any of the SGAs or antihistamines ($P > .05$).

These findings suggest that a decrease in VO_2 does not appear to be related to any changes in physical activity as a measure of the level of sedation. This should not be surprising because the physical activity is only one contributor to daily caloric requirements.

Effects of Noncompetitive Fat Oxidation Inhibition

CPT1 is the rate controlling step in fat oxidation. It is negatively regulated by intracellular concentrations of malonyl CoA. In previous studies, we found that olanzapine increased the FFA metabolic rate and this was associated with a rapid decline in malonyl CoA.[13] Recent studies have suggested that increased FFA oxidation in diabetes may be "too much of a good thing" and contributes to diabetes pathology.[38–42] CPT-1 inhibitors tend to enhance glucose metabolism.[17,21–23,43] We questioned whether CPT-1 blockade could prevent the SGA-induced increase in fat metabolism, which might in turn convert the animals back to using more glucose as a fuel. To test this, we used 2 CPT-1 noncompetitive antagonists, etomoxir and TDGA. A previous study showed that two 30 mg/kg doses of a CPT-1 inhibitor within 24 h produces an effective noncompetitive CPT-1 blockade.[28]

In pilot studies, CPT-1 blockade did not increase the dark-cycle RER significantly after treatment with either etomoxir (figure 6) or TDGA (data not shown). In one previous study, 2 doses of etomoxir (30 mg/kg) raised light-cycle RER measurements from 0.77 to 0.89 in fasted male C57BL/6J mice tested in the light cycle,[28] conditions that are ideal to observe an increase in the RER. Such food-deprived conditions are typical for calorimetry in humans as well. However, our studies were performed on ad libitum-fed mice during the dark cycle in order to raise the RER close to 0.89 or above in order to provide ideal conditions for observing RER lowering. Under these conditions, not observing a further rise in RER may represent a ceiling affect.

In the first experiment, we provided 2 doses of etomoxir (both 30 mg/kg) 15 and 3 h before dark-cycle administration of 2.5 mg/kg olanzapine or vehicle (ETX-ETX-OLZ; figure 6). The ETX-ETX-OLZ treatment was compared to a vehicle treatment in which animals received vehicle instead of etomoxir but still received 2.5 mg/kg of olanzapine (VEH-VEH-OLZ; figure 6) . Notably, the lower 2.5 mg/kg dose of olanzapine did not affect VO_2 in earlier studies (figure 4A) but did lower RER (figure 2A). Unexpectedly, on the morning following this treatment regimen, some of the animals were found to have died during the night. Those living were in a moribund state with body temperatures approaching room temperature (figure 6C). Analysis of the calorimetry data (table 2 and figure 6) showed that shortly after olanzapine treatment, VO_2 in the surviving ETX-ETX-OLZ–treated mice had dropped by ~90% within hours. The RER (figure 6A) also decreased but was for a much smaller VO_2 (figure 6B) and VCO_2 (not shown). This lowering of the VO_2 with the RER of ~0.7 implies that the animals could not switch to an alternate fuel after ETX-ETX-OLZ treatment and were mostly metabolizing fat using the residual CPT-1 activity that the etomoxir treatment had failed to block.

To further investigate the effects of CPT-1 blockade on SGA metabolic responses, we explored the effects of a presumptive partial CPT-1 blockade (ie, only a single dose of either etomoxir or TDGA 3 h before SGA or vehicle). Here again, lower doses of SGAs that had minimal effects on VO_2 by themselves were used (figure 7). Figures 7A and B show that treatment with a single dose of either etomoxir or TDGA followed by vehicle (ETX-VEH or TDGA-VEH) had no effect on VO_2 compared with 2 consecutive vehicle treatments (VEH-VEH). However, the combination of a single dose of either etomoxir or TDGA followed by 2.5 mg/kg olanzapine led to reduced VO_2 and body temperature (figures 7A–C). Thus, etomoxir and TDGA were equally effective at causing this adverse VO_2 lowering when combined with olanzapine. Although all of the mice treated with the lower cumulative dose CPT-1 inhibitor and SGA combination treatment survived through the next morning, their moribund state and reduced body temperature (figure 7C) continued, requiring humane euthanasia the next day.

The activity data in tables 1 and 2 were acquired at different times, with a different cohort of mice, so we did not

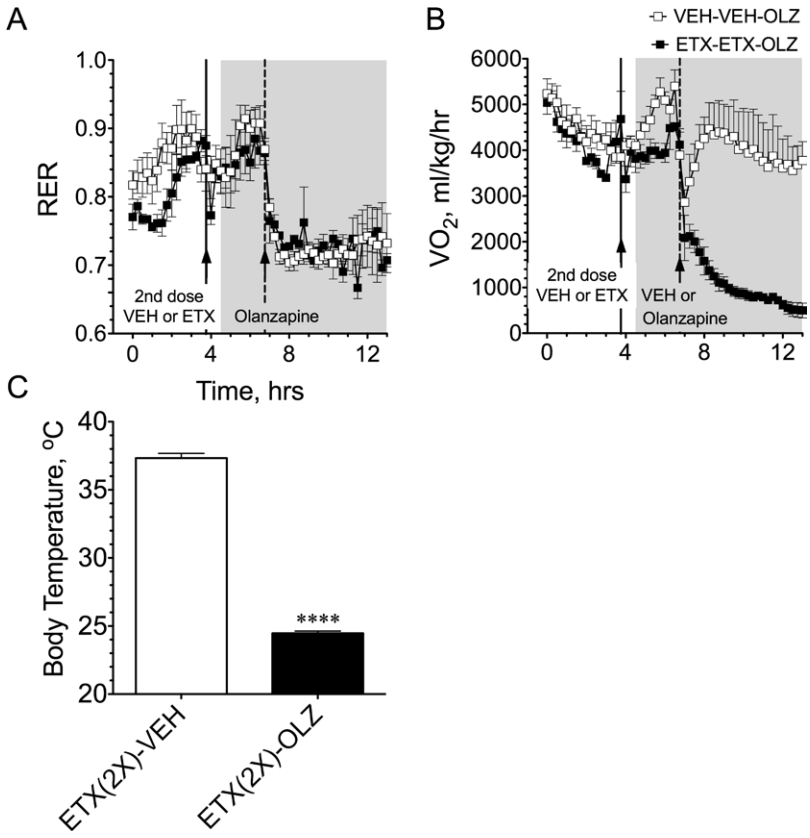

Fig. 6. Effects of 2 doses of etomoxir (ETX) in combination with olanzapine (OLZ) on rates of oxygen consumption, respiratory exchange ratio, and body temperature. Male C57BL/6J mice acclimated to single housing, gavage treatment, and calorimetry cages as in figures 2 and 3. ETX (30 mg/kg) or vehicle (VEH) were given twice via oral gavage 15 h (not shown) and 3 h before a single dose of (A–C) 2.5 mg/kg OLZ or (C) VEH at 2100 h. Mean ± SE of the (A) respiratory exchange ratio and (B) rates of oxygen consumption are shown ($n = 4$ grp). Repeated measures ANOVA detected differences between VEH-VEH-OLZ and ETX-ETX-OLZ between 7.5 and 13 h ($P < .0001$ to $P < .05$) for rates of oxygen consumption. No differences were detected between VEH-VEH-OLZ and ETX-ETX-OLZ for respiratory exchange ratio ($P > .05$). Gray background indicates dark cycle and white background indicates light cycle times. Mice had access to food and water ad libitum before and after gavage. Rectal body temperature (C) was measured in remaining live mice the following light cycle (0900 h) when the mice treated with ETX and OLZ (ETX-ETX-OLZ, $n = 3$) were observed to be deceased or in a moribund state compared with OLZ- or VEH-treated mice. Asterisks indicate statistically different from vehicle at $P < .0001$.

Table 2. Six-Hour Cumulative Physical Activity (Light Array Beam Breaks) After First Treatment With Etomoxir or Vehicle Followed by Second Treatment With Second-Generation Antipsychotics or Vehicle as Indicated

Treatment 1	Dose (mg/kg)	Treatment 2	Dose (mg/kg)	Activity on *X*-axis	Activity on *Z*-axis	*n*
Vehicle	0	Vehicle	0	7575 ± 1914	2076 ± 680	6
Etomoxir	30	Olanzapine	2.5	920 ± 180**	273 ± 54*	4
Vehicle	0	Vehicle	0	7349 ± 2094	2009 ± 818	6
Etomoxir	30	Risperidone	2.5	1370 ± 594*	402 ± 118	6
Vehicle	0	Vehicle	0	7236 ± 1729	1204 ± 437	6
Etomoxir	30	Clozapine	3	1393 ± 266**	244 ± 51*	6
Vehicle	0	Vehicle	0	7813 ± 1753	1353 ± 469	6
Etomoxir	30	Aripiprazole	1	3207 ± 825*	503 ± 165	7
Vehicle	0	Vehicle	0	7089 ± 1689	1305 ± 574	6
Etomoxir	30	Vehicle	0	4529 ± 809	1123 ± 296	10

Note: Activity was measured using infrared beam breaks during the dark cycle. Data are mean ± SE for 6-h cumulative activity after oral gavage at 2100 h as in table 1. Etomoxir, TDGA, or vehicle was given at 1800 h (Treatment 1), followed by second-generation antipsychotics or vehicle at 2100 h (Treatment 2). Data are mean ± SE for 6-h cumulative activity after oral gavage at 2100 h. Asterisks indicate number of beam breaks different from vehicle (*$P < .05$, **$P < .01$).

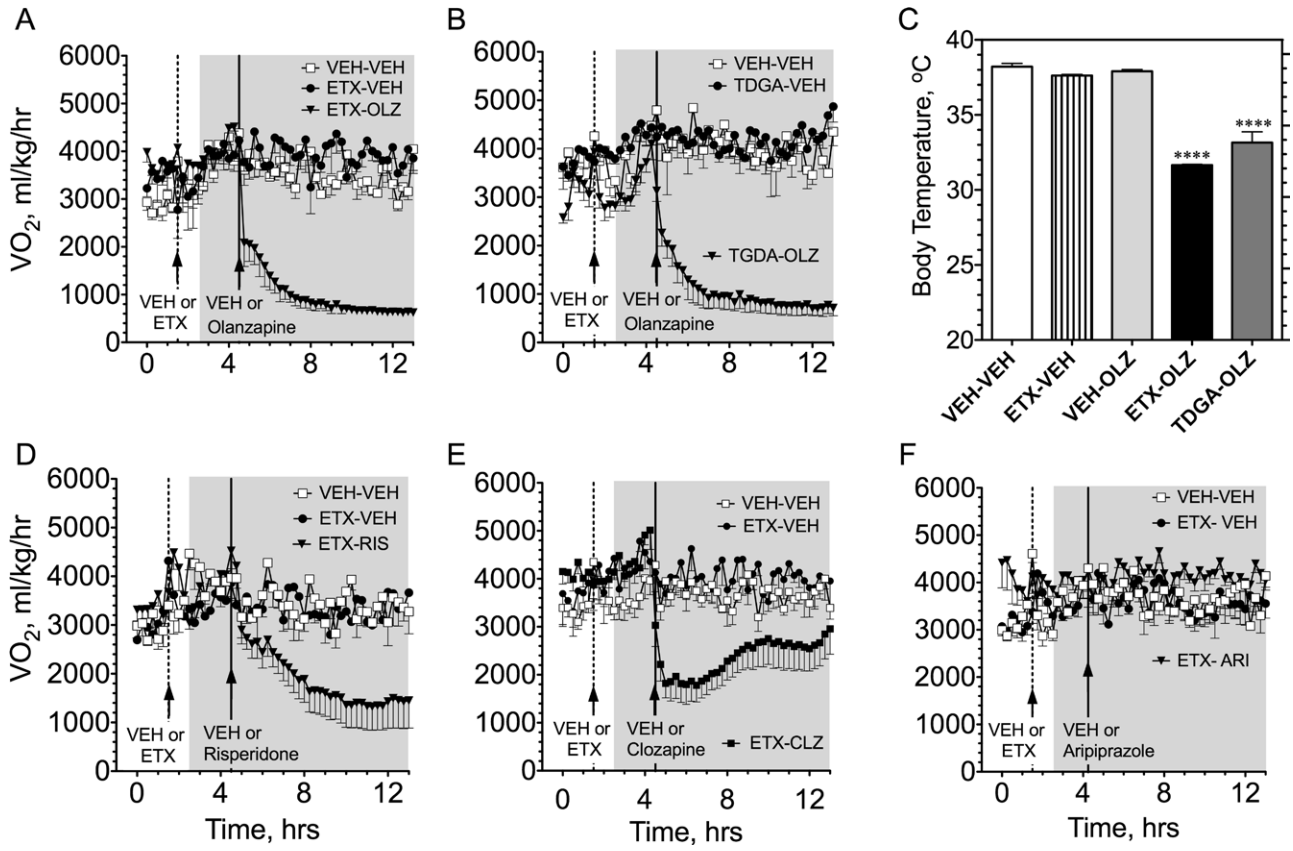

Fig. 7. Effects of a single dose of CPT-1 inhibitor (ETX or TDGA) and lower doses of second-generation antipsychotics on rates of oxygen consumption and body temperature. (A) One oral dose of ETX (30 mg/kg) or (B) TDGA (30 mg/kg) was given 3 h before 2.5 mg/kg olanzapine (OLZ) at 2100 h. Gavage times are indicated by arrows (n = 3–8/grp). Repeated measures ANOVA revealed that the rates of oxygen consumption of ETX-OLZ–treated mice was significantly lower than vehicle (VEH) between 4.75 and 13 h ($P < .01$ to $P < .0001$) and the rates of oxygen consumption of TDGA-OLZ–treated mice was lower between 4.5 and 13 h ($P < .0001$). Rectal body temperature (C) was measured during the light phase of the light/dark cycle (0900 h), 12 h after OLZ or VEH. Asterisks indicate statistically different from VEH at $P < .0001$. Dark-cycle rates of oxygen consumption was also measured after one 30 mg/kg dose of ETX followed by (D) 2.5 g/kg risperidone (RIS), (E) 3 mg/kg clozapine (CLZ), or (F) 1 mg/kg aripiprazole (ARI) at 2100 h (n = 3–8/grp). Repeated measures ANOVA indicated treatment differences between VEH and ETX-RIS–treated mice between 6.75 and 13 h ($P < .0001$ to $P < .05$), ETX-CLZ–treated mice between 5.25 and 9.25 h ($P < .01$ to $P < .05$), and ETX-ARI–treated mice between 7 and 7.75 h ($P < .05$).

attempt to compare these data statistically. Nevertheless, the vehicle activity counts were frequently higher in table 2 compared with table 1. This may be attributable to having 2 vehicle gavage treatments spaced 3 h apart in table 2 compared with only 1 gavage in table 1 despite previous acclimation to gavage and the metabolic cages in both cases. Nevertheless, the combination of 2.5 mg/kg olanzapine and a single dose of etomoxir led to reduced physical activity (table 2). The reduction in X-axis physical activity after the etoxomir and olanzapine combination treatment appears greater than that observed with olanzapine alone (compare tables 1 and 2). However, the earlier caveat applies here again; the data in these 2 tables were not controlled for the time of year they were performed or the cohort of animals.

The detrimental effects of olanzapine after CPT-1 blockade were also observed when risperidone and clozapine were used as the SGA. Doses of risperidone and clozapine that had little effect on VO_2 by themselves (figure 4) had dramatic effects on VO_2 after a single dose of etomoxir (figures 7D and E). As with olanzapine, risperidone and clozapine still lowered RER after etomoxir treatment. However, in the latter case, the RER was for a much lower VO_2. The physical activity was also decreased after the combination of risperidone or clozapine and etoxomir (table 2).

In contrast to the other SGAs, effects on VO_2 observed in animals treated consecutively with etomoxir and aripiprazole were minimal (figure 7F). Despite this lack of effect on VO_2 the combination treatment of etomoxir followed by aripiprazole did lead to a significant decrease in physical activity. This is similar to the effects on physical activity of etomoxir combined with the other SGAs (table 2).

Discussion

In this study, olanzapine acutely and dose dependently increased accumulation of glucose in the plasma of male

C57BL/6J mice consistent with our previous results and those of Assie et al.[16] in rats. This was associated with dose-dependent changes in fat oxidation as reflected in rapidly falling RER values, consistent with our previous studies showing that olanzapine acutely increases fat oxidation in most peripheral tissues of the rat.[13] However, in our previous studies, we also showed that olanzapine impairs lipolysis and increases lipogenesis in adipose tissue. These factors appeared to underlie lower FFA concentrations. As mentioned, switching peripheral tissues to fat oxidation while impairing FFA mobilization would seem to be an unsustainable situation from a metabolic standpoint. Our current study also supports this idea. In the current experiments, olanzapine, risperidone, and clozapine rapidly and dose dependently lowered RER values, consistent with increased fat oxidation. These compounds also affected whole-body cellular respiration as reflected in the VO_2. However, this effect occurred at higher doses than those needed to observe effects on RER, and it was possible to identify doses that did not have a significant effect on VO_2 but lowered RER. These findings are consistent with the idea that switching fat metabolism without a supporting increase in FFA rate of appearance is not sustainable from an energy-balance perspective. Aripiprazole did not affect VO_2 and had smaller effects on RER. Overall, aripiprazole has greatly reduced incidence of metabolic side effects compared with olanzapine, risperidone, and clozapine; for review, see Ref. 44. As mentioned already, the effects of olanzapine on RER and VO_2 occurred in conjunction with a dose-dependent accumulation of glucose in the plasma; dose-related increases in blood glucose have also been observed previously in rats acutely treated with olanzapine, risperidone, and clozapine, but not aripiprazole.[16]

H1-histamine receptors have been linked to some of the metabolic side effects of atypical antipsychotics.[5,34–36] However, 2 example antihistamines tested here, astemizole and terfenadine, did not cause any effects on RER or VO_2. It seems unlikely, therefore, that histamine receptors are involved in these responses.

As mentioned earlier, there are differences between the metabolic changes elicited by SGAs and metabolic syndrome. One difference concerns the glucose intolerance. In contrast to traditional glucose intolerance associated with insulin resistance, olanzapine does not appear to impair insulin-stimulated increases in skeletal muscle AKT activation during a euglycemic clamp.[13] Another difference is that although impaired lipolysis in the rodent model has been used to help explain SGA-induced adiposity,[11] it represents a sharp difference from traditional type II diabetes where FFA utilization and mobilization rise in the early stages and later. After olanzapine treatment, glycerol rates of appearance are not affected in humans,[17] and in rats, basal and isoproterenol-stimulated glycerol concentrations are lower, not higher,[13] despite increased FFA oxidation. Given these differences, it may not be productive to interpret the acute metabolic effects of SGAs using the traditional framework of diabetes pathogeny. Indeed, the metabolic unsustainability of olanzapine increasing fat oxidation while impairing intracellular lipolysis is certainly disconcerting from a physiological or diabetes pathophysiological perspective. However, these findings could explain the decrease in VO_2 we observed with higher doses of SGAs. If some SGAs are switching the major fuel in mice to fat and preventing fat mobilization, that should lead to exhaustion of plasma FFA (FFA lowering). We observed this previously with olanzapine in rodents,[11,13] and in humans FFA lowering is also observed.[10,12,17] SGAs have a very short half-life in rodents, so it is tempting to speculate that with longer exposures facilitated by higher doses, plasma concentrations of FFA may reach a critical concentration that is insufficient to maintain energy requirements leading to the observed drops in the VO_2 at higher SGA doses. It does not seem likely that the VO_2 lowering is secondary to sedation because decreased physical activity was also observed in animals treated with aripiprazole, which did not affect VO_2, and ANCOVA testing failed to reveal an interaction between these variables after any treatment. Furthermore, physical activity only constitutes approximately 15% to 30% of daily energy expenditure with resting metabolic rate comprising 60% to 75%.[45] With such a small contribution to daily energy expenditure, it is not surprising that no interaction of physical activity with VO_2 was detected.

We initially viewed the converse effects of olanzapine on fat oxidation and glucose intolerance as a potentially reversible substrate competition due to an affect that primarily increased fat oxidation.[13] Fatty acids and glucose are the major fuels for oxidative phosphorylation in skeletal muscle. Early studies in muscle demonstrated that provision of fatty acid as a fuel decreased glucose oxidation,[46] and later, the ability of FFA to diminish glucose uptake and glycolysis was demonstrated.[19] The converse relationship also exists; increasing glucose oxidation diminishes fat oxidation.[47] This relationship between glucose and fat oxidation has been termed the Randle Cycle.[19] Consistently, previous studies have shown that TDGA could increase glucose oxidation in vitro and enhance clearance or decrease production of glucose in vivo.[17,21–23] However, when we tested the ability of CPT-1 inhibitors to prevent SGA-induced shifts in fat oxidation, the results were unexpected. CPT-1 blockade, instead, synergistically and dramatically lowered VO_2 and body temperature and caused a moribund state or death when combined with olanzapine. Similar results occurred with risperidone and clozapine, but not aripiprazole. Thus, with higher doses of olanzapine, risperidone, or clozapine or with a combination of lower doses and even partial CPT-1 blockade, there does not seem to be sufficient oxidative capacity for the mice to meet their

energy demands, accounting for the falling VO_2. Our findings imply that the SGAs are not simply making the conditions ideal to promote FFA oxidation. FFAs are apparently being increasingly oxidized because the SGAs are preventing glucose from being used as a fuel, and the degree of this effect increases with dose that is consistent with that of Assie et al.[16]

Thus, the usual substrate competition countermeasures that should occur to promote glucose oxidation when fat oxidation is blocked appear to be impaired after SGA treatment. Alternatively, SGAs may be impairing a step preceding the points of this counter regulation in glucose metabolism. These usual counter-regulatory steps are thought to include pyruvate dehydrogenase and the regulation of phosphofructokinase by intracellular citrate concentrations (muscle citrate was lowered by SGA treatment in our previous study).[13] Alternatively, SGAs could interfere with glucose transport. Consistently, risperidone and clozapine were reported to decrease glucose transport in "preneuronal" PC12 and L6 muscle cells.[48–50] Similarly, risperidone, clozapine, and olanzapine decrease glucose transport in differentiated 3T3L-1 adipocytes.[51] A caveat is that subsequent studies questioned the clinical relevance of the SGA concentrations used in those in vitro experiments.[52] Furthermore, we have shown that multiple tissues with different glucose transporters have elevated FFA metabolic rates.[13] This would mean that SGAs would have to affect multiple glucose transporters to bring about these effects. Although possible, it is unlikely, and that may raise a caution flag about this idea. An alternative could be that olanzapine, risperidone, and clozapine interfere with an early step in glycolysis to bring about these effects or work through a central mechanism that impacts peripheral glucose metabolism. These are testable hypotheses we are exploring.

In conclusion, our findings indicate that the mechanism underlying the effects of SGAs on RER and VO_2 are not likely to involve H1-histamine receptors. Our findings suggest potential mechanisms for how SGAs conversely affect plasma FFA and glucose. Further studies are needed to investigate the potential sites responsible for these effects including glucose transport and early steps in glycolysis. CPT-1 is currently considered a potential therapeutic target for diabetes drug development. This seems reasonable based on previous studies on etomoxir or TDGA.[17,21–23] However, if ongoing efforts with CPT-1 inhibitors lead to new drugs in the future, our studies would seem to suggest that those taking SGAs should avoid them.

Funding

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health grant (DK084428).

Acknowledgments

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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