Antipsychotic drug-induced weight gain mediated by histamine H\textsubscript{1} receptor-linked activation of hypothalamic AMP-kinase

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The atypical antipsychotic drugs (AAPDs) have markedly enhanced the treatment of schizophrenias but their use has been hindered by the major weight gain elicited by some AAPDs. We report that orexigenic AAPDs potently and selectively activate hypothalamic AMP-kinase, an action abolished in mice with deletion of histamine H1 receptors. These findings may afford a means of developing more effective therapeutic agents and provide insight into the hypothalamic regulation of food intake.

The atypical antipsychotic drugs | obesity | hypothalamus

The antipsychotic actions of classic neuroleptics revolutionized the therapy of schizophrenia, but their use has been impeded by side effects such as extrapyramidal symptoms, tardive dyskinesia, a high incidence of nonresponders, and the failure of negative symptoms such as apathy to respond. The atypical antipsychotic drugs (AAPDs), pioneered by clozapine, represent an important advance in improving negative symptoms, benefiting patients who do not respond to the typical drugs, and displaying fewer side effects (1–5). A major limitation of AAPDs is pronounced weight gain, predominantly mediated by increased food intake (6–10). Weight gain elicited by AAPDs is primarily related to increased food intake, although there may also be metabolic alterations (11–13).

To directly address central systems that mediate appetite and weight gain, we have explored hypothalamic AMPK phosphorylation, which activates the enzyme (14, 15). In the periphery, AMPK activation is associated with decreased lipid formation, because AMPK phosphorylates acetyl-CoA carboxylase (ACC) inhibiting the generation of malonyl-CoA. Malonyl-CoA is a substrate for fatty acid synthase so that inhibition of ACC diminishes formation of fatty acids and lipid (14–16). In the hypothalamus, AMPK acts in a seemingly reciprocal fashion to regulate food intake (15, 17–19). Kahn and collaborators (20) showed that AMPK activity in the arcuate and paraventricular hypothalamic nuclei is inhibited by orexigenic agents such as leptin and augmented by the orexigenic agouti-related protein (AGRP) (20).

We now show that orexigenic AAPDs selectively and potently stimulate hypothalamic AMPK, which has been linked to the regulation of food intake (20), and reverse the actions of the orexigenic hormone leptin. This action involves the histamine H1 receptor (H1R), because clozapine augmentation of AMPK is abolished in B6.H1Rtm1Wat (H1RKO) mice, and orexigenic potencies of neuroleptics correlate with their affinities for H1R.

**Results**

In hypothalamic slices, clozapine and olanzapine markedly enhance levels of phospho-AMPK, and quetiapine, which is also orexigenic, produces similar effects (Fig. 1A). However, risperidone, ziprasidone, haloperidol, and aripiprazole, which are much less orexigenic (Table 1), fail to stimulate AMPK (Fig. 1B). Increased AMPK phosphorylation is observed as early as 5 min after treatment with clozapine or olanzapine (Fig. 1 C and D).

The drug actions are potent and substantial with EC\textsubscript{50} values for both of \(\sim 10\) nM and with 6- and 3.5-fold maximal increases, respectively, with clozapine and olanzapine (Fig. 1 E–H).

Clozapine also potently and selectively augments hypothalamic AMPK in intact animals. As little as 1 mg/kg of clozapine markedly stimulates levels of phospho-AMPK (Fig. 2A) as well as AMPK catalytic activity, with 5 mg/kg producing a 3.5-fold augmentation of activity (Fig. 2B). The increase of phospho-AMPK and AMPK catalytic activity is relatively selective for the hypothalamus, because clozapine (1 mg/kg) fails to increase phospho-AMPK levels in the cerebellum and liver [see supporting information (SI) Fig. 5.4 and B], and AMPK catalytic activity is not affected in the cerebral cortex or cerebellum by clozapine (1 mg/kg) (Fig. 2C). At 5 mg/kg, clozapine elicits a 20% increase in cortical AMPK activity, much less than the quadrupling of hypothalamic AMPK activity, whereas no increase is apparent in the cerebellum (Fig. 2D). The effect of clozapine is maximal 3 h after drug administration and gradually decreases to basal levels in 24 h (SI Fig. 5F).

Kahn and colleagues (20) reported that the anorexigenic peptide leptin reduces hypothalamic AMPK activity, which we confirm. Clozapine reverses reductions in hypothalamic phospho-AMPK elicited by leptin (Fig. 3A) and insulin (20) (SI Fig. 6). In intact mice, leptin (3 mg/kg) reduces hypothalamic phospho-AMPK (Fig. 3B) and catalytic activity (Fig. 3C), and clozapine reverses these actions.

The arcuate and paraventricular hypothalamic nuclei display the greatest alterations of AMPK activity in response to feeding stimuli (20). In immunohistochemical experiments phospho-AMPK is selectively augmented in these two nuclei with clozapine (1 and 5 mg/kg), whereas much lesser effects are evident in the cerebral cortex (SI Fig. 7A and B). By contrast, ziprasidone fails to alter phospho-AMPK in the paraventricular nucleus (SI Fig. 8).

We wondered whether the influence of AAPDs on hypothalamic AMPK is secondary to actions of the drugs on specific neuropeptide receptors that have been implicated in appetite regulation. Clozapine and olanzapine (10 and 100 nM) fail to influence ligand binding to receptors for leptin, α-MSH, and neuropeptide Y (data not shown).

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The authors declare no conflict of interest.

Abbreviations: AAPD, atypical antipsychotic drug; H1R, H1 receptor.

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Relative potencies of AAPDs in blocking H1R have been reported to correlate with their orexigenic potencies (21, 22), which we confirm (Table 1). Moreover, in hypothalamic slices, the H1R antagonist triprolidine stimulates phospho-AMPK to the same extent as clozapine both in hypothalamic slices (Fig. 3).

Table 1. Neuroleptic affinities for the H1R correlate with orexigenic actions

<table>
<thead>
<tr>
<th>Drugs</th>
<th>IC50, nM</th>
<th>Orexigenic effects</th>
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<tbody>
<tr>
<td>Clozapine</td>
<td>9</td>
<td>++++</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>13</td>
<td>+++</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>40</td>
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<tr>
<td>Risperidone</td>
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<tr>
<td>Ziprasidone</td>
<td>150</td>
<td>–</td>
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<tr>
<td>Haloperidone</td>
<td>2,000+</td>
<td>–</td>
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<tr>
<td>Aripiprazole</td>
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Receptor binding was assayed by using rat brain membranes incubated with [3H]mepyramine and 12 concentrations of drugs ranging from 30 pM to 10 μM, in triplicate. Data are means of three independent determinations that varied <10%. Orexigenic action of drugs was obtained from published literature, indicating reproducible differences among AAPDs in eliciting weight gain when administered at comparable therapeutic doses (5, 8, 14).

Fig. 2. AAPDs activate AMPK in intact animals. (A and B) Mice received clozapine (1 or 5 mg/kg) and were killed at 3 h. Hypothalami were removed and tissue lysates analyzed for phospho-AMPK or α2-AMPK activity. (C and D) Mice received clozapine (1 or 5 mg/kg) and were killed at 3 h. Various parts of brain were isolated, and α2-AMPK activity was assayed. Bars represent the mean ± SE of three independent lysates performed in triplicate. *, Student’s t test (n = 5).

Discussion
Our findings indicate that the appetite stimulation–weight gain associated with AAPDs is mediated by activation of hypothalamic AMPK linked to blockade of the histamine H1R. AMPK stimulation parallels the orexigenic actions of the drugs, with clozapine and olanzapine producing the most marked effects.
The drug actions are very potent, with substantial effects evident at 5 nM concentration. They are selective, with effects restricted largely to the arcuate and paraventricular nuclei of the hypothalamus. Orexigenic potencies of AAPDs parallel their affinities for histamine H1Rs, and stimulation by AAPDs of AMPK is lost in H1R-deleted mice. These findings are in accord with studies implicating central histamine (23, 24) and AMPK (20) in weight control as well as the orexigenic role of the paraventricular and arcuate nuclei (25, 26). Moreover, mice, like humans, manifest weight gain in response to AAPDs, although inhibition of locomotor activity sometimes impairs characterization of orexigenic actions (27).

Numerous mechanisms have been advanced to explain the orexigenic influences of AAPDs. Because therapeutic actions of the drugs have been linked to serotonin receptors, these have also been hypothesized to mediate orexigenic effects. Thus, agonists at 5HT2C receptors, such as fenfluramine and m-chlorophenylpiperazine, are anorexigenic (28), whereas mice with targeted deletion of 5HT2C receptors are obese (29). Orexigenic potencies of neuroleptics correlate significantly with affinity for 5HT2C receptors, although there are notable exceptions, such as ziprasidone, which is not orexigenic yet has high receptor affinity (30). Neuroleptics elicit antipsychotic actions by blocking dopamine D2 receptors. Although blockade in vitro of these receptors does not correlate with orexigenic potencies, positron emission tomographic studies reveal a relationship between obesity and D2 receptor occupancy in humans (31). Moreover, blocking D2 sites in the lateral hypothalamus increases feeding behavior in rodents (32). Some anorectic drugs act by augmenting synaptic levels of norepinephrine in the hypothalamus (33), and affinity of drugs for noradrenergic α2 receptors correlates with orexigenic potency (34). Although these correlations suggest some role for serotonin, norepinephrine, and dopamine in obesity, the present study establishes definitively that orexigenic AAPDs act via histamine H1 receptors and AMPK. Our findings predict that H1R-deleted mice should be resistant to the orexigenic actions of AAPDs and that H1 antihistamines should be orexigenic. In rats (35) and humans (36), H1 antihistamines have been reported to be orexigenic. Because these drugs typically are used sporadically and in substantially lower doses than AAPDs, effects on weight are less prominent for H1 antihistamines.

Weight gain elicited by AAPDs can be massive and associated with the “metabolic syndrome” leading to diabetes (5, 7, 37, 38). Thus, the orexigenic actions of AAPDs, especially olanzapine and clozapine, have precluded their use in large numbers of patients. Ignorance of the mechanism of these orexigenic actions has hindered efforts to develop alternative therapeutic agents. Evaluation of candidate drugs for influences on H1R and hypothalamic AMPK provides a straightforward approach to developing better drugs and may advance our understanding of the hypothalamic regulation of food intake.
**Methods**

**Drug Preparation.** All drugs were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Clozapine was dissolved in 0.1 M HCl (0.8 ml) and neutralized by 0.1 M NaOH (0.7 ml). The drug was diluted with 8.5 ml of saline solution, and the appropriate doses were administered to mice. For control mice, the same solution was injected without a drug. For *in vitro* assays, drugs were dissolved in DMSO.

**Hypothalamic Slices.** Hypothalami from 8- to 10-week-old mice were cut at 0.4-mm intervals in sagittal and coronal planes by using a McIlwain tissue chopper. The slices were dispersed in artificial cerebrospinal fluid buffer.

**Immunohistochemistry.** Phospho-AMPK immunohistochemistry was performed as described (39, 40), and all solutions before and including the primary antibody incubation contained 2 mM sodium fluoride. C57BL/6 mice or B6.129P-HHim1Iwar (H1R knockout) mice (8–10 weeks of age) were perfused with 4% paraformaldehyde maintained at 37°C. Organs were postfixed for 2 h at room temperature and cryoprotected overnight at 4°C (30% sucrose in PBS). Free-floating sections (45 μm) were quenched with 3% H2O2 in water for 10 min at room temperature, washed in TBST (16 mM Tris, pH 7.4/140 mM sodium chloride/0.1% Tween 20), and antigen retrieved for 30 min in a 70°C water bath (10 mM sodium citrate in TBST-T). Sections were blocked (5% NGS in TBST-T) for 1 h at room temperature and incubated with a mouse anti-phospho-AMPKα antibody (Cell Signaling Technologies, Danvers, MA) diluted 1:200 into the blocking solution overnight at 4°C. Subsequent washes were conducted in TBST, and labeling was visualized with the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). Images were quantified with the AlphaEaseFC program.

**Effect of Neuroleptics on H1R Binding.** The IC50 values of neuroleptics on H1R were determined as described (41). Briefly, rats were killed by decapitation and the forebrains removed. Brains were homogenized in 30 vol of Na-K phosphate buffer, pH 7.5, and centrifugated at 48,000 × g for 10 min. The tissue was resuspended in buffer and centrifuged an additional three times. Tissue was resuspended in buffer at 15 mg/ml.

Tissue (0.2 ml) was added to tubes containing 25 μl of drug and 25 μl of [3H]mepyramine (30 nM). Nonspecific binding was determined in the presence of 1 μM tripolidine. Tubes were incubated for 1 h at 25°C, and the samples were filtered over 0.5% poly(ethyleneimine)-coated filters washed with 2 × 5 ml of cold 50 mM NaCl.

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**References**