Histamine H3 receptors (H3Rs) are located on the presynaptic membranes and cell soma of histamine neurons, where they negatively regulate the synthesis and release of histamine. In addition, H3Rs are also located on nonhistaminergic neurons, acting as heteroreceptors to regulate the releases of other amines such as dopamine, serotonin, and norepinephrine. The present study investigated the effects of H3 ligands on appetite and body-weight regulation by using WT and H3R-deficient mice (H3RKO), because brain histamine plays a pivotal role in energy homeostasis. The results showed that thioperamide, an H3R inverse agonist, increases, whereas imetit, an H3 agonist, decreases appetite and body weight in diet-induced obese (DIO) WT mice. Moreover, in DIO WT mice, but not in DIO H3RKO mice, imetit reduced fat mass, plasma concentrations of leptin and insulin, and hepatic triglyceride content. The anorexigenic effects of imetit were associated with a reduction in histamine release, but a comparable reduction in histamine release with α-fluoromethylhistidine, an inhibitor of histamine synthesis, increased appetite. Moreover, the anorexigenic effects of imetit were independent of the melanocortin system, because imetit comparably reduced appetite in melanocortin 3 and 4 receptor-deficient mice. The results provide roles of H3Rs in energy homeostasis and suggest a therapeutic potential for H3R agonists in the treatment of obesity and diabetes mellitus.

autoreceptor | heteroreceptor | appetite regulation

Obesity has recently become a matter of great concern, because it is considered one of the highest risk factors for diabetes mellitus, hyperlipidemia, and arteriosclerosis. To maintain body weight, caloric intake and energy expenditure must be balanced, and excessive caloric intake is a leading cause of obesity (1). There is growing evidence that the brain receives and integrates information related to energy status from peripheral tissues and that appetite is under the control of numerous neurotransmitters and hormones, such as neuropeptide Y, melanocortin, leptin, and ghrelin (2, 3).

Histamine is a classical inflammatory mediator in peripheral tissues and also functions as a neurotransmitter in the brain. Histamine plays a pivotal role in various physiological functions, such as feeding behavior and energy homeostasis (4). Intracerebroventricular administration of histamine consistently decreases appetite in several species (4). Mice with genes disrupted for histamine H1 receptor or histidine decarboxylase (HDC), a rate-limiting enzyme for histamine synthesis, are prone to becoming obese on a high-fat diet or at advanced age (5–8). Furthermore, several antipsychotic drugs with high affinities for H1 receptors are known to cause weight gain in rodents and humans (9). These results suggest a significant role for histamine and H1 receptors in feeding behavior and body-weight regulation.

Histamine H3 receptors (H3Rs) were pharmacologically identified more than a decade ago and recently cloned (10, 11). They are predominantly expressed in the brain, where they negatively regulate histamine release, acting as presynaptic autoreceptors (10). Therefore, the therapeutic potential of H3R antagonists/ inverse agonists for treating obesity has been extensively discussed (12, 13). Our previous study using H3R-deficient (H3RKO) mice demonstrated a crucial role for H3Rs in appetite and body-weight regulation (14). However, enhanced histamine release was accompanied by obese and hyperphagia phenotypes in H3RKO mice. Moreover, the effects of pharmacological blockade of H3Rs on appetite and energy homeostasis have remained controversial (6, 13, 15–19).

In this study, we address the role of H3Rs in appetite and body-weight regulation using selective pharmacological tools and H3RKO mice. This study demonstrates that activation of H3Rs in mice decreases food intake and increases energy expenditure. Furthermore, chronic dosing with an H3 agonist reduces body weight, fat mass, hyperleptinemia, and hyperinsulinemia in diet-induced obese (DIO) mice.

Results

Chronic Dosing with Imetit but Not with Thioperamide Reduced Body Weight and Ameliorated Hyperinsulinemia and Hyperleptinemia in DIO Mice. We investigated whether chronic dosing with thioperamide, an H3R inverse agonist (20), or imetit, an H3 agonist (21), could modulate body weight in mice. Surprisingly, twice daily dosing with thioperamide did not decrease but, rather, increased food intake and body weight in mice (Fig. 1). The weight gain was slight but statistically significant. We next addressed the effects of chronic dosing with imetit on food intake and body-weight regulation in DIO mice. Imetit significantly decreased food intake and body weight in DIO WT mice (Fig. 2A and C). The body-weight reduction of the imetit-treated group was sustained during the experiment, even though the reduction in food intake disappeared by the end of the experiment. The food- and weight-reducing effects of imetit were negligible in DIO H3RKO mice (Fig. 2B and D), demonstrating that the weight loss was mediated by H3Rs. The fat mass and plasma concentration of leptin were also significantly decreased in the imetit-treated DIO WT mice but not in the imetit-treated DIO H3RKO mice at day 10 (Table 1). Moreover, the plasma concentration of insulin decreased in the imetit-treated DIO WT mice, suggesting that insulin resistance was ameliorated. Further biochemical analysis of the imetit-treated DIO WT mice showed that hepatic triglyceride content was decreased but that plasma

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Abbreviations: H3R, histamine H3 receptor; α-FMH, α-fluoromethylhistidine; DIO, diet-induced obese; HDC, histidine decarboxylase; M3/4R, melanocortin 3 and 4 receptor; t-MH, tele-methylhistamine; RQ, respiratory quotient.

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concentrations of triglyceride, free fatty acid, cholesterol, and hepatic cholesterol content were not changed (Table 2). Intracerebroventricular administration of Rα-methylhistamine, another H3 agonist, also significantly reduced food intake and body-weight gain in DiO C57BL/6J mice (Fig. 9, which is published as supporting information on the PNAS web site). These results clearly indicate a role for H3Rs in the maintenance of body weight and provide evidence that H3R agonists, but not H3R inverse agonists, reduce adiposity in mice.

Regulation of Food Intake and Energy Expenditure by Imetit. The above findings were surprising; however, they may explain the obese phenotypes observed in H3RKO mice (14). To address the antiobesity effects of imetit more precisely, we examined the acute phenotypes observed in H3RKO mice (14). To address the antiobesity effects of imetit, we measured histamine release. Histamine is synthesized from histidine by the rate-limiting enzyme histidine decarboxylase, which is also the rate-limiting enzyme in the synthesis of dopamine. These results clearly indicate a role for H3Rs in the maintenance of body weight and provide evidence that H3R agonists, but not H3R inverse agonists, reduce adiposity in mice.

Table 1. Effects of chronic dosing with imetit in DiO mice on body composition and the plasma concentrations of glucose, insulin, and leptin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fat, %</th>
<th>Lean, %</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
<th>Leptin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT Vehicle</td>
<td>45.9 ± 0.9</td>
<td>53.2 ± 0.9</td>
<td>169.0 ± 9.1</td>
<td>7.5 ± 0.9</td>
<td>148.4 ± 21.5</td>
</tr>
<tr>
<td>Imetit</td>
<td>39.2 ± 2.0*</td>
<td>58.3 ± 1.6</td>
<td>1641.1 ± 6.4</td>
<td>4.7 ± 0.8*</td>
<td>99.2 ± 7.2*</td>
</tr>
<tr>
<td>H3RKO Vehicle</td>
<td>47.6 ± 2.0</td>
<td>51.4 ± 1.5</td>
<td>160.9 ± 9.7</td>
<td>6.0 ± 1.5</td>
<td>99.0 ± 7.0</td>
</tr>
<tr>
<td>Imetit</td>
<td>46.3 ± 2.0</td>
<td>52.7 ± 1.4</td>
<td>166.7 ± 10.5</td>
<td>4.7 ± 0.9</td>
<td>102.8 ± 11.3</td>
</tr>
</tbody>
</table>

*Significance of drug effects was determined by Student’s t test. *P < 0.05 vs. vehicle.

Imetit Decreased Food Intake Independently of Histaminergic Tone Modulation. The anorexigenic effects of imetit and the orexigenic effects of thioperamide were also observed and were even more significant when administered in the cerebroventricle in WT mice, but not in H3RKO mice (Fig. 3C), indicating that brain H3Rs are responsible for the anorexigenic effects of imetit. Collectively, these results indicate that the effects of these drugs on food intake are highly dependent on H3Rs, although they show moderate affinity for receptors other than H3Rs (12). Oral dosing with imetit did not elicit conditioned taste aversion (Fig. 4) and did not affect gross behavior and locomotor activity in either WT or H3RKO mice (activity count in WT/H3RKO mice: vehicle, 2009 ± 467/2023 ± 278; 5 mg/kg of body weight, 2400 ± 620/2523 ± 536; 20 mg/kg body weight, 1587 ± 251/1953 ± 429; P > 0.05 vs. respective vehicle control). An indirect calorimetry study indicated that imetit significantly increased O2 consumption (VO2) and reduced respiratory quotient (RQ) (Fig. 5). Because VO2 reflects energy expenditure, and a reduction in RQ represents a shift of energy source from carbohydrate to lipid, these results suggest that imetit increases energy expenditure and uses more lipid as an energy source. Collectively, these data suggest that imetit reduces adiposity not only by decreasing appetite but also by increasing energy expenditure and lipid use.

Table 2. Effects of chronic dosing with imetit in DiO WT mice on the plasma concentrations of triglycerides, free fatty acids, and cholesterol and hepatic triglyceride and cholesterol content

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>TG, mg/dl</td>
</tr>
<tr>
<td>Vehicle</td>
<td>21.6 ± 1.7</td>
</tr>
<tr>
<td>Imetit</td>
<td>27.2 ± 3.8</td>
</tr>
</tbody>
</table>

*Significance of the drug effects was determined by Student’s t test. *P < 0.05 vs. vehicle. TG, triglycerides; FFA, free fatty acids; Cho, cholesterol.
by HDC, released after the depolarization of nerve endings, and metabolized by histamine N-\textgamma-\textbeta-methyltransferase to tele-methylhistamine (t-MH), a major metabolite of histamine in the brain. Hence, the level of t-MH is used as an index of released histamine (22). In this study, oral administration of imetit increased histamine and decreased t-MH (Fig. 6 A and B), indicating that histamine release was inhibited and that consequently histamine was accumulated in nerve terminals. Next, we manipulated histaminergic tone by inhibiting HDC using \(\alpha\)-Fluoromethylhistidine (\(\alpha\)-FMH), an irreversible inhibitor of HDC (23). This treatment almost halved the brain content of histamine, indicating that neuronal histamine is almost completely depleted (because half of the brain histamine is stored in nonneuronal cells that are resistant to \(\alpha\)-FMH) (24). Accordingly, t-MH was significantly decreased after the \(\alpha\)-FMH treatment (Fig. 6 A and B), further indicating that histamine release was reduced. The reduction in t-MH was comparable between these treatments (imetit, 62%; \(\alpha\)-FMH, 54% of vehicle level), indicating that they comparably inhibit histamine release. Moreover, simultaneous administration of imetit and \(\alpha\)-FMH did not reduce t-MH any further when compared with either compound alone (Fig. 6B). Collectively, these results suggest that imetit and \(\alpha\)-FMH comparably and almost completely inhibit histamine release from the same source, i.e., neuronal cells. In contrast, we observed a significant difference in the effects of these compounds on appetite; whereas imetit reproducibly decreased nocturnal food intake, \(\alpha\)-FMH did not reduce food intake (in 4 h, Fig. 6C) but significantly increased 12-h food intake (vehicle, 2.6 \pm 0.1 g; \(\alpha\)-FMH, 3.5 \pm 0.1 g; \(P < 0.05, n = 11–13\) mice). Moreover, \(\alpha\)-FMH did not influence the anorexigenic effects of imetit (Fig. 6C). These data suggest that imetit decreases appetite independently of histaminergic tone modulation.

**Effects of Imetit on Other Catecholamines.** Multiple lines of evidence have shown that H3Rs regulate the releases of not only histamine but also other amines, such as norepinephrine, dopamine, and serotonin (4). Because theses amines also regulate appetite, we addressed the effects of imetit (20 mg/kg of body weight) on these amines and metabolites in C57BL/6J mice. The results showed that imetit significantly increased homovanillic acid (a dopamine metabolite) and decreased normethanephrine (a norepinephrine metabolite) (Table 3). In contrast, \(\alpha\)-FMH had no effect over these amines and metabolites, suggesting that the reduction of histamine release does not contribute to the observed changes with imetit. In addition, imetit did not change either of the substances when administered in H3RKO mice. These results suggest that imetit might regulate appetite by regulating the release of dopamine and/or norepinephrine.

**H3R and the Melanocortin Systems Regulate Appetite Independently.** The melanocortin system plays an important role in the regulation of appetite and body weight (25). Evidence has shown that it mediates several important signals such as leptin, insulin, ghrelin, serotonin, and cholecystokinin (25–27). Therefore, we addressed the relationship of H3R and the melanocortin system using melanocortin 3 and 4 receptor (MC3/4R)KO mice. As reported (28), MC3/4RKO mice are hyperphagic and severely obese compared with WT mice (WT, 19.5 \pm 0.3 g; MC3/4RKO, 49.0 \pm 0.9 g; \(n = 22–26; P < 0.05, \text{Student’s } t \text{ test} \)). The anorexigenic effects of imetit, however, were almost comparable between WT mice and MC3/4RKO mice when compared relative to respective vehicle control (Fig. 7A; 5 mg/kg of body weight; WT, 82 \pm 6\% MC3/4RKO, 85 \pm 5\% 20 mg/kg of body weight; WT, 65 \pm 4\%; MC3/4RKO, 64 \pm 6\%, \(P < 0.05, \text{Student’s } t \text{ test} \)). Moreover, MTII, an MC3/4R agonist (29), comparably reduced food intake in WT and H3RKO mice (Fig. 7B). These results indicate that H3R and the melanocortin system regulate appetite independently.

**Discussion**

Energy homeostasis is a complex process composed of multiple interacting homeostatic and behavioral pathways, including sa-
tiety, cognitive and motivational systems, glucose and lipid homeostasis, and the activity of the hypothalamic–adrenal–pituitary axis (2, 3, 30). Recent studies using genetically engineered mice have provided enormous insight into energy homeostasis, and a number of new drug targets for controlling body weight have been identified. However, some transgenic mice, including H3RKO mice, have failed to display the phenotypes expected from the previous pharmacological studies (31).

This study demonstrates an antiobesity role for H3R agonists and provides a mechanism whereby H3Rs regulate appetite and body weight. Imetit reduced adiposity in DIO mice by inhibiting appetite and increasing energy expenditure and lipid use. The antiobese effects of the H3 agonist were also confirmed by using a different H3 agonist, R-α-methylhistamine, indicating that the antiobese properties are not specific to imetit. In contrast, a slight but significant increase in body weight in lean mice and a trend of weight gain in DIO mice were observed after chronic dosing with thioperamide (Fig. 10, which is published as supporting information on the PNAS web site). In addition, the obese phenotypes of H3RKO mice also suggest an antiobesity role for H3R agonists. The results presented here are surprising, because H3R inverse agonists have been considered an attractive mechanism to treat obesity (12, 13) (discussed below). In addition to the weight-reducing effect, imetit significantly decreased the plasma concentration of insulin and hepatic triglyceride content. Recent evidence has shown that excessive accumulation of triglyceride in nonadipose tissues, such as liver and skeletal muscle, may be causative in insulin resistance and subsequent emergence of diabetes mellitus (32). Thus, it is likely that the enhanced lipid use, as well as the anorexigenic effects of imetit, led to a significant improvement in insulin resistance. These results indicate that H3R agonists might be beneficial for the treatment of obesity and diabetes mellitus.

Elevation of histamine levels in the brain, by either direct administration of histamine or systemic administration of its precursor, histidine, results in appetite suppression, and a number of pharmacological studies have suggested the involvement of H1 receptors (5, 8, 13, 15–18). This finding was confirmed by this study, which shows that inhibition of histamine release by α-FMH increased appetite slightly at 4 h and significantly at 12 h. In contrast, comparable inhibition of histamine release by imetit resulted in appetite suppression, suggesting that imetit decreases appetite independently of histaminergic tone modulation. Moreover, H3RKO mice are hyperphagic and obese from a young age, which is paradoxically accompanied by enhanced histamine release (14). The hyperphagia was exclusively observed in the nocturnal period, which differs from the phenotypes observed in H1RKO mice, late-onset obesity and hyperphagia only in the diurnal period (8). These observations further indicate that compensatory changes in H1 receptors observed in H3RKO mice (14) are not fundamental causes for hyperphagia. Collectively, it is highly likely that the predominant role of H3Rs in the regulation of appetite is independent of either their modulation of histaminergic tone or subsequent changes in the activity of H1 receptors.

There is no doubt that histamine plays a crucial role in sleep–wake control. The genetic disruption or pharmacological inhibition of HDC in mice results in a deficit in controlling wakefulness (33). Moreover, several H3R agonists, including imetit, although still controversial, increase slow-wave sleep by inhibiting histamine release (34–36). Thus, it might be argued that the anorexigenic effects of imetit might be due to this hypnotic action. However, this is unlikely, because imetit did not significantly reduce locomotor activity compared with vehicle, and comparable inhibition of histamine release with α-FMH resulted in hyperphagia. Moreover, imetit increased energy expenditure and lipid use. Therefore, we conclude that the hypnotic action, if any, is not a primary cause of the anorexigenic effects of imetit.

Although H3Rs are known to act as presynaptic autoreceptors, they are also located on nonhistaminergic terminals, acting as heteroreceptors to regulate the release of other neurotrans-

Table 3. Effects of imetit or α-FMH on the brain levels of neurochemicals in C57BL/6J and H3RKO mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
<th>5-HT</th>
<th>5-HIAA</th>
<th>NE</th>
<th>NM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1833 ± 41</td>
<td>81.6 ± 0.9</td>
<td>278 ± 13</td>
<td>894 ± 31</td>
<td>301 ± 15</td>
<td>810 ± 13</td>
<td>20.8 ± 1.0</td>
</tr>
<tr>
<td>Imetit</td>
<td>1989 ± 70</td>
<td>81.3 ± 3.6</td>
<td>313 ± 5*</td>
<td>940 ± 38</td>
<td>329 ± 22</td>
<td>795 ± 17</td>
<td>16.7 ± 0.2*</td>
</tr>
<tr>
<td>α-FMH</td>
<td>1878 ± 28</td>
<td>86.3 ± 4.7</td>
<td>272 ± 8</td>
<td>905 ± 21</td>
<td>326 ± 12</td>
<td>840 ± 16</td>
<td>18.1 ± 1.3</td>
</tr>
<tr>
<td>H3RKO mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1021 ± 20</td>
<td>17.7 ± 1.9</td>
<td>116 ± 3</td>
<td>845 ± 6</td>
<td>398 ± 14</td>
<td>692 ± 29</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>Imetit</td>
<td>994 ± 26</td>
<td>19.6 ± 0.4</td>
<td>116 ± 3</td>
<td>845 ± 12</td>
<td>403 ± 23</td>
<td>752 ± 47</td>
<td>9.2 ± 1.1</td>
</tr>
</tbody>
</table>

Dosing was at 20 mg/kg of body weight. The amount of amines is expressed in nanograms per gram of tissue. Significance of the drug effects was determined by ANOVA, followed by Dunnett’s test (C57BL/6J mice) or Student’s t test (H3RKO mice). *P < 0.05 vs. vehicle. DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; NE, norepinephrine; NM, normethanephrine.
producers stimulate food intake in rats reported from five groups (13, 15–18, 37), no effect from thioperamide and other H3R inverse agonists on appetite and energy homeostasis remain controversial. The reported results vary considerably depending on experimental paradigm, administration route, or strains/species used. These results include anorexigenic effects on spontaneous food intake in rats reported from five groups (13, 15–18, 37), no effect from two groups (19, 38), and orexigenic effects from one group (39). Eating is very sensitive to malaise, stress, pain, and other normal behaviors, which can disrupt normal appetite. Because knockout mice are powerful tools for validating the in vivo selectivity of compounds (40), we carefully addressed feeding behavior and energy expenditure of H3R ligands in WT and H3RKO mice. We also observed that intracerebroventricular administration of thioperamide increased, whereas that of imetit is related food intake in rodents independently from its modulation of histaminergic tone. Furthermore, chronic administration of an H3R agonist reduced adiposity and ameliorated hyperinsulinemia and hyperleptinemia in DiO mice. These results suggest the therapeutic potential of H3R agonists in the treatment of obesity and diabetes mellitus.

Materials and Methods

Animals. H3RKO mice were generated as reported (14) and backcrossed for five generations (N5) into a C57BL/6J background. Male littermate mice were used for all acute dosing studies with H3RKO mice (6–9 months of age). Mice obtained from intracolony crossing of H3RKO or WT parents (N5 on C57BL/6J) were used for the chronic dosing study (18–20 months of age). MC3/4RKO mice were generated as reported (28), and female MC3/4RKO mice (3–4 months of age) and age-matched control C57BL/6J female mice (The Jackson Laboratory, Bar Harbor, ME) were used. In other studies, male C57BL/6J mice (2–4 months of age) were purchased from CLEA Japan (Tokyo, Japan). All animals were individually housed at 25°C with 12 h light–dark cycles (0700–1900 hours light cycle) with ad libitum access to water and regular chow for lean mice and rats (CE2; CLEA Japan) or medium-high-fat diet for DiO mice (Oriental BioService, Kanto, Japan). Animals were fully acclimatized to handling before experiments to minimize stress. All experimental procedures followed the Japanese Pharmacological Society Guideline of Animal Use.

Compounds. Imetit was purchased from Tocris Cookson (Ellisville, MO), and thioperamide and α-FMH were from Sigma–Aldrich (St. Louis, MO). Thioperamide and imetit were dissolved in 0.5% methylcellulose when orally administered (5 ml/kg of body weight) and in PBS containing 0.5% BSA when intracerebroventricularly administered (1 μl per head). α-FMH was dissolved in saline and i.p. administered at 10 ml/kg of body weight.

Measurement of Food Intake. Imetit or thioperamide was administered to animals between 1700 and 1800 hours, and α-FMH was administered 1 h before imetit dosing. Food intake was measured by weighing food at 1900 and 2300 hours, and the differences in weight were assumed to be the amount of consumed food.

Antiobesity Studies. Mice were classified into subgroups with matched average body weight and daily food intake before compound dosing. Body weight and food intake were measured daily. In the thioperamide study, C57BL/6J lean mice were dosed with vehicle or thioperamide twice daily (5 ml/kg). In the imetit study, WT or H3RKO DiO mice fed a medium-high-fat diet were dosed with vehicle or imetit twice daily. On day 10, mice were fasted for 2 h, and blood was collected from the orbital cavity for measurements of glucose, leptin, and insulin. Plasmas for measurements of triglyceride, total cholesterol, and free fatty acid (FFA) were collected from the heart under anesthesia with isoflurane (4%). Total lipids were extracted from 50 ml of liver homogenate (41) and dried under nitrogen gas. Measurements for each plasma and liver parameter were performed by using commercially available kits for glucose, triglycerides, and cholesterol (Kyowa Medex, Tokyo, Japan), leptin and insulin (Morinaga, Tokyo, Japan), and FFA (Wako Pure Chemical Industries, Osaka, Japan). Fat and lean mass were measured by using an NMR analyzer (Minispec; Bruker, Billerica, MA) at the end of experiment.

Surgery. A stainless guide cannula was stereotaxically implanted into the lateral ventricle in mice. Stereotaxic coordinates were...
0.4 mm posterior to the bregma, 0.8 mm lateral to the midline, and 2.0 mm from the surface of the skull.

**Conditioned Taste Aversion.** Mice were subjected to a conditioned taste-aversion test according to a previous report (42). Thirteen mice were given 1-h access to water at the same time each day until their intakes became constant. On training day 1, each mouse was given 1-h access to either one of two novel flavors in two bottles (20% sucrose flavored with grape or cherry Kool-Aid (Kraft, Toronto, ON, Canada), with 6 or 7 of each group receiving each flavor) instead of water. Immediately after access to the flavor, each mouse received an injection of either 0.15 M LiCl or saline (2% of body weight). On the next day, the mice had 1-h access to water, followed by saline injection. On training day 3, each mouse was given 1-h access to alternate flavor in two bottles (mice that had received grape flavor on day 1 received cherry, and mice that had received cherry flavor on day 1 received grape) and received alternate injection (mice that had received LiCl on day 1 received saline, and mice that had received saline on day 1 received LiCl). Each mouse received an identical flavor/LiCl-saline sequence on days 5 and 7 and were given 1-h access to water on days 5 and 7. Hence, each mouse experienced 2 days of exposure to each flavor and paired injection, so that consumption of one of the flavors was always associated with LiCl and that of the other was always associated with saline in each mouse. On day 9, mice were given 1-h access to both flavors (each in one bottle), and the intake of each flavor was measured for 1 h. Imetit and 0.5% methylcellulose were administered orally instead of LiCl and saline, respectively.

Student’s r test indicated a significant effect of LiCl on the consumption of Kool-Aid intake ($P < 0.05$ vs. the intake of saline-paired flavor).

**Measurement of Energy Expenditure.** Energy expenditure was measured by using indirect calorimetry (Columbus Instruments, Columbus, OH). In brief, C57BL/6J lean mice were acclimatized to the test chambers for 3 days, and O$_2$ consumption (VO$_2$) and CO$_2$ production (VCO$_2$) were measured for 1 min at 10-min intervals, with reference air collected every 10 min. The RQ was obtained by dividing VCO$_2$ by VO$_2$. Data were averaged between 2 and 8 h after dosing. Experiments were conducted between 0900 and 1700 hours. Mice were allowed to eat and drink freely during the experiments.

**Locomotor Activity.** C57BL/6J lean mice were dosed with imetit between 1700 and 1800 hours, and locomotor activity was measured by using an infrared radiation sensor between 1900 and 2300 hours (Neuroscience, Tokyo, Japan). Mice were acclimatized to the test cages >4 h before the experiment, with ad libitum access to food and water.

**Measurement of Monoamines.** Mice were dosed with compounds between 0900 and 1400 hours and killed by decapitation at 4 h after administration. Brains were quickly frozen in liquid nitrogen, and histamine, t-MH, and other neurotransmitters were measured as reported (43, 44).

**Statistics.** Unpaired Student’s t test or ANOVA followed by Dunnett’s test was used to compare two or more than two groups against vehicle control. $P < 0.05$ was considered significant.

We thank Satoshi Mashiko, Akira Gomori, Hiroko Matsushita, Ryuichi Moriya, Makoto Ito, and Yuki Takahashi for technical assistance.