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Central H3R activation by thioperamide does not affect energy balance

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Abstract

The central histamine 3 receptor (H3R) is a presynaptic autoreceptor that regulates neuronal release and synthesis of histamine, and is thought to play a key role in controlling numerous central nervous system (CNS)-mediated parameters, including energy homeostasis. Thioperamide, the prototypical selective H3R antagonist, was used to examine the role that H3R plays in regulating energy balance in vivo. Thioperamide was administered either intraperitoneally or orally to rats and the pharmacokinetic parameters were examined along with central H3R binding and histaminergic system activation. Food intake and metabolic parameters of either route of thioperamide administration were likewise examined. In a dose-dependent manner, both the intraperitoneal and oral route of administration resulted in similar ex vivo binding curves and tele-methylhistamine dose–response curves despite the route of administration. However, only intraperitoneal administration of 30 mg/kg thioperamide resulted in a significant decrease in 24-h food intake (60% lower than control) and respiratory quotient (RQ), while the oral route of delivery did not. Moreover, the decrease in RQ with the 30 mg/kg ip administration also decreased energy expenditure (EE) thus resulting in an unchanged energy balance. The decrease in food intake and EE was coupled with a conditioned taste aversion with the 30-mg/kg ip administration. These data indicate that the activation of the central H3R system by thioperamide does not play a direct role in decreasing food intake or altering energy homeostasis.

Keywords: Histamine 3 receptor; Thioperamide; Respiratory quotient; Energy expenditure; Tele-methylhistamine

1. Introduction

Although histamine was discovered over 80 years ago, much remains to be learned about the biology of systems modulated by this monoamine and the family of receptors it binds. Four histamine receptor subtypes (H1R, H2R, H3R and H4R) have been identified to date, with peripheral H1R being recognized as mediating the classical inflammatory responses of histamine and H2R playing a primary role in control of gastric acid secretion. However, histamine has become recognized as an important neurotransmitter in the last decade. Recent evidence has shown that modulation of the central histamine levels or central H1R (Orthen-Gambill, 1988; Sakata et al., 1988, 1991, 1997; Doi et al., 1994; Masaki et al., 2001) can affect a number of behavioral and physiological pathways, including energy homeostasis. The ability of leptin to inhibit food intake may be regulated through the histaminergic system (Yoshimatsu et al., 1993, 1999; Morimoto et al., 1999, 2000). All these previous data strongly suggest a role for the central histaminergic system in regulating energy homeostasis.

Histamine release and levels in the brain appear to be primarily regulated by the newly discovered histamine 3 receptor (H3R), suggesting it as the primary receptor involved in controlling numerous central nervous system (CNS)-mediated parameters by histamine, including energy homeostasis (Arrang et al., 1983; Werf et al., 1987). The pharmacological characterization (Arrang et al., 1983), cloning (Lovenberg et al., 1999) and knockout (Takahashi et al., 2002; Toyota et al., 2002) of the primarily centrally located H3R histamine receptor has led to a limited understanding of its function in the brain as a presynaptic auto- and heteroreceptor regulating the release of histamine as well as other monoamines (Oishi et al., 1990; Lovenberg et al., 1999). The H3R antagonist thioperamide has been reported to increase brain histamine levels (Garbarg et al., 1989a,b; Taylor et al., 1992; Jansen et al., 1998) and decreases food intake when administered centrally (Sakata et al., 1991, 1997; Ookuma et al., 1993; Naruse et al., 1995;

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Itoh et al., 1998, 1999) and interperitoneally (Mollet et al., 2001). This effect can be blocked with the H3R agonists Imetit and Immepip (Merali and Banks, 1994). Although this could suggest a central role for H3R in energy utilization and that H3R antagonists would be useful agents in controlling body weight, the recently identified histamine H4R confounds the issue because thioperamide appears to also be an antagonist at this receptor (Nakamura et al., 2000). Likewise, the recently published paper on the H3R knockout (KO) animals resulting in an obese phenotype indicates that the H3R may not play a critical role in creating a negative energy balance (Toyota et al., 2002).

In this manuscript, we examined the pharmacokinetics, ex vivo binding and turnover of central histamine (tele-methylhistamine) and compared it to food intake and other metabolic parameters in rats when thioperamide was administered by two different routes. We observed from these experiments that there is no correlation between thioperamide brain levels, ex vivo binding to cerebral H3R receptors or central histaminergic activation (tele-methylhistamine) with a decrease in food intake or alteration in energy balance. These experiments suggest that thioperamide does not alter energy balance through a central H3R-mediated mechanism and brings into question the hypothesis that H3R antagonists may be useful in the treatment of obesity.

2. Materials and methods

2.1. Animals

For all studies, male Long–Evans rats (Harlan Sprague–Dawley, Indianapolis, IN) that have been raised from weaning on a Teklad (TD 95217)-adjusted fat diet were used. Weight ranges were from 450 to 520 g. Animals were maintained on a 12-h light/dark photoperiod (10 a.m. lights off, 10 p.m. lights on). The animals were housed individually throughout the study at 74 °F ambient temperature, had free access to the TD diet and had free access to water (unless otherwise noted). Rats were well acclimated to having vehicle administered orally (10% acacia) or intraperitoneally (0.9% saline) 2–3 days in advance of the test day. All animal protocols used in the study were in accordance with the NIH *Guide for Care and Use of Laboratory Animals* and approved by the Lilly Research Labs Institutional Animal Care and Use Committee.

2.2. Pharmacokinetic studies

The pharmacokinetic profile of thioperamide (Tocris Cookson, Ballwin, MO) was measured following a single intraperitoneal and oral dose. Rats ($n = 4$/time group) were administered a single 10- and 30-mg/kg ip or po dose of thioperamide dissolved in vehicle. Blood samples were collected serially via retro-orbital bleeding in separate groups of rats at 1 and 2, 4 and 6, and 8 and 24 h. The blood samples were collected in heparinized tubes and centrifuged. Brains were collected at the 2-, 6- and 24-h time points. Prior to removal of the brain, the animals were perfused with saline to remove residual blood. Plasma and brain samples were frozen at −20 °C until analysis. Each plasma sample was extracted with acetonitrile followed by centrifugation. An aliquot was transferred and analyzed by LC/MS/MS to determine the concentration of thioperamide. The standard curve range was 2–5000 ng/ml. Acetonitrile was added to each brain in a volume equal to twice the mass of each brain. The samples were then homogenized, centrifuged and an aliquot was transferred for analysis. The standard curve range was 2–5000 ng/g.

2.3. Ex vivo binding and tele-methylhistamine

In the first experiment, a dose response of thioperamide was generated with both intraperitoneal and oral administration (0, 3, 10 and 30 mg/kg, $n = 3$/dose) at 1.5 h in fed rats to examine both central penetration (ex vivo binding) and histamine release/turnover (tele-methylhistamine). In the second experiment, a time-course response (1.5 and 6 h) for both parameters was examined using the highest dose of thioperamide (30 mg/kg, $n = 3$/time point) in fed rats. For both experiments, 30 min before lights off, animals were administered compound (orally or intraperitoneally) or vehicle for control. At the appropriate time after administration of thioperamide, animals were rapidly euthanized with CO_2, decapitated and brains were removed. Both hemispheres of the cerebral cortex were then isolated and frozen immediately on dry ice. The cerebral cortex was kept on dry ice and processed immediately or stored at −70 °C if not immediately processed. Ex vivo binding was performed according to Taylor et al. (1992). Briefly, the tissue was homogenized in 7.5 volumes (v/w) of ice-cold HEPES buffer. Binding of [3H](*R*)-alpha-methylhistamine (0.8 nM, Amersham Biosciences, Piscataway, NJ) to the brain homogenates was determined in duplicate at 25 °C in HEPES buffer with a total incubation volume of 1 ml. Incubations were started by the addition of 100 μl of homogenate suspension. Specific binding was defined as the difference between total binding and binding in the presence of a specific H3R antagonist. With this procedure, no binding of thioperamide to H4R in the brain can be detected. This was determined with a Lilly proprietary H3R antagonist with no activity on the H4 receptor as determined by in vitro competitive binding assays with 6 nM of [3H]-histamine and HEK293 cell membranes expressing the cloned human H4 receptor. After 60 min, the incubations were filtered through Whatman GF/B filters presoaked in 0.1% polyethylenimine using a Millipore filtration apparatus. The filters were washed three times with 4 ml of buffer and the amount of radioactivity bound to the filters was measured in a Beckman scintillation counter. Specific inhibition of H3R ex vivo binding was expressed as a percent of that in saline-injected animals. To determine the effect of thioperamide on cerebral cortex

histamine release/turnover, tele-methylhistamine levels were measured via radioimmunoassay (RIA) methods in the other cerebral hemisphere. It has been well documented that an increase in histamine release from thioperamide results in an increase in tele-methylhistamine, and is a useful tool to understand changes in histamine release (Barnes et al., 2001). The hemisphere was homogenized in ice-cold 0.4 N perchloric acid (5 ml/g of tissue). The suspension was then centrifuged (3K rpm, 4 °C) and the supernatant was decanted off the pellet. An aliquot of the supernatant was then neutralized in five volumes of phosphate-buffered saline (v/v) and frozen at −70 °C until assayed. Tele-methylhistamine in each sample was determined using a double antibody RIA (Pharmacia, Kalamazoo, MI) and each sample was run in duplicate according to the kit instructions.

2.4. Food intake and metabolic measurements

Food intake (4, 6 and 24 h) was measured to assess the acute affects of thioperamide on feeding ($n = 6–7$/group). Vehicle or thioperamide (10 and 30 mg/kg po or ip) was administered 30 min before lights off and food hoppers were weighed at the appropriate times. Daily body weights were also measured. Because only the higher dose of thioperamide was found to significantly decrease food intake (see Results), separate experiments were conducted to examine if thioperamide (30 mg/kg po or ip) could alter 24-h energy expenditure (EE) and respiratory quotient (RQ) by indirect calorimetry using an open circuit calorimeter (Oxymax, Columbus Instruments, Columbus, OH). Rats ($n = 5$/group) were placed in calorimeter chambers with free access to food and water during the experiment. The instrument was calibrated before each experiment using gas mixtures with known percentages of CO_2, N_2 and O_2. Gas sampled from each of the chambers was first dried by a condenser then passed through magnesium perchlorate to further dry the air. The volume of oxygen consumed (VO_2) and carbon dioxide expired (VCO_2) was measured using a paramagnetic oxygen sensor and a spectrophotometric CO_2 sensor. Such measurement was obtained hourly for approximately 23 h allowing the rats a basal period to acclimate to the chambers during the first hour before initiation of the treatment.

2.5. Conditioned taste aversion

To assess the likelihood that thioperamide administration resulted in a potentially aversive consequence, a two-bottle conditioned taste aversion test was run. Rats were deprived of water overnight. On the conditioning day, the rats were exposed to a novel taste stimulant (0.15% saccharin) for 30 min. They were then immediately administered either vehicle (orally or intraperitoneally) and thioperamide (10 or 30 mg/kg po or ip, $n = 7$/group) resulting in five different dose combinations, vehicle/vehicle, vehicle/10 mg/kg, vehicle, 30 mg/kg, 10 mg/kg/vehicle, 30 mg/kg/vehicle (oral/intraperitoneal doses, respectively). A positive control group was also included which was administered lithium chloride (127 mg/kg ip/vehicle po). Water was returned to the cages immediately after the procedure was completed. Two days after conditioning, the animals were given a 24-h two-bottle choice test between a saccharin bottle and a water bottle. Bottle placement was randomized on the left or right side of cage to prevent place preference. Solution intake over 24 h was measured to the nearest gram.

2.6. Calculations and statistics

All data are expressed as mean ± S.E.M. The C_{max} and T_{max} values were reported from observed data. The $T_{1/2}$ values were obtained by linear regression of the terminal phase of log−linear concentration-time profiles. $AUC_{0-8\ h}$ values were calculated by linear trapezoidal approximation. RQ was calculated as the ratio of VCO_2 to VO_2. EE was calculated as the product of calorific value (CV) and VO_2 per kilogram of body weight, where $CV = 3.815 + 1.232 \cdot RQ$ (Elia and Livesey, 1992). Total caloric expenditure during a 24-h period was calculated to determine daily fuel utilization. To calculate proportion of protein, fat and carbohydrate that is oxidized during that 24-h period, we used Flatt's (1991) proposal assuming that protein oxidation was equivalent to protein intake for adult stable animals. Using formulae and constants derived by Elia and Livesey (1992), we calculated the percent of daily fuel utilization derived from carbohydrate and fat. The saccharin preference ratio was determined by dividing the saccharin intake by total fluid intake and all groups were normalized against the vehicle/vehicle-treated group. Statistical analyses were performed by one-way ANOVA followed by Tukey's test for multiple comparisons using Sigmastat 2.03.

3. Results

3.1. Pharmacokinetic profile

After the administration of 10 and 30 mg/kg thioperamide via intraperitoneal dosing, the mean maximum plasma concentration was observed at 1 h (4987 ± 149 and 18,661 ± 932 ng/ml, respectively; Fig. 1). The area under the curve from 0 to 24 h, $AUC_{0-24\ h}$ was calculated to be 14.8 ± 0.9 and 113.7 ± 7.3 μg h/ml following the 10- and 30-mg/kg ip dose, respectively (Table 1). Similar to the intraperitoneal administration, the 10-mg/kg po administration resulted in maximal plasma concentration being measured at 1 h postdose (4644 ± 918 ng/ml) whereas the 30-mg/kg po dose resulted in a maximal plasma concentration at 4 h (13,820 ± 1814 ng/ml; Fig. 1). The $AUC_{0-24\ h}$ was calculated to be 20.2 ± 3.2 and 136.0 ± 12.6 μg h/ml following the 10- and 30-mg/kg po dose, respectively (Table 1). Mean maximum plasma concentrations and $AUC_{0-24\ h}$ values were approximately the same for both the 10- and 30-mg/kg doses regardless

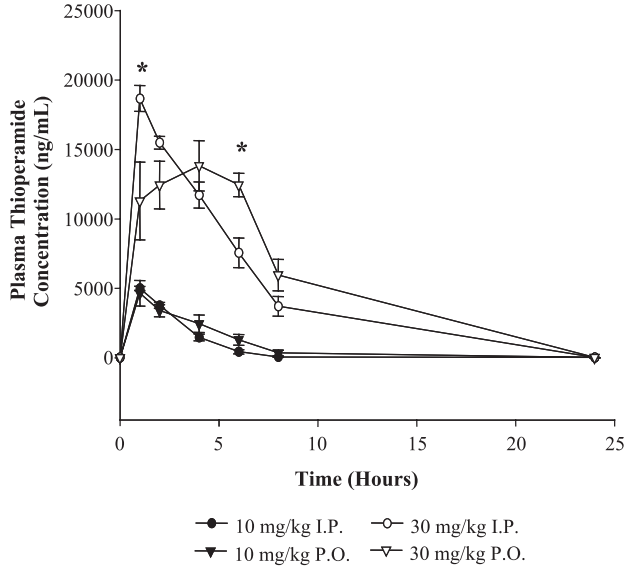

Fig. 1. Mean plasma concentrations of thioperamide following a single 10- or 30-mg/kg oral or interperitoneal dose. Values are means ± S.E.M. for $n = 4$/group. *P<.05 vs. other 30 mg/kg group.

of the route of administration. Mean brain concentrations of thioperamide at 2 h postdose were slightly higher with the intraperitoneal route of administration than the oral administration ($P \leq .05$), whereas the 6-h total brain levels of thioperamide was higher following oral administration ($P \leq .05$). The calculated $AUC_{0-24\ h}$ values from the brain concentration curve were approximately the same for the 30-mg/kg dose groups (8.62 ± 1.5 and 10.1 ± 1.0 μg h/ml) following intraperitoneal and oral administration, respectively.

3.2. Ex vivo H3R binding and tele-methylhistamine level dose response

With intraperitoneally administered thioperamide, [3H](*R*)-alpha-methylhistamine binding showed a significant ($P \leq .05$) dose-dependent inhibition from 23.4 ± 3.8% at 3 mg/kg to 76.4 ± 3.3% at 30 mg/kg (Fig. 2A). Likewise, oral administration of the same doses showed a remarkably similar pattern, with the 30-mg/kg dose inhibiting binding 85.7 ± 1.9%. Because inhibition of [3H](*R*)-alpha-methylhistamine binding indicates that thioperamide reached cerebral cortex H3 receptors, the activation of the histaminergic system by thioperamide was next examined using tele-methylhistamine levels in the cerebral cortex. The intraperitoneal administration of thioperamide showed a robust dose-dependent rise on cerebral cortex tele-methylhistamine levels from 52.8 ± 9.2 ng/g at the 3-mg/kg dose to 69.9 ± 8.7 ng/g at the 30-mg/kg dose (Fig. 2B), demonstrating an increase in central histamine turnover with increasing doses of intraperitoneal thioperamide. A similar increase and levels of tele-methylhistamine were obtained when the same doses of thioperamide were administered via the oral route.

Table 1
Pharmacokinetic parameters for thioperamide following a single 10- or 30-mg/kg oral or intraperitoneal dose

Parameter	10 mg/kg ip	30 mg/kg ip	10 mg/kg po	30 mg/kg po
AUC (μg* h/ml)	14.8 ± 0.9	113.7 ± 7.3 *	20.2 ± 3.2	136.0 ± 12.6 *
C_{max} (μg/ml)	5.0 ± 0.1	18.7 ± 0.9 *	4.6 ± 0.9	13.8 ± 1.8 *
T_{max} (h)	1	1	1	4
$T_{1/2}$ (h)	2.59	1.69	1.0	nc
Brain at 2 h (ng/g)	320 ± 99[#]	1055 ± 180 *	134 ± 30[#]	476 ± 82 *,[#]
Brain at 6 h (ng/g)	39 ± 11[†]	493 ± 127 *	91 ± 20	784 ± 87 *
Brain at 24 h (ng/g)	BQL	3 ± 1	BQL	3 ± 1

BQL = below quantification limit, nc = not calculated.
* $P \leq .05$ vs. 10 mg/kg dose.
[#] $P \leq .05$ vs. 30 mg/kg ip dose.
[†] $P \leq .05$ vs. 30 mg/kg po dose.

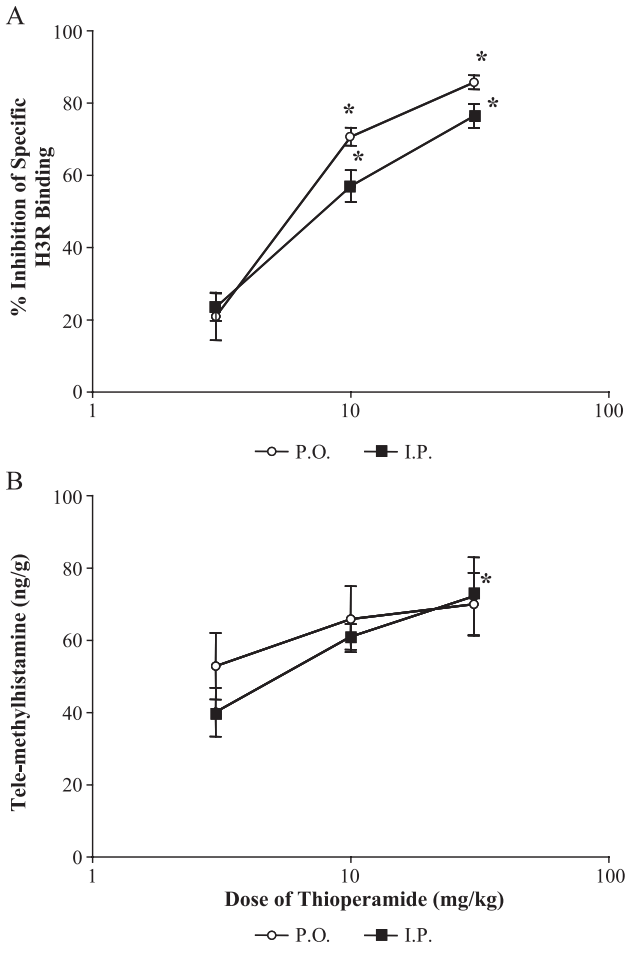

Fig. 2. Dose–response relationship of thioperamide binding to cerebral cortex H3Rs and the turnover of histamine in the cerebral cortex. (A) Percent inhibition of specific [3H](*R*)-alpha-methylhistamine (0.8 nM) in the cerebral cortex of rats vs. log doses of thioperamide. (B) Increase in cerebral cortex tele-methylhistamine levels vs. log doses of thioperamide. Values are means ± S.E.M. for $n = 3$/group. *P<.05 vs. 30 mg/kg group.

3.3. Time-course H3R binding and tele-methylhistamine levels

To investigate what effect time had on both the ability of thioperamide to penetrate the CNS and to activate the histaminergic system, a time-course study was performed. intraperitoneally administered thioperamide (30 mg/kg) inhibited [3H](*R*)-alpha-methylhistamine binding in the cerebral cortex 85.0 ± 2.48% (compared to the vehicle-dosed group) 1.5 h after dosing and dropped slightly to 70.9 ± 3.2% at 6 h postdosing (Fig. 3A). Likewise, oral thioperamide (30 mg/kg) resulted in a similar decline in binding from 81.0 ± 2.8% (1.5 h) to 74.0 ± 4.4% (6 h). Similarly, cerebral cortex tele-methylhistamine increased to 94.2 ± 10.8 ng/g (1.5 h) and 130.0 ± 7.2 ng/g (6 h) after orally administered thioperamide (30 mg/kg) and to 94.1 ± 13.3 ng/g (1.5 h) and 140.1 ± 14.3 ng/g (6 h) after 30 mg/kg ip thioperamide (Fig. 3B). These levels were significantly higher when compared to the vehicle group (40.8 ± 5.5 ng/g at 1.5 h and 51.3 ± 0.7 ng/g at 6 h).

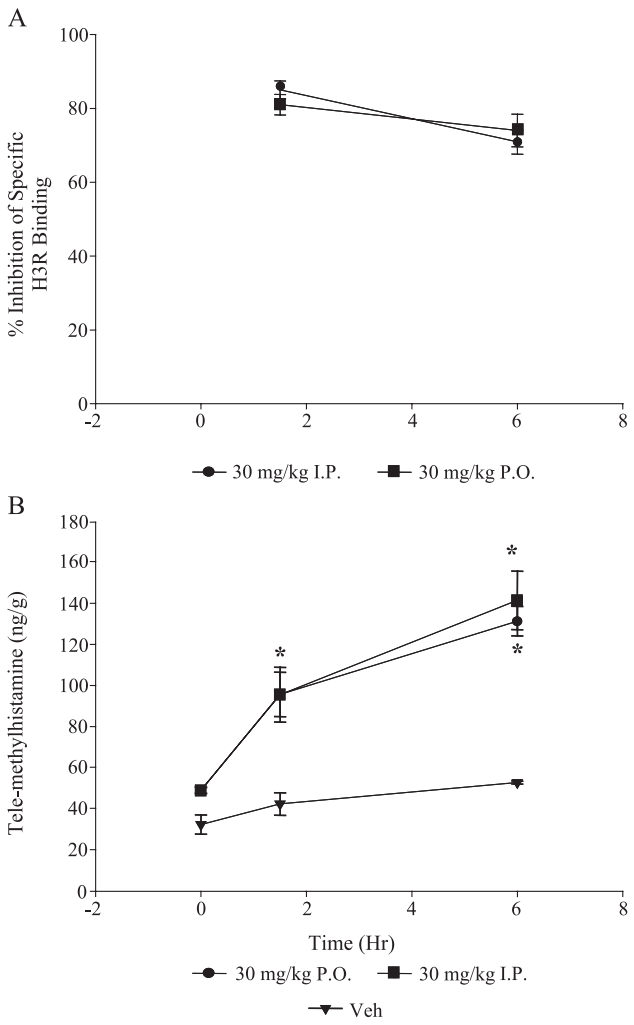

Fig. 3. Time course of thioperamide binding to cerebral cortex H3Rs and the turnover of histamine in the cerebral cortex. (A) Time course of percent inhibition of specific [3H](*R*)-alpha-methylhistamine (0.8 nM) binding to cerebral cortex H3R after thioperamide (30 mg/kg) administration 30 min prior to the onset of the dark cycle. (B) Time course of cerebral cortex tele-methylhistamine levels after vehicle or thioperamide (30 mg/kg) administration 30 min prior to the onset of the dark cycle. Values are means ± S.E.M. for $n = 3$/dose group. *P<.05 vs. vehicle group.

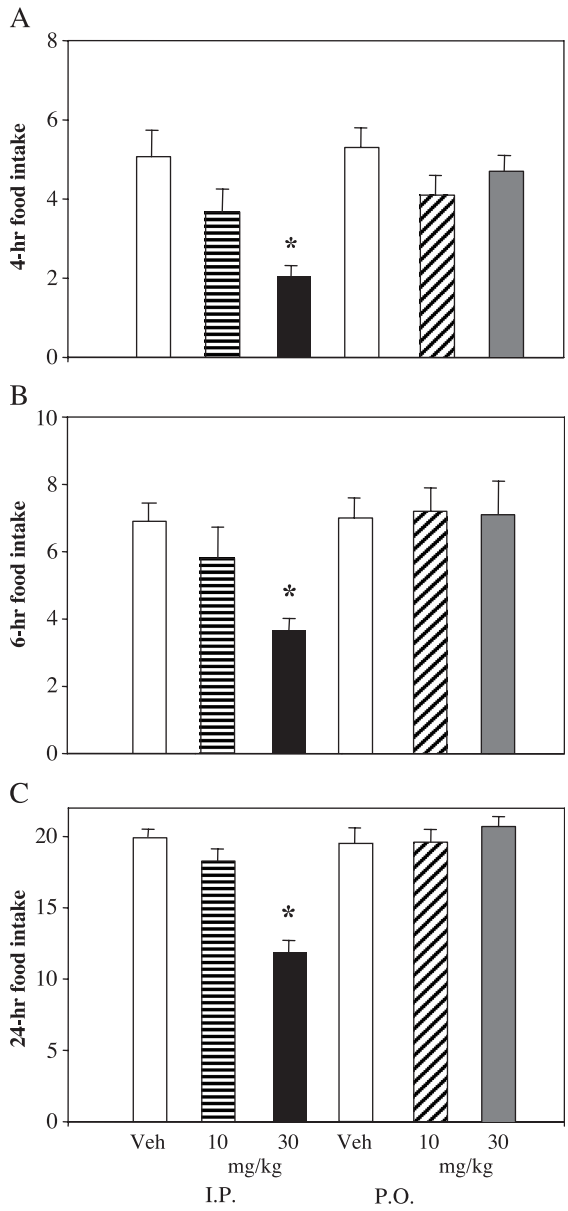

Fig. 4. Food intake 4 h (A), 6 h (B) and 24 h (C) after vehicle or thioperamide (30 mg/kg) administration 30 min prior to the onset of the dark cycle in diet-induced obese, male Long–Evans rats. Values are means ± SEM for $n = 6–7$/group. * $P \leq .05$ vs. vehicle group.

3.4. Food intake and metabolic parameters

Vehicle-treated animals consumed 5.1 ± 0.7 g of food 4 h postintraperitoneal injection, 6.9 ± 0.3 g at 6 h and 19.9 ± 0.2 g in 24 h (Fig. 4). With the intraperitoneal

administration of thioperamide at 30 mg/kg, food intake was inhibited at the 4-h time point (2.1 ± 0.3 g, $P \leq .05$), the 6-h time point (3.7 ± 0.4 g, $P \leq .05$) and the 24-h time point (11.9 ± 0.9 g, $P \leq .05$). The 10-mg/kg dose showed a similar but blunted decline in food intake that was not statistically different from vehicle controls. With the oral administration of thioperamide at the same two doses, there was no decrease in food intake detected.

In a separate set of experiments examining metabolic changes due to thioperamide administration, it was found that the baseline RQ showed no difference between the vehicle and the 30-mg/kg ip thioperamide groups (0.86 ± 0.2, Fig. 5A). With the administration of 30 mg/kg ip thioperamide, the RQ transiently fell to 0.79 ± 0.2 and remained significantly lower over the course of the experimental period (average: 0.86 ± 0.1, $P \leq .05$) while the RQ steadily rose in the vehicle-treated group (average: 0.90 ± 0.01). Moreover, there was a significant reduction in EE after the 30-mg/kg ip thioperamide administration (5.82 ± 0.07 kcal/kg/h) when compared to the vehicle group (6.59 ± 0.12 kcal/kg/h, Fig. 5B). At the end of the experiment, food intake was found to be significantly suppressed with 30 mg/kg ip thioperamide (17.1 ± 1.4 g) when compared to the vehicle-

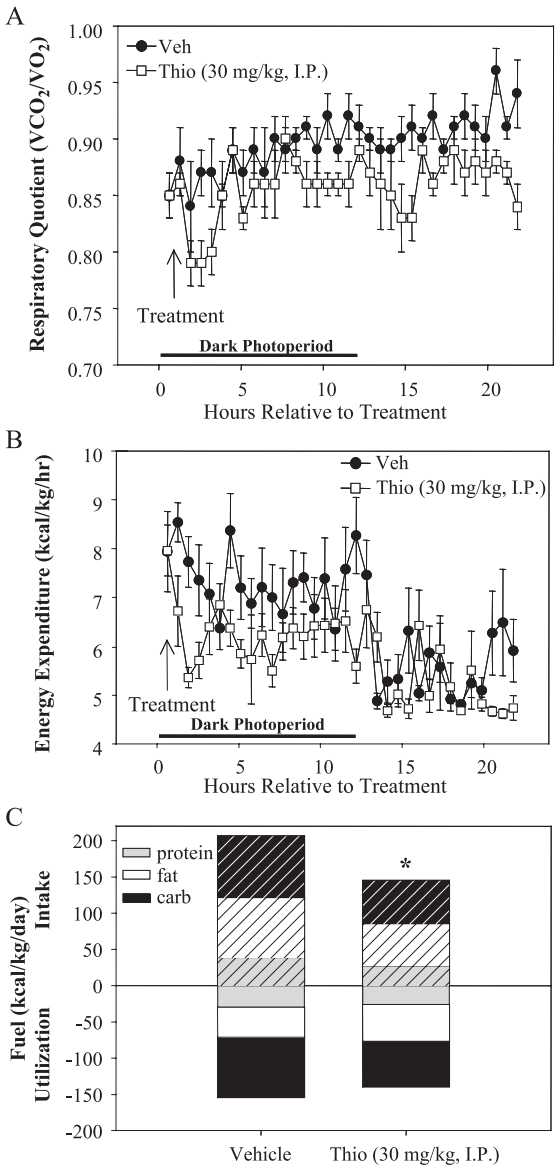

Fig. 5. Metabolic effects after vehicle or thioperamide (30 mg/kg ip) administration. (A) Changes in RQ, (B) EE and (C) energy balance with either vehicle or thioperamide (30 mg/kg ip). Values are means ± S.E.M. for $n = 5$/group. * $P \leq .05$ vs. vehicle group.

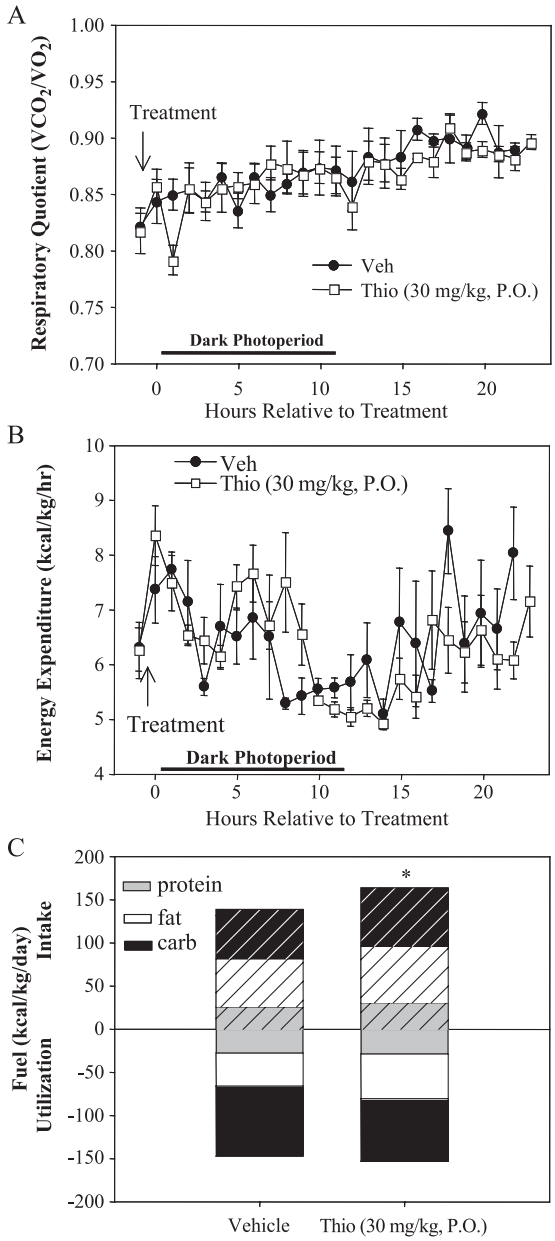

Fig. 6. Metabolic effects after vehicle or thioperamide (30 mg/kg po) administration. (A) Changes in RQ, (B) EE and (C) energy balance with either vehicle or thioperamide (30 mg/kg po). Values are means ± S.E.M. for $n = 5$/group. * $P \leq .05$ vs. vehicle group.

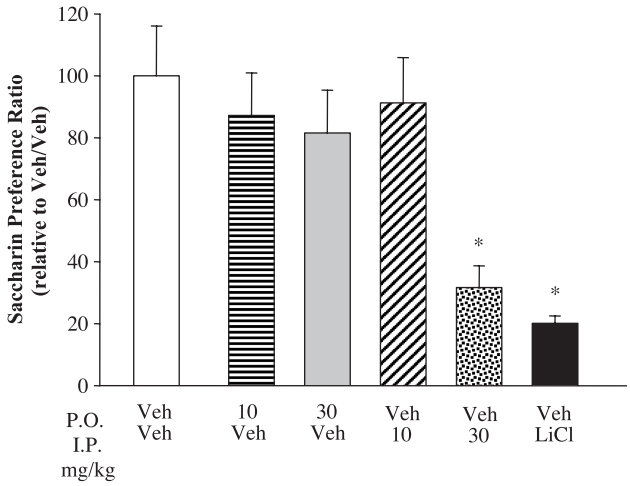

Fig. 7. Saccharin preference ratio over 24 h. Rats previously had the 0.15% saccharin solution paired with dosing regiment as specified in the graph (po/ip mg/kg dose, respectively). A two-bottle choice test (saccharin or water) was conducted 48 h after the initial pairing of the dosing regiment and saccharin solution. Values are means ± S.E.M. for $n = 7$/group. * $P \leq .05$ vs. vehicle/vehicle group.

treated control group (23.0 ± 0.2 g). Because food intake and EE both decreased over the course of the experiment in the 30-mg/kg ip thioperamide group, a nearly equal energy balance (5.64 kcal/kg/day, Fig. 5C) was found while the control group had a slightly positive energy balance of 48.69 kcal/kg/day.

Examining the metabolic effects with the oral route of administration, baseline RQs showed no difference between the vehicle and the 30-mg/kg po thioperamide groups (0.81 ± 0.2, Fig. 6A). With the administration of thioperamide at 30 mg/kg po, RQ transiently fell for 1 h then returned to values (average: 0.87 ± 0.1, Fig. 6A) that were not different from the vehicle group (average: 0.87 ± 0.01). Moreover, there was no effect on EE after 30 mg/kg po thioperamide administration (6.42 ± 0.13 kcal/kg/h) when compared to the vehicle controls (6.37 ± 0.07 kcal/kg/h, Fig. 6B). Food intake with oral thioperamide (20.0 ± 1.0 g) was slightly greater when compared to the vehicle-treated control group (17.2 ± 0.7 g, $P \leq .05$). This resulted in a nearly equal energy balance in both the vehicle-treated control group and the oral thioperamide group (− 8.22 and 19.2 kcal/kg/day, respectively, Fig. 6C).

3.5. Conditioned taste aversion

There was a significant effect of the 30-mg/kg ip thioperamide dose and LiCl to cause a conditioned taste aversion upon reexposure to the saccharin solution (Fig. 7). The rats that had this treatment consumed significantly less of the saccharin solution (9 ± 4and 7 ± 1 g, respectively, $P \leq .05$) compared to any of the other groups (average: 36 ± 8 g). Total fluid intake was not significantly different in any of the groups (48 ± 6 g/24 h).

4. Discussion

Numerous pieces of evidence have implicated histamine as an important neurotransmitter modulating energy homeostasis. Reports of the H3R antagonist thioperamide decreasing food consumption are consistent with this concept in that it binds to H3R, increases histamine levels in the brain (Garbarg et al., 1989a,b; Taylor et al., 1992; Jansen et al., 1998) and decreases food intake when administered centrally (Sakata et al., 1991, 1997; Ookuma et al., 1993; Naruse et al., 1995; Itoh et al., 1998, 1999) and intraperitoneally (Mollet et al., 2001). However, with the discovery of the histamine H4R receptor, the potential for promiscuous binding of the currently studied histamine H3R receptor antagonists, such as thioperamide, to other monoamime receptors (Leurs et al., 1995) could cause the decrease in food intake. Therefore, we examined various pharmacological measurements to try to demonstrate centrally mediated histamine H3R receptor mechanism for inhibition of food consumption by thioperamide. Our data clearly demonstrate that the intraperitoneal and oral route of thioperamide administration resulted in similar pharmacokinetic profiles, inhibition of H3R binding and activation of histamine release as indicated by tele-methylhistamine. This indicates that via either route of administration, central penetration and activation of the histaminergic system appear to be equivalent. Moreover, the results obtained with thioperamide's inhibition in binding of [3H](*R*)-alpha-methylhistamine and rise in tele-methylhistamine are in line with that found in the literature (Garbarg et al., 1989a,b).

The current pharmacokinetic study indicates that the AUC values were approximately 27% and 17% greater, following oral administration of thioperamide compared to intraperitoneal administration at the same doses (10 and 30 mg/kg, respectively). As suspected, the intraperitoneal route of administration with the 30-mg/kg dose resulted in a quicker peak in the plasma exposure and 55% greater brain concentration of thioperamide at 2 h. The results from the pharmacokinetic study are consistent with thioperamide entering the circulation faster with the intraperitoneal route, resulting in an initial greater brain concentration. Moreover, the resultant peak in plasma concentration at 1 h and higher brain concentrations at 2 h could provide the potential explanation of why only this route and dose of thioperamide (30 mg/kg ip) significantly decreased food intake, but is unlikely in the face of the other data presented in the current manuscript. The 6-h thioperamide brain levels of the 30-mg/kg po group (784 ng/g) were nearly equivalent to the 2-h brain levels (1055 ng/g) found in the 30-mg/kg ip group (P = ns). Because there was no reduction in food intake or decrease in energy balance found with the 30-mg/kg po thioperamide dose, this indicates that the brain concentration of thioperamide most likely does not alter energy homeostasis. Likewise, the central H3R specific binding and the activation of central histamine release, as indicated by the increase in tele-

methylhistamine levels, illustrate that with either route of administration of thioperamide, the effect is similar for these parameters. Nonetheless, oral administration of thioperamide did not result in an inhibition of food intake or a discernable long-lasting effect on any metabolic parameter, such as RQ, while 30 mg/kg ip thioperamide administration did exhibit a reduction in food intake. However, along with the reduction in food intake with the 30-mg/kg ip route of administration, EE decreased which resulted in a nearly equal energy balance (5.64 kcal/kg/day).

The central penetration and activation of the central histaminergic system by thioperamide, and not effecting overall energy balance, is a striking result when compared to the previous studies demonstrating that central (Sakata et al., 1991, 1997; Ookuma et al., 1993; Naruse et al., 1995; Itoh et al., 1998, 1999) and intraperitoneal (Mollet et al., 2001) administration of thioperamide results in a robust decrease in food intake. Moreover, other studies examining changes in histamine release and behavioral responses invoked by thioperamide have used lower doses (Lin et al., 1990; Jansen et al., 1998; Giovannini et al., 1999; Orsetti et al., 2001). This could indicate that only a selective change in the hypothalamic histamine system, via intracerebroventricular administration, results in a decrease in food intake or it could be due to the unwanted side effect of another receptor system (Leurs et al., 1995). However, the latter appears unlikely because H3R KO mice do not respond to centrally administered thioperamide (Toyota et al., 2002). To answer the question, future investigations will need to examine if centrally administered thioperamide causes a similar change in EE when food intake decreases. Because H4R is localized in the gut and not in brain tissue, and the central ex vivo binding data in this manuscript is consistent with these findings, the ability of intraperitoneal thioperamide to inhibit food intake could be brought about via an effect on the gut histaminergic system and consequently, gut motility/function (Bertaccini et al., 1991; Coruzzi et al., 2001) or by a side effect of increased histamine levels in the visceral tissue. Moreover, the decrease in EE found with the 30-mg/kg ip thioperamide dose coupled with the same dose being able to invoke a conditioned taste aversion indicates that visceral illness or injection site irritation from intraperitoneal administration of thioperamide is the most likely cause of the decrease in food intake, and not a centrally mediated effect.

However, both routes of administration resulted in a very transient decrease in RQ, which could indicate that a centrally mediated histaminergic effect was present. Moreover, the transient change may indicate that the central H3R was desensitized rapidly by the increase in thioperamide concentration. Future studies will be needed to examine if effects on gut motility (Coruzzi et al., 2001) or changes in the sensitivity of the receptor system brought about by thioperamide could explain these results. Likewise, intraperitoneal injection of thioperamide into the H3R KO mice or a more pharmacologically selective H3R antagonist could be used to examine if the H4R system is responsible for the decrease in food intake with this route of administration.

In conclusion, we find that the pharmacokinetic profile, the central H3R specific ex vivo binding and increase in histamine release (tele-methylhistamine) brought about by thioperamide is remarkably similar with either the intraperitoneal or oral route of administration. However, only intraperitoneal administration of thioperamide at 30 mg/kg was able to affect food intake, but this effect was offset by a decrease in EE while invoking a conditioned taste aversion, both consistent with visceral illness. The data indicate that the effect of thioperamide on the central H3R system appears not to play a role in the reduction of food intake or alterations in energy balance.

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