Preclinical Evaluation of Melanin-Concentrating Hormone Receptor 1 Antagonism for the Treatment of Obesity and Depression

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ABSTRACT

The mammalian neuropeptide, melanin-concentrating hormone, interacts with two G protein-coupled receptors, melanin-concentrating hormone receptor (MCHR) 1 and MCHR2; however, only MCHR1 is expressed in rats and mice. In the present study, we evaluated MCHR1 antagonism in preclinical models believed to be predictive of antiobesity and antidepressant activity. Central activity of the selective MCHR1 antagonist, GW803430 [6-(4-chloro-phenyl)-3-[3-methoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-3H-thieno[3,2-d]pyrimidin-4-one], was evaluated using ex vivo binding with autoradiography. Effective doses of GW803430 (1 and 3 mg/kg p.o.) were correlated with antiobesity activity in a 14-day study of diet-induced obese rats. GW803430 was evaluated subsequently for antidepressant-like effects in mice and rats. Acute and subchronic administration reduced immobility time in the mouse forced-swim test at doses of 3 (acute) and 3 and 10 (chronic) mg/kg p.o., an effect that was absent in MCHR1(−/−) mice. Combined subeffective doses of GW803430 (0.3 and 1 mg/kg p.o.) and imipramine (5 mg/kg) produced a robust antidepressant-like response. The compound was also active in the tail suspension test at a dose of 10 mg/kg p.o. GW803430 (30 mg/kg p.o.) significantly reduced submissive behaviors at weeks 2 and 3, a model of submissive behavior that may predict antidepressant onset. GW803430 decreased marble burying in mice at doses of 3, 10, and 30 mg/kg p.o., an assay that detects anxiolytic-like effects. Thus, GW803430 produces robust antiobesity and antidepressant-like effects in rats and mice at doses that compete for central MCHR1 in vivo. As such, MCHR1 should be considered as a promising target for future drug discovery efforts.

Melanin-concentrating hormone (MCH) was originally discovered as a 17-amino acid peptide hormone mediating the lightening of fish skin color (Kawauchi et al., 1983). MCH was identified subsequently in the mammalian hypothalamus as a 19-amino acid cyclic peptide (Vaughan et al., 1989). In the brain, cell bodies containing MCH are found exclusively in the lateral hypothalamus and zona incerta, with projections to the dorsal and ventral striatum, prefrontal cortex, nucleus of the solitary tract, and the parabrachial nucleus (Skofitsch et al., 1985; Bittencourt et al., 1992). Immunoreactive fibers containing MCH are localized in subregions of the hypothalamus that are important for feeding and endocrine regulation and the limbic system where MCH is thought to regulate mood-related behaviors. Central administration of MCH increases food consumption by rodents (Qu et al., 1996), and chronic administration increased weight gain and susceptibility to diet-induced obesity (Gomori et al., 2003). Genetic overexpression of MCH in mice produces obesity and insulin resistance (Ludwig et al., 2001), whereas MCH gene deletion results in resistance to weight gain on a palatable diet (Kokkotou et al., 2005). Aged MCH(−/−) mice are resistant to the aging-related weight gain and weigh 25 to 30% less than control mice (Jeon et al., 2006). MCH produces its effects through two G protein-coupled receptors, MCHR1 and MCHR2 (for review, see Pissios et al., 2006). Although MCHR2 is expressed in humans with a similar distribution pattern compared with MCHR1 (An et al., 2001; Hill et al., 2001; Sailer et al., 2001), a functional MCHR2 is not expressed in rats and mice, and so little is known about its physiological function. The highest densities...
of MCHR1 are found in the nucleus accumbens and caudate putamen of rats and mice (Hervieu et al., 2000; Saito et al., 2000, 2001). MCHR1 is localized to other brain regions implicated in the etiology of mood disorders, including the locus ceruleus, amygdala, and hippocampus (Hervieu et al., 2000). MCHR1(−/−) mice are lean compared with MCHR1(+/+) controls but exhibit increased locomotor activity and hyperphagia (Chen et al., 2002; Marsh et al., 2002; Smith et al., 2005, 2008; Zhou et al., 2005). MCHR1 antagonism decreases feeding and adiposity, indicating the importance in regulating body weight and composition (for review, see Kowalski and Sasikumar, 2007). Although some work has identified mood-related phenotypes in the MCHR1(−/−) mice (Roy et al., 2006, 2007; Smith et al., 2006), the pronounced increase in locomotor activity observed in these mice made those evaluations challenging. Therefore, exploration of the effects of MCHR1 antagonism may provide more reliable and consistent data.

An emerging body of literature supports a role for MCH and MCHR1 in the endocrine and behavioral responses to stress. Central injection of MCH activates the endocrine stress response as evidenced by elevated plasma levels of adrenocorticotropic hormone and corticosterone (Kennedy et al., 2003; Smith et al., 2006). In a number of stress-based animal models, several investigators reported that central administration of MCH produces anxiolytic-like effects in the elevated plus-maze, open-field, and Vogel tests (Monzón and De Barioglio, 1999; Kela et al., 2003). However, effects of MCH in the elevated plus test have ranged from anxiogenic (Smith et al., 2006) to no effect (Duncan et al., 2005). MCHR1 gene deletion results in anxiolytic- and antidepressant-like phenotypes (Roy et al., 2006; Smith et al., 2006). When normal mice are subjected to a chronic mild stress paradigm, MCHR1 expression in the hippocampus is up-regulated (Roy et al., 2007), and this is reversed by fluoxetine administration. In addition, MCHR1 antagonists produce anxiolytic-like actions in the social interaction test, elevated plus maze, stress-induced hyperthermia, and guinea pig maternal separation (Borowsky et al., 2002; Chaki et al., 2005; Smith et al., 2006). Antidepressant-like behaviors were reported with MCH antagonism (Borowsky et al., 2002), and this antidepressant action may differ from the monoamine reuptake inhibitors in that it does not require neurogenesis (David et al., 2007).

The aggregate weakness of the current literature is that a variety of MCHR1 antagonists were evaluated in different models with different routes of administration. MCHR1 occupancy by the antagonists was not established in those studies to verify MCHR1 blockade in vivo and MCHR1(−/−) mice were not used to verify target mediated activity of the compounds. In the present study, we establish effective doses of the potent and selective MCHR1 antagonist, GW803430 (Hertzog et al., 2006), in vivo and evaluated its activity in models predictive of antiobesity and antidepressant efficacy. We also establish that these activities are dependent on MCHR1 using MCHR1(−/−) mice.

Materials and Methods

Animals. All experiments were performed according to the policies of the Animal Care and Use Committee of Eli Lilly and Company, in conjunction with the American Association for the Accreditation of Laboratory Animal Care-approved guidelines and the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). Animals were individually housed in an environmentally controlled facility where food and water were available ad libitum.

Drugs. The potent and selective MCHR1 antagonist, GW803430 (Hertzog et al., 2006), was synthesized at Lilly Research Laboratories (Indianapolis, IN). GW803430 has a reported high affinity (pIC50 = 9.3) for the MCHR1 and was found to be selective (>100×) over a battery of G protein-coupled receptors, ion channels, and enzymes (Hertzog et al., 2006). GW803430 was suspended in 1% acetic acid/0.5% carboxymethylcellulose/0.125% Tween 80 in double-distilled H2O or 10% acacia and given by oral administration. Imipramine (IMI; Sigma-Aldrich, St. Louis, MO) was dosed intraperitoneally using 0.9% NaCl as the vehicle. Chlordiazepoxide HCl (Sigma-Aldrich) was dosed intraperitoneally in 0.9% NaCl. Compounds were dosed in a volume of 1 (rats) or 10 (mice) ml/kg.

Rat Ex Vivo Autoradiography. Male Sprague-Dawley rats, 225 to 250 g, were gavaged with vehicle (1 ml/kg) or GW803430 in vehicle. Four hours after dosing, the rats were decapitated and the forebrains were removed, frozen on dry ice, and stored at −70°C. Twelve micron tissue sections were cut on a cryostat and thaw mounted onto gelatin-coated microscope slides. Slides were incubated in modified Krebs’ phosphate buffer with 0.0375% bacitracin (Fluka, Buchs, Switzerland) and 0.4% bovine serum albumin (Sigma-Aldrich), pH 7.4, containing 30 pM [125I]S036057 (PerkinElmer Life and Analytical Sciences, Walbaum, MA; 2200 Ci/mmol) for 90 min at 25°C. Non-specific binding was assessed by the addition of 10 μM GW803430 to the incubation media. Sections were rinsed twice for 10 min each in buffer, then dipped twice in cold distilled water and dried quickly with a stream of cool air. Sections were exposed with [125I]-Microscale Standards (GE Healthcare, Chalfont St. Giles, UK) on filmless phosphorimaging plates (FujiFilm, Tokyo, Japan) for 3 days. Quantitation was facilitated using MCID Elite (MCID, Linton, Cambridge, UK) image analysis. Curve fitting and ED50 determinations were accomplished using GraphPad Prism software (GraphPad Software Inc., San Diego, CA).

Rat Feeding. Diet-induced obese (DIO) Long Evans male rats (Harlan, Indianapolis, IN; starting average body weight, 535.6 ± 6.7 g) were acclimated (single housed) for at least 2 weeks in a temperature (25°C)- and light-controlled (12-h light/dark; lights on at 10:00 PM) environment. The animals were housed individually throughout the study and permitted to eat high-fat/high-sucrose palatable diet (TD95217; Harlan Teklad, Madison, WI) and drink ad libitum throughout the study. Rats were randomized to four groups, and each group comprised five animals. Rats were treated daily by oral gavage with vehicle (saline, 1 ml/kg) or 0.1, 1, or 3 mg/kg GW803430. Body weight and food intake were measured daily at 9:00 AM. All data are shown as the mean ± S.E.M., and multiple group statistical comparisons were made using ANOVA repeated measures followed by Dunnett’s test, using GraphPad Prism software.

Feeding in MCHR1(+/+) and MCHR1(−/−) Mice. Adult, male MCHR1(+/+) and MCHR1(−/−) mice were derived by heterozygous intercross and had a mixed 129SvJ × C57BL/6 background (Chen et al., 2002). Genotyping was performed by PCR analysis on mouse-tail DNA. MCHR1(−/−), MCHR1(+/+), and C57BL/6NTac (C57) mice were 3 to 5 months old and weighed 25 to 35 g during testing. Animals were maintained on a 12:12-h light/dark cycle (lights on 10:00 PM–10:00 AM) and individually housed throughout the study at ambient temperature. All mice had free access to water and a high fat/high sucrose diet (TD95217; Harlan Teklad). After 2 weeks of acclimation to the facility, naïve mice were treated by oral gavage at 1 h before onset of dark period with vehicle or 3 mg/kg GW803430 for 4 days. Body weight of the mice and weight of food consumed in the last 24 h were measured daily. Statistical analyses were performed by two-way ANOVA followed by Tukey’s test.

Sweetened-Condensed Milk Consumption in MCHR1(+/+) and MCHR1(−/−) Mice. Animals were maintained on a 12-h light/
dark cycle (lights on 10:00 PM–10:00 AM) and individually housed throughout the study at ambient temperature. All mice had free access to water and a high-fat/high-sucrose diet (TD95217; Harlan Teklad). After 2 weeks of acclimation to the facility, naive mice were given 1:2 diluted sweetened-condensed milk for 2 h at the onset of the dark period. On day 4, 2 h before presentation of sweetened-condensed milk, mice were treated by oral gavage at 1 h before onset of the dark period with vehicle or 10 mg/kg GW803430. Sweetened-condensed milk consumed each day was measured. Statistical analyses were performed by one-way ANOVA followed by Tukey’s t test.

**Mouse Forced-Swim Test.** Male, NIH Swiss mice (purchased from Harlan) weighing 20 to 25 g were housed in plastic cages (40.6 × 20.3 × 15.2 cm) with 10 to 12 mice/cage in a vivarium at least 7 days before the experiments. This mouse strain was used in this assay because they are the standard strain for which our laboratory has optimized the assay (Bai et al., 2001). In one experiment, MCHR1(−/−) mice and their littermate MCHR1(+/+) controls were used (see description of mice above). This experiment was carried out to ascertain whether the antidepressant-like effects of GW803430 would be absent in mice without MCHR1. Mice were maintained on a 12-h light/dark cycle (6:00 AM/6:00 PM), and all procedures were performed between 12:00 PM and 4:00 PM. Animals were removed from the vivarium to the testing area in their home cages and allowed to adapt to the new environment for at least 1 h before testing. The forced-swim test was performed using the original method described by Porsolt et al. (1977). In brief, mice were placed individually in clear plastic cylinders (10 cm in diameter by 25 cm in height) filled to 6 cm with 22 to 25°C water for 6 min. The duration of immobility was recorded during the last 4 min of a 6-min trial. A mouse was regarded as immobile when floating motionless or making only those movements necessary to keep its head above the water. Dose-effect functions for GW803430 were carried out by dosing mice by mouth, 4 h before testing. Imipramine was used as a comparator standard (15 mg/kg p.o., 30 min before). Statistical analyses were performed by ANOVA followed by Dunnett’s test.

**Tail-Suspension Test.** This assay detects the activity of antidepressants and is used here as a modification of the procedure of Steru et al. (1985). Male, C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) weighing approximately 25 g were suspended by their tails from the edge of a shelf 80 cm above the floor. This mouse strain was used because it has been the most well characterized under these assay conditions (Bai et al., 2001). Immobility, defined as the mice hanging passively without movements to right themselves, was recorded during a 5-min trial. Dose-effect functions for GW803430 were carried out by dosing mice p.o., 4 h before testing. Imipramine was used as a comparator standard (15 mg/kg p.o., 30 min before). Statistical analyses were performed by ANOVA followed by Dunnett’s test.

**Locomotor Activity.** Because general increases in motor activity could result in false-positive findings in the forced-swim test, tail suspension test, and in the marble-burying assay (below), investigation of the effects of GW803430 was undertaken. Locomotor activity of male, NIH Swiss mice was assessed in Plexiglas locomotor activity chambers (40.6 × 20.3 × 15.2 cm) in a 20-station photobeam activity system (San Diego Instruments, San Diego, CA) with seven photocells per station in a lighted room. Dose-effect functions for GW803430 were carried out by dosing mice by mouth 4 h before testing. For these experiments, mice were dosed 3 h before placement into the locomotor chambers and returned to their home cage. The mice were subsequently placed individually into the locomotor arena. Locomotion was recorded (for a 30-min session) as successive breaks of the photobeams after a 60-min habituation period. The effect of d-amphetamine sulfate (1 mg/kg i.p.) was used as a comparator standard psychomotor stimulant and was dosed immediately before the locomotor recording period of 30 min.

**Results**

**Establishing Effective Doses of GW803430 Using Rat Ex Vivo Autoradiography.** Initial studies were performed to establish doses of GW803430 that effectively inhibited...
binding to the MCHR1 receptor. To accomplish this, animals were administered the compound by the oral route and sacrificed 4 h after administration. The brains from the animals were subjected to ex vivo autoradiography to assess how effectively the compound concentrations within the brain tissue inhibited the binding of $^{125}\text{I}-\text{S036057}$ to the MCHR1 receptor. In these studies, the binding to three brain regions was assessed using image analysis techniques. The $ED_{50}$ for these brain regions ranged from 0.05 to 0.56 mg/kg (Fig. 1). Based on these results, doses of 1 mg/kg or greater would be expected to effectively antagonize a majority of the rat brain MCHR1 receptors in vivo.

**Effect of GW803430 on Food Intake and Body Weight.**

Having established the effective doses of GW803430, we evaluated whether the doses capable of inhibiting binding ex vivo were capable of affecting food consumption and body weight in DIO rats. In these studies, rats were administered the compound for 14 days, during which food consumption and body weight were monitored. Compared with vehicle controls, there was a decrease in body weight at 0.1 mg/kg, but this did not reach significance ($F_{1,16} = 5.22, p = 0.09$) (Fig. 2A). A dose of 0.1 mg/kg produced a slight, but not significant, reduction in cumulative food consumption, although

![Fig. 1. Dose-dependent inhibition of MCHR1 binding by orally administered GW803430 ex vivo. Brains from rats that received GW803430 were subjected to quantitative autoradiography. Receptor binding to three brain regions was assessed, and $ED_{50}$ values were calculated from the dose-response curves (left). Results in the graph are the mean (n = 5). NS, nonspecific binding. Right, representative set of autoradiograms shown as a pseudocolor reconstruction.](image)

![Fig. 2. MCHR1 antagonist, GW803430, decreases cumulative body weight and food intake in DIO male Long Evans rats. DIO rats were orally treated with vehicle (●, 1 ml/kg, 10% acacia/0.15% saccharin/1% lactic acid) or GW3430 at 0.1 (○), 1 (□), or 3 (△) mg/kg. The treatment lasted for 14 days as once daily. The results are means ± S.E.M. of five animals per group. A, effect of GW803430 on cumulative body weight changes in DIO rats. *, $p < 0.05$ versus vehicle control group; †, $p < 0.05$ versus GW803430 at 0.1 mg/kg dose group (one-way ANOVA repeated measures, Tukey’s multiple comparison test). B, effect of GW803430 on daily food intake in DIO rats. GW803430 produced a significant reduction in daily food intake compared with vehicle at all doses ($p < 0.001$, one-way ANOVA repeated measures, Tukey’s multiple comparison test). C, effect of GW803430 on cumulative food intake over the 14-day period. *, $p < 0.05$ versus vehicle control group (one-way ANOVA, Dunnett’s post hoc test).](image)
the reduction in daily food consumption was significantly decreased ($F_{1,16} = 9.344, p < 0.001$). Doses of 1 and 3 mg/kg produced a more substantial reduction in daily ($p < 0.001$) (Fig. 2B) and cumulative ($p < 0.05$) food intake (Fig. 2C). Food consumption and body weight were not significantly different comparing 1 and 3 mg/kg dose groups, though the 3 mg/kg dose group trended toward a slightly greater body weight decrease.

To establish that the feeding effect was mediated by MCHR1 antagonism, MCHR1(+/+) and MCHR1(−/−) animals were treated for 4 days with GW803430. MCHR1(+/+) animals treated with compound exhibited a substantial decrease in food intake and body weight compared with the MCHR1(+/+) mice treated with vehicle (Fig. 3). Vehicle-treated MCHR1(−/−) mice exhibited lower food intake compared with vehicle-treated MCHR1(+/+) mice ($F_{1,24} = 5.75, p < 0.05$). GW803430 treatment resulted in significant decreases in cumulative food intake on days 2, 3, and 4 ($F_{1,24} = 15.36, p < 0.001$) and reductions in body weights on days 1, 3, and 4 ($F_{1,24} = 21.47, p < 0.001$) in MCHR1(+/+) mice. However, treatment of the MCHR1(−/−) mice with GW803430 did not result in a difference in body weight or food intake compared with vehicle treatment.

In a subsequent study, the effects of MCHR1 antagonism on sweetened-condensed milk consumption was evaluated using MCHR1(+/+) and MCHR1(−/−) mice. In these studies, the mice were allowed access to sweetened-condensed milk for 1 h/day. During the first 3 days, a stable baseline was established, and on the 4th day, the animals were treated with 10 mg/kg GW803430. During all 4 days of evaluation, the MCHR1(−/−) mice exhibited decreased milk consumption. With compound administration, there was no statistically significant difference of the treatment in either MCHR1(+/+) or MCHR1(−/−) (Fig. 4).

**Effects of GW803430 in the Mouse Forced-Swim Test.** To understand the potential role of MCHR1 antagonism in models used to predict antidepressant effects, we evaluated the effects of GW803430 in the mouse forced-swim test. In these studies, we evaluated the effects of the compound after acute and subchronic administration. With acute administration, GW803430 produced a dose-dependent reduction in immobility time that was statistically significant at 3 mg/kg ($F_{4,34} = 4.91, p < 0.005$) (Fig. 5A). When administered for 5 consecutive days, GW803430 produced a dose-dependent reduction in the immobility time, with the 3 and 10 mg/kg doses reaching statistical significance ($F_{3,21} = 4.74, p < 0.005$) (Fig. 5B). To better define the mechanism of action of this antidepressant-like effect of GW803430, compound was studied under the forced-swim test in both MCHR1(+/+) and MCHR1(−/−) mice. Although GW803430 (10 mg/kg) decreased immobility in MCHR1(+/+) mice, this effect was absent in MCHR1(−/−) mice (Fig. 6A). ANOVA revealed a significant genotype by drug condition interaction ($F_{1,26} = 90.9, p < 0.005$) and nonsignificant overall effects of drug treatment ($F_{1,26} = 0.72$) and of genotype ($F_{1,26} = 0.22$). Imipramine significantly decreased immobility in both MCHR1(+/+) and MCHR1(−/−) mice ($F_{1,27} = 68.8, p < 0.0001$). There were no significant differences between strains ($F_{1,27} = 3.1, p = 0.57$) and no drug × strain interactions ($F_{1,27} = 0.33, p = 0.09$) (Fig. 6B). Exploring this effect further, we evaluated the effect of combining subeffective doses of the MCHR1 antagonist with a subeffective dose of imipramine (Fig. 7). Combining a 1 mg/kg dose of GW 803430 with a 5 mg/kg dose of imipramine produced a reduction in immobility time that was significantly different from effects of either drug alone ($F_{1,26} = 4.12, p < 0.05$).
The Effects of GW803430 in the Mouse Tail Suspension and Rat Dominance Assays. To extend the generality of the findings on GW803430 in the forced-swim test, GW803430 was also evaluated in the mouse tail-suspension test. GW803430 decreased immobility in this assay with a minimal effective dose of 10 mg/kg (F\textsubscript{4,38} = 7.02, p < 0.001) (Fig. 8). To further substantiate the antidepressant-like effects of MCHR1 antagonism, we evaluated the effects of GW803430 in the social dominance test. This paradigm is important in that: 1) it detects actions of antidepressant agents after subchronic but not after acute dosing, and 2) it is believed to reflect the delayed onset of antidepressant action. In this study, a 30 mg/kg dose was administered for 3 weeks, and the animals were assessed before treatment and at 1-week intervals for 3 weeks during treatment. In this study, GW803430 produced a reduction in submissive behavior (Fig. 9A; F\textsubscript{5,14} = 4.71, p = 0.018) that reached statistical significance (p < 0.05) 2 and 3 weeks after treatment. These data are consistent with our previous unpublished findings with significant antidepressant effects of fluoxetine and of imipramine (both studied at 10 mg/kg/day) that was detected at 3 weeks postdosing. In this study, body weight was also evaluated, and a statistically significant reduction (p < 0.05) was observed 14, 21, and 25 days after treatment (Fig. 9B).

Effects of GW803430 in the Mouse Marble-Burying Assay. Marble-burying of mice was also studied as a method for assessing the potential anxiolytic-effects of GW803430. GW803430 dose-dependently decreased marble-burying of mice, with a dose as low as 3 mg/kg being effective (F\textsubscript{3,35} = 20.6, p < 0.0001) (Fig. 10). Although the benzodiazepine anxiolytic chlordiazepoxide also decreased marble burying at 30 mg/kg, significant increases in rotorod deficits were ob-
Effects of GW803430 on Mouse Locomotor Activity. Locomotor activity of mice was evaluated to ascertain potential behavioral effects that might confound interpretation of the preceding data sets. Mice habituated to a locomotor monitoring arena were dosed with GW803430 or d-amphetamine served; in contrast, GW803430 was without effect on rotorod performance at the same equieffective dose.

In the present study, we have evaluated the effects of the specific MCHR1 antagonist, GW803430, on feeding and body weight gain in rats. Dominant-submissive pairs of rats were established as described under Materials and Methods based on time spent drinking from a common vessel containing sweetened-condensed milk. After a baseline determination, submissive rats received daily oral doses of GW803430 (30 mg/kg), and dominant rats received vehicle (2 ml/kg). The compound produced a time-dependent reduction in the difference in drinking time between the dominant and submissive rats (A) and a reduction in body weight of the submissive rat (B). The results are means ± S.E.M. of 11 pairs of animals. *, p < 0.05.

Discussion

GW803430 (3–30 mg/kg p.o.) did not significantly alter locomotor activity of mice (3–30 mg/kg p.o.); in contrast d-amphetamine (1 mg/kg i.p.) stimulated locomotion (Fig. 11) (F(4,43) = 13.4, p < 0.0001).

Fig. 8. Oral administration of GW803430 decreased immobility in the mouse tail-suspension test. Each point represents the mean ± S.E.M. of seven to eight mice. *, p < 0.05 by Dunnett’s test.

Fig. 9. Oral administration of GW803430 reduced the dominance level in the rat social dominance test. Dominant-submissive pairs of rats were established as described under Materials and Methods based on time spent drinking from a common vessel containing sweetened-condensed milk. After a baseline determination, submissive rats received daily oral doses of GW803430 (30 mg/kg), and dominant rats received vehicle (2 ml/kg). The compound produced a time-dependent reduction in the difference in drinking time between the dominant and submissive rats (A) and a reduction in body weight of the submissive rat (B). The results are means ± S.E.M. of 11 pairs of animals. *, p < 0.05.

Fig. 10. GW803430 dose-dependently decreased marble-burying behavior of mice without affecting rotorod performances. Chlordiazepoxide (CDAP) is shown as a positive control. Each point represents the mean ± S.E.M. of eight mice. *, p < 0.05; **, p < 0.01. Marble burying was assessed by Dunnett’s test, and rotorod failures were assessed by Fisher’s exact test.

GW803430 (mg/kg, po)

Fig. 11. GW803430 did not significantly alter habituated locomotor activity of mice in contrast to the effects of 1 mg/kg d-amphetamine. Each bar represents the mean ± S.E.M. of eight mice (12 for vehicle control). **, p < 0.01 as assessed by ANOVA followed by post hoc Dunnett’s multiple comparison test.
weight in rats. Using ex vivo autoradiography, we established that this antagonist inhibits MCHR1 binding in three areas of the brain and exhibits substantial occupancy of the MCHR1 in these brain regions at doses of 1 mg/kg and greater. In subsequent studies using DIO rats, 14-day administration of the MCHR1 antagonist produced a robust and dose-dependent decrease in food intake and body weight. These results are consistent with previous results reported with this compound using DIO AKR/J mice (Hertzog et al., 2006). Given that treatment decreases feeding and body weight through off-target mechanisms, we evaluated the effects of 10 mg/kg GW803430 in MCHR1(+/+) and MCHR1(−/−) mice for 4 days. Using this paradigm, the compound should be effective in the MCHR1(+/+) but not the MCHR1(−/−) mice if the compound is producing its effects via MCHR1 antagonism. Consistent with the literature (Chen et al., 2002), untreated MCHR1(−/−) mice exhibited a slower rate of body weight gain than the untreated MCHR1(+/+) mice. When treated with compound for 4 days, the MCHR1(+/+) mice exhibited a robust decrease in body weight, whereas the MCHR1(−/−) did not. Therefore, GW803430 produced significant reductions in food intake and body weight via MCHR1 antagonism. In addition, these reductions occurred at doses that produced substantial MCHR1 antagonism, a relationship that further strengthens the validation of MCHR1 as the target protein for initiating these biological effects.

In recent years, MCH has been recognized as an important regulator of body weight. MCH- and MCHR1 (Chen et al., 2002; Marsh et al., 2002)-deficient mice exhibit decreased body weight compared with their MCHR1(+/+) littermates. The MCH knockout mice are lean and exhibit decreased food consumption. The MCHR1(−/−) mice are also lean but are hyperphagic, and their body weight reduction is secondary to increased metabolism and hyperlocomotion. In preclinical models, chronic intracerebroventricular administration of an MCHR1 antagonist reduced feeding, reduced body weight, and improved hypercholesterolemia, hyperinsulinemia, and hyperglycemia in DIO mice (Mashiko et al., 2005). A number of nonpeptide MCHR1 antagonists produce weight loss in preclinical models (for review, see Kowalski and Sasicumar, 2007; Luthin, 2007). Combined with the present data, these data add to the growing literature linking MCH receptor mechanisms to the regulation of food intake and body weight.

In a subsequent study, the ability of GW803430 to inhibit sweetened-condensed milk consumption was evaluated. This model was used to evaluate the effects of the compound on highly palatable food consumption before conducting the submissive behavior model that relies on sweetened milk consumption to assess dominant and subordinate relationships. During the baseline evaluation, there was a modest difference between the MCHR1(+/+) and MCHR1(−/−) mice, with the knockouts exhibiting reduced consumption. These results are surprising because the MCHR1(−/−) animals are reported to be hyperphagic (Chen et al., 2002). It is interesting that GW803430 had no effect on the consumption of sweetened condensed milk when administered before the feeding session on the fourth day in either mouse line compared with vehicle controls. Based on these results, it would not be anticipated that MCHR1 receptors participate in reward-based or palatable food consumption in mice. These results also decrease the likelihood that direct effects of the compound on sweetened milk consumption would be a confounding variable in the dominance assay.

These results seem to follow a consistent pattern with many of the other phenotypes seen in the MCHR1(−/−) mice that are not replicated by MCHR1 antagonists including hyperphagia, hyperlocomotion, and increased metabolism, suggesting significant developmental compensation has occurred in these mice. We have shown previously that the increased locomotor activity seen in the MCHR1(−/−) mice is due to a D1 receptor up-regulation compared with MCHR1(+/+) mice (Smith et al., 2005), but this was not observed when animals were treated with GW803430 for up to 3 weeks (data not shown). Therefore, it is likely that both the hyperphagia and increased metabolism are a consequence of the increased locomotor activity seen in the MCHR1(−/−) mice but not seen with MCHR1 antagonism.

Having established effective doses of GW803430 using both an occupancy measure and a pharmacodynamic response (feeding in the diet-induced obese rat), we assessed the ability of this compound to affect behavioral and biochemical measures in rat and mouse mood disorder models. To evaluate the potential of MCHR1 antagonism in the treatment of depression, we used the forced-swim test, a validated model of antidepressant action (Porsolt et al., 1977). In the mouse forced-swim test, GW803430 produced a dose-dependent antidepressant-like signature, as evidenced by decreases in immobility time. This effect reached statistical significance at a minimal effective dose of 3 mg/kg. Efficacy was also observed after 5-day administration of the compound, with a similar dose-response relationship. To ensure that these antidepressant-like effects of GW803430 were mediated through the MCHR1 receptor, we conducted similar studies in MCHR1(+/+) and MCHR1(−/−) mice. Although GW803430 was active in MCHR1(+/+) mice, when evaluated in MCHR1(−/−) mice, no statistically significant effect of the compound was observed, verifying that the effect was mediated through MCHR1 antagonism. It should be noted that the MCHR1 receptor +/+ mice were slightly more immobile under baseline conditions, and the drug effect was slightly less than in the NIH Swiss outbred mice (compare to Fig. 5), suggesting strain differences. Further substantiation that MCHR1 might be a relevant antidepressant drug target came from drug interaction studies. When combined, subeffective doses of GW803430 and imipramine produced a robust antidepressant-like effect in the forced-swim test that was significantly different from either compound alone. Thus, there is potential for the use of MCHR1 antagonists in combination with other antidepressants to improve efficacy and to reduce the side effects of conventional antidepressants. The generalization of the findings of antidepressant-like activity by an MCHR1 mechanism was enhanced by the concurrence of efficacy of GW803430 in another assay used to predict antidepressant efficacy. Thus, GW803430 was also active in the mouse tail-suspension test. In this test, a different strain of mouse was also used and, hence, provides additional assurance of the generality of the antidepressant-like effects of GW803430.

To further expand on the observed antidepressant-like activity of the MCHR1 antagonist in the mouse models, we used a dominance/submissive paradigm that has been used previously to model the delayed antidepressant onset of monoamine reuptake inhibitors (Malatynska et al., 2002). In this model, animal pairs were selected based on dominant and subordinate relationships. Once these relationships have been established, they will continue in a quantitative manner for at least 7 weeks (Malatynska et al.,
2007). Test compounds were administered daily to subordinate animals for 3 weeks. During the period of compound administration, the subordinate animals exhibited increased sweetened milk consumption (feeding time), consistent with an antidepressant-like action. In addition, it is important to note that we saw no effect of acute compound treatment on sweetened-condensed milk consumption in mice, indicating that effects on milk consumption per se should not be a confounding variable in the dominance assay. It is interesting that the antidepressant-like effect in this assay became apparent at 1 week and statistically significant at 2 weeks, indicating MCHR1 antagonists may have a more rapid onset of action than conventional agents. These findings contrast with data on the antidepressant fluoxetine in this model where we have previously observed antidepressant-like effects only after 3 weeks of daily dosing (10 mg/kg/day, data not shown). Investigators (Knapp et al., 2002) have previously reported 2 weeks of dosing under the conditions of their assay for 10 mg/kg fluoxetine. Full dose-response comparisons side-by-side under the same conditions will be essential for establishing differences in onset times.

Based on these results, the MCHR1 antagonist GW803430 produces antidepressant-like effects in three distinct models, in two species, and in different mouse strains. The antidepressant-like effects of GW803430 are not associated with locomotor stimulation, as reported in MCHR1 knockout mice (see Introduction). Because locomotor stimulation could result in false-positive findings in the mouse forced-swim and tail-suspension tests, these results further substantiate the potential antidepressant activity of this mechanism. Further support for this conclusion comes from the fact that antidepressant-like effects were observed at doses consistent with MCHR1 occupancy and that, in the mouse forced-swim test, the behavioral effects were absent in MCHR1 knockout mice. The ability of the monoamine-based antidepressant imipramine to enhance the antidepressant-like efficacy of GW803430 also adds to the target validation for MCHR1 for mood disorders and raises the possibility of MCHR1 blockade to be used as an adjunct in treatment-refractory patients.

First line therapies for mood disorders also have anxiolytic effects (e.g., selective serotonin uptake inhibitors). Engaging both antidepressants along with anxiolytic effects, therefore, should be a desirable feature of a new antidepressant. A host of data has accumulated that supports the anti-anxiety-like effects of MCHR1 antagonist (Borowsky et al., 2002; Chaki et al., 2005; David et al., 2007; Smith et al., 2008). We used marble burying to assess potential anxiolytic-like effects of the MCHR1 antagonists GW803430 (Borsini et al., 2002; Li et al., 2006). In this assay, GW803430 potently decreased marble burying at doses without any effect on motor behavior measured on the rotordor. The benzodiazepine anxiolytic chlordiazepoxide also decreased marble burying but only did so at a dose (30 mg/kg) that is effective in other anxiolytic tests in mice (Witkin et al., 2004) and at doses that produce motor-impairing effects (see also Li et al., 2006).

In a recent study, David et al. (2007) evaluated the MCHR1 antagonist SNAP 9487 in several models of anxiety and depression. In this study, the investigators reported anxiolytic-like activity in the mouse elevated plus maze and light/dark paradigm but did not see a significant effect in the mouse forced-swim test. They did see a robust anxiolytic-like effect of the compound in a novelty-suppressed feeding test that is proposed to be predictive of anxiolytic and antidepressant effects of compounds. Although the authors documented the presence of the compound in the brain, they did not use a receptor occupancy measure or a null control like the MCHR1-deficient mouse. In contrast to the present study, the authors also did not observe a decrease in home cage feeding with chronic administration. It will be difficult to resolve these discrepancies without understanding the relative receptor occupancy of SNAP 9487.

In summary, we have demonstrated antiobesity and antidepressant-like effects of a specific MCHR1 antagonist. These activities are produced at doses consistent with substantial MCHR1 occupancy and are not present in mice with the MCHR1 gene deleted. Therefore, MCHR1 antagonism is an attractive mechanism to target for future antidepressant and antiobesity drug discovery.

References


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