Preclinical Evaluation of Melanin-Concentrating Hormone Receptor 1 (MCHR1) Antagonism for the Treatment of Obesity and Depression

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Nonstandard abbreviations: GW803430, 6-(4-Chloro-phenyl)-3-[3-methoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-3H-thieno[3,2-d]pyrimidin-4-one; MCH, melanin-concentrating hormone; MCHR1, melanin concentrating hormone receptor 1; MCHR2, melanin concentrating hormone receptor 2; S036057: Tyr-(8-amino-3,6-dioxo octanoyl)-Arg-Cys-Met-Leu-Gly-Arg-Val-Phe-Arg-Pro-Cys-Trp; SNAP 9487: N-[3-(1-[[4-(3,4-difluorophenoxy)phenyl]methyl](4-piperidyl))-4-methylphenyl]-2-methylpropanamide.
Abstract

The mammalian neuropeptide, melanin-concentrating hormone (MCH) interacts with two G-protein coupled receptors, MCHR1 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 anti-obesity and antidepressant activity. Central activity of the selective MCHR1 antagonist, 6-(4-Chloro-phenyl)-3-[3-methoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-3H-thieno[3,2-d]pyrimidin-4-one (GW803430), was evaluated using ex vivo binding with autoradiography. Effective doses of GW803430 (1 and 3 mg/kg, p.o.) were correlated with anti-obesity activity in a 14 day study of diet-induced obese rats. Subsequently, GW803430 was evaluated 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 mg/kg, p.o. (acute) and 3 and 10 mg/kg, p.o. (chronic), 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 anti-obesity 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.
Introduction

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). Subsequently, MCH was subsequently identified 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 (Bittencourt et al., 1992; Skofitsch et al., 1985). 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) while 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 25-30% 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)). While MCHR2 is expressed in humans with a similar distribution pattern as compared to 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.,
MCHR1 antagonism decreases feeding and adiposity indicating the importance in regulating body weight and composition (for review see (Kowalski and Sasikumar, 2007)). While some work has identified mood-related phenotypes in the MCHR1-/- mice (Roy et al., 2007; Roy et al., 2006; 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 (ACTH) 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 (Kela et al., 2003; Monzon and De Barioglio, 1999). 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-like 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 upregulated (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 anti-obesity and antidepressant efficacy. We also establish that these activities are dependent on MCHR1 using MCHR1-/- mice.
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 those of the National Institutes of Health. Animals were individually housed in an environmentally controlled facility where food and water were available ad libitum.

Drugs and Melanin-Concentrating Hormone. 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 (>100x) 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 ddH₂O or 10% acacia and given by oral administration. Imipramine (IMI; Sigma Chemical Co St. Louis, MO) was dosed i.p. using 0.9% NaCl as the vehicle. Chlordiazepoxide HCl (Sigma Chemical Co.) was dosed i.p., in 0.9% NaCl. Compounds were dosed in a volume of 1ml/kg (rats) or 10 ml/kg (mice).

Rat Ex Vivo Autoradiography. Male Sprague Dawley rats, 225-250 g, were gavaged with vehicle (1ml/kg) or GW803430 in vehicle. Four hours after dosing, the rats were decapitated and the forebrains 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) and 0.4% bovine serum albumin (Sigma Aldrich, St. Louis, MO).
Louis, MO) (pH 7.4) containing 30 pM $^{125}$I- Tyr-(8-amino-3,6-dioxoctanoyl)-Arg-Cys-Met-Leu-Gly-Arg-Val-Phe-Arg-Pro-Cys-Trp (S36057, Perkin Elmer/NEN, Boston, MA, 2200 Ci/mmol) for 90 min at 25° C. Nonspecific binding was assessed by the addition of 10 µM GW803430 to the incubation media. Sections were rinsed twice for 10 minute each in buffer, then dipped twice in cold distilled water and dried quickly with a stream of cool air. Sections were exposed with $^{125}$I-Microscale Standards (Amersham, Pharmacia Biotech, Piscataway, NJ) on Phosphorimaging plates (Fuji) for 3 days. Quantitation was facilitated using the MCID Elite (St. Catherines, ON) image analysis. Curve fitting and ED$_{50}$ determinations were accomplished using Graphpad Prism software (San Diego, CA).

**Rat Feeding.** Diet-induced obese (DIO) Long Evans male rats (Harlan Sprague Dawley, Indianapolis, IN (starting average body weight was 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, 12-h dark; lights on at 2200 h) environment. The animals were housed individually throughout the study and permitted to eat high fat/high sucrose palatable diet (TD 95217; Teklad) and drink *ad libitum* throughout the study. Rats were randomized to four groups and each group was comprised of five animals. Rats were treated daily by oral gavage with vehicle (saline, 1 ml/kg), 0.1, 1, or 3 mg/kg of GW803430. Body weight and food intake were measured daily at 0900 h. All data are shown as the mean ± SEM, 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 x
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 3-5 months old and weighed 25-35 g during testing. Animals were maintained on a 12:12 light : dark cycle (lights on 22:00h – 10:00h) 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, Madison, WI). After 2 weeks acclimation to the facility, naïve mice were treated by oral gavage at 1 hour prior to 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 hours were measured daily. Statistical Analyses were performed by two-way ANOVA followed by Tukey’s t-test.

_Sweetened-Condensed Milk Consumption in MCHR1+/+ and MCHR1-/- mice._

Animals were maintained on a 12 hr light/dark cycle (lights on 22:00h – 10:00h) 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 Takled, Madison, WI). After 2 weeks acclimation to the facility, naïve mice were given 1:2 diluted sweetened-condensed milk for 2 hours at onset of dark period. On day 4, 2 hours before presentation of sweetened-condensed milk, mice were treated by oral gavage at 1 hour prior to onset of 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 Sprague-Dawley, Indianapolis, IN) weighing 20-25g were housed in plastic cages (40.6×20.3×15.2 cm) with 10-12 mice/cage in a vivarium at least 7 days before the
experiments. This mouse strain was used in this assay since they are the standard strain for which our lab 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 12hr light/dark cycle (0600/1800 hours), 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. (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 22-25°C water for 6 min. The duration of immobility was recorded during the last 4 minutes of a 6-minute 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 p.o., 4 hrs prior to testing. Imipramine was used as a comparator standard (15 mg/kg, p.o., 30 min prior). 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 (Steru et al., 1985). Male, C57BL/6 mice (Jackson Labs) weighing about 25 g were suspended by their tails from the edge of a shelf 80 cm above the floor. This mouse strain was used since 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 were recorded during a 5 min trial. Dose-effect functions for GW803430 were carried out by dosing mice p.o., 4 hrs prior to testing. Imipramine was used as a comparator standard (15 mg/kg, p.o., 30 min prior). Statistical Analyses were performed by ANOVA followed by Dunnett’s test.

**Locomotor Activity.** Since 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 were 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 p.o., 4 hrs prior to testing. For these experiments, mice were dosed 3 hrs prior to 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 prior to the locomotor recording period of 30 min.

**Social Dominance Test.** Similar to (Malatynska et al., 2002), experimentally-naïve male Sprague-Dawley rats, weighing 160-180 grams at the start of the study, (Harlan Sprague Dawley) were assigned randomly to pairs and the paired animals remained separated through the course of the experiment, except during the 5-minute testing periods. Rats were maintained on a 12hr light/dark cycle (0600/1800 hours), and
all procedures were performed between 12:00 PM and 4:00 PM in ambient room light. The testing apparatus was constructed from transparent plastic material and consisted of two identical chambers (24 x 17 x 14 cm) connected by a round tunnel (4.5 cm diameter x 52 cm long). A container (10 ml beaker) of sweetened milk (9 % sucrose) was placed in an opening in the floor at the mid-point of the tunnel. Animals were food deprived over-night prior to the first test session. During the testing period, each member of a pair was placed in the different chambers of the testing apparatus, the gates were opened, and the time spent drinking was recorded for each animal for a 5 minute period. At the end of the 5-minute testing period, animals were returned to their home cage and given free access to food for 1 hour. The animals were also given free access to food from Friday afternoon (following the test session) to Sunday morning, when they were once again food-deprived. During the first week of the testing (acclimation week), drinking time was not scored. During the second week of testing (selection week), the time spent drinking was recorded. Pairs of animals that passed the following criteria continued into the drug administration phase of the experiment: (1) the difference between the average daily drinking scores of the two animals was significant (two-tailed t-test, p < 0.05); and, (2) the dominant animal’s score was at least 25% greater than the submissive animal’s score. Any pairs not passing the selection criteria were dropped from the study. The animal of a pair with the higher drinking score was labeled as “dominant” and the animal of a pair with lower drinking score was labeled as “submissive”. The submissive rat of each pair was treated with the test compound while the dominant rat of each pair was treated with vehicle for the next 21 days. The 5-minute test was repeated once daily, except weekends, for 21 days following the beginning of the drug treatment. Dominance levels
were calculated as the difference in daily drinking scores between paired rats. The daily
dominance level values were averaged over each week and statistical comparisons were
made by analysis of repeated measurements coupled with Dunnet’s test. Body weight
was measured weekly and analyzed with Student’s t-test.

**Marble-burying and rotorod performance.** Marble-burying behavior in mice has
been reported to be inhibited by anxiolytics and is one of the only assays that might
detect anxiolytic activity of antidepressant agents after acute dosing (Borsini et al., 2002).
We used the method described by Li et al. (Li et al., 2006) which utilized male, NIH
Swiss mice (as described above in mouse forced swim methods). In these experiments,
20 marbles were placed atop 5 mm sawdust bedding within a plastic tank. After 30 min,
the number of marbles buried was counted (2/3 buried by sawdust). Prior to this test,
mice were placed on a rotating rod (6 rotations/min) and rotorod failures were defined if
a mouse fell off the rod on two occasions within a two min test period. No pretraining on
either test was performed. Dose-effect functions for GW803430 were carried out by
dosing mice p.o., 4 hrs prior to testing. Chlordiazepoxide HCl (i.p, 30 min prior) was
used as a comparator standard. The highest dose of 30 mg/kg was used as this is the
minimal effective dose in the marble-burying assay (Li et al., 2006) and the mouse Vogel
conflict test with food (Witkin et al., 2004). Effects of drugs on marble-burying were
assessed by ANOVA followed by Dunnett’s test. Drug effects on rotorod performance
were evaluated by Fisher’s exact probability test.
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 hours 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 $^{125}$I-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 (Figure 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 diet-induced obese (DIO) rats. In these studies, rats were administered the compound for 14 days during which food consumption and body weight were monitored. Compared to vehicle controls, there was a decrease in body weight at 0.1 mg/kg but this did not reach significance ($F_{1,16}=5.22$, $p=0.09$) (Figure 2A). A dose of 0.1 mg/kg produced a slight, but not significant reduction in cumulative food consumption though 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) (Figure 2B) and cumulative (p<0.05) food intake (Figure 2C). Food consumption and body weight were not significantly different when 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 GW 803430. MCHR1+/+ animals treated with compound exhibited a substantial decrease in food intake and body weight when compared the MCHR1+/+ mice treated with vehicle (Figure 3). Vehicle treated MCHR1-/- mice exhibited lower food intake when compared to vehicle treated MCHR1+/+ mice (F1,24=5.75, p<0.05). GW803430 treatment resulted in significant decreases in cumulative food intake on days 2, 3 and 4 (F1,24=15.36, p<0.001) and reductions in body weights on days 1, 3 and 4 (F1,24= 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 when compared to 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 one hour a day. During the first three days, a stable baseline was established and on the fourth day, the animals were treated with 10 mg/kg GW803430. During all four 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-/- (Figure 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 ($F_{4,34} = 4.91, p < 0.005$) (Figure 5A). When administered for five consecutive days, GW803430 produced a dose-dependent reduction in the immobility time with the 3 and 10 mg/kg doses reaching statistical significance ($F_{3,31} = 4.74, p < 0.005$) (Figure 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 in MCHR1-/− mice. Although GW803430 (10 mg/kg) decreased immobility in MCHR1+/+ mice, this effect was absent in MCHR1-/− mice (Figure 6A). ANOVA revealed a significant genotype by drug condition interaction ($F_{1,26} = 90.9, p < 0.005$), and non-significant 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 x strain interactions ($F_{1,27} = 0.33, p = 0.09$) (Figure 6B).

Exploring this effect further we evaluated the effect of combining subeffective doses of the MCHR1 antagonist with a subeffective dose of imipramine (Figure 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 than effects of either drug alone ($F_{1,28} = 4.12, p < 0.05$).
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 ($F_{4,38} = 7.02, p < 0.001$) (Figure 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) because it is believed to reflect the delayed onset of antidepressant action. In this study, a 30 mg/kg dose was administered for three weeks and the animals were assessed prior to treatment and at one week intervals for three weeks during treatment. In this study, GW803430 produced a reduction in submissive behavior (Figure 9A; $F_{3,14}=4.71, p=0.018$) 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 post dosing. 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 (Figure 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 ($F_{3,35} = 20.6, p < 0.0001$)
(Figure 10). Although the benzodiazepine anxiolytic chlordiazepoxide also decreased marble-burying at a dose (30 mg/kg), significant increases in rotorod deficits were observed; in contrast, GW803430 was without effect on rotorod performance at the same equieffective dose.

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 as a positive psychomotor stimulant standard. GW803430 (3-30 mg/kg, po) did not significantly alter locomotor activity of mice (3-30 mg/kg, po); in contrast d-amphetamine (1 mg/kg, i.p.) stimulated locomotion (Figure 11) (F4,43 =13.4, p<0.0001).
Discussion

In the present study, we have evaluated the effects of the specific MCHR1 antagonist, GW803430 on feeding and body 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 diet-induced obese (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 treatments can reduce feeding and body weight through off target mechanisms, we evaluated the effects of 10 mg/kg GW803430 in MCHR1+/+ and MCHR1-/-mice for four 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 four days, the MCHR1+/+ mice exhibited a robust decrease in body weight while 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 which 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 when compared to 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 i.c.v. 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 reviews see (Kowalski and Sasikumar, 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 prior to 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 since the MCHR1-/- animals are reported to be hyperphagic (Chen et al., 2002). Interestingly, GW803430 had no effect on the consumption of sweetened condensed milk when administered prior to the feeding session on the fourth day in either mouse line when compared to 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 previously shown that the increased locomotor activity seen in the MCHR1-/- mice is due to a D1 receptor upregulation when compared to MCHR1+/+ mice (Smith et al., 2005), but this was not observed when animals were treated with GW803430 for up to three 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 following 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 figure 5 A and B) 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 than 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 generality 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 utilized a dominance/submissive paradigm that has previously been used 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 seven weeks (Malatynska et al., 2007). Test compounds were administered daily to subordinate animals for three 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. Interestingly, the antidepressant-like effect in this assay became apparent at one week and statistically significant at two 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 two 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-/- mice (see Introduction). Since 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 antidepressant along with anxiolytic effects should therefore be a desirable feature of a new antidepressant. Indeed, a host of data has accumulated that supports the anti-anxiety-like effects of MCHR1 antagonism (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 rotorod. 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 and coworkers (David et al., 2007) evaluated the MCHR1 antagonist N-[3-([4-(3,4-difluorophenoxy)phenyl]methyl)(4-piperidyl))-4-methylphenyl]-2-methylpropanamide (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. While the authors documented the presence of the compound in
the brain, they did not use a receptor occupancy measure nor did they use 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 anti-obesity and antidepressant 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 anti-obesity drug discovery.
References


ATC0065 and ATC0175: nonpeptidic and orally active melanin-concentrating hormone receptor 1 antagonists. J Pharmacol Exp Ther 313: 831-839.


Legends for Figures

**Figure 1.** Dose-dependent inhibitor 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 calculated from the dose response curves (left hand side). Results in the graph are the mean (N=5). NS = nonspecific binding. A representative set of autoradiograms are shown on the right hand side as a pseudocolor reconstruction.

**Figure 2.** MCHR1 antagonist, GW803430, decreases cumulative body weight and food intake in diet-induced obese (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 Mean ± SEM of 5 animals per group. A. The effect of GW803430 on cumulative body weight changes in DIO rats. *p<0.05 versus vehicle control group; + p<0.05 versus GW3430 at 0.1 mg/kg dose group (One-way ANOVA repeated measures, Tukey’s multiple comparison test). B. The effect of GW803430 on daily food intake in DIO rats. GW803430 produced a significant reduction in daily food intake when compared to vehicle at all doses (p<0.001, One-way ANOVA repeated measures, Tukey’s multiple comparison test). C. The effect of GW803430 on cumulative food intake over the 14 day period. *p<0.05 versus vehicle control group (One-way ANOVA, Dunnet’s post-hoc test).
Figure 3. Cumulative food intake (A) and Body weight change (B) in male MCHR1+/+ and MCHR1-/- mice during treatment with 10 mg/kg GW803430. Rx denotes the initiation of compound dosing. Data are the mean ± SEM of 7 mice per group. *, p < 0.05 compared with MCHR1+/+ group. The results are Mean ± SEM of 5 animals per group.

Figure 4. GW803430 treatment does not affect sweetened-condensed milk (SCM) consumption in MCHR1+/+ and MCHR1-/- mice. Animals were allowed to establish baseline consumption for three days and were treated with vehicle or 10 mg/kg GW803430 in vehicle on the fourth day. Data are normalized to SCM consumption by the vehicle treated MCHR1+/+ mice. Data are the mean ± SEM of 6 mice per group.

Figure 5. The MCHR1 antagonist GW803430 reduces immobility time in the mouse forced swim test. Mice were pretreated orally with various doses of GW803430 one hour prior to being subjected to the forced swim test. A. Acute administration of GW803430 produced a significant reduction in immobility time at a dose of 3 mg/kg. This reduction was similar to that produced by acute administration of imipramine (IMI). B. Repeated oral administration produced a significant reduction in immobility time at doses of 3 and 10 mg/kg. *p<0.05. The results are mean ± SEM of 8 animals per group.

Figure 6. A. GW803430 (3 mg/kg, p.o.) produced a significant reduction in forced swim time in the MCHR1 +/+ but not the MCHR1-/- mice. B. Imipramine (IMI, 15 mg/kg,
i.p.) significantly decreased immobility in both MCHR1 +/+ and MCHR1−/− mice. The results are Mean ± SEM of 8 animals per group.

**Figure 7.** Subeffective doses of GW803430 (GW) and imipramine (IMI) when combined produce a significant decrease in immobility time in the forced swim test. GW803430 was administered 4 hours prior to testing and IMI was administered 30 minutes prior to testing. *p<0.05 compared to vehicle (veh) (Dunnett’s test), #p<0.05 compared to 1 mg/kg of GW803430, $p = 0.056$ compared to IMI 5 alone. Results are Mean ± SEM of 8 animals per group except for IMI at 15 mg/kg where N=4.

**Figure 8.** Oral administration of GW803430 decreased immobility in the mouse tail-suspension test. Each point represents the mean ± S.E.M. of 7-8 mice. * p < 0.05 by Dunnett’s test.

**Figure 9.** Oral administration of GW803430 reduced the dominance level in the rat social dominance test. Dominant-submissive pairs of rats were established as described in the Methods section based on time spent drinking from a common vessel containing sweetened-condensed milk. Following 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 Mean ± SEM of 11 pairs of animals. * p<0.05.
**Figure 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 ± SEM of 8 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.

**Figure 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 ± SEM of 8 mice (12 for vehicle control). *p < 0.01 as assessed by ANOVA followed by post-hoc Dunnett’s multiple comparison test.
GW803430 (mg/kg, p.o.)

**Caudate Putamen**
- ED$_{50}$ = 0.05 mg/kg

**Nucleus Accumbens--Core**
- ED$_{50}$ = 0.56 mg/kg

**Nucleus Accumbens--Shell**
- ED$_{50}$ = 0.15 mg/kg

% of Control

---

**mg/kg p.o.**

- Vehicle
  - 0.01
  - 0.03
  - 0.1
  - 0.3
  - 1
  - 3

NS

Figure 1
Figure 2

A. Cumulative Body Weight (g) over Time (day)

B. Daily Food Intake (g) over Time (day)

C. Cumulative Food Intake (g) over GW803430 (mg/kg, PO)
Figure 3

A

Cumulative Food Intake (g)

Treatment Day

MCHR1 +/+ Vehicle
MCHR1 +/+ GW803430
MCHR1 -/- Vehicle
MCHR1 -/- GW803430

B

Cumulative Weight Change (g)

Treatment Day

MCHR1 +/+ Vehicle
MCHR1 +/+ GW803430
MCHR1 -/- Vehicle
MCHR1 -/- GW803430

Rx
Figure 4

% of WT-Vehicle

Vehicle
10mg/kg GW803430

MCHR1 +/+  MCHR1 -/-

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A

Acute Dosing

Immobility (s)

vehicle  |  0.3 mg/kg  |  1 mg/kg  |  3 mg/kg  |  IMI 15
-------|------------|-----------|-----------|------
150    | 120        | 100       | 110       | 80

GW803430 (mg/kg, p.o.)

B

Five Day Dosing

Immobility (s)

vehicle  |  1 mg/kg  |  3 mg/kg  |  10 mg/kg
-------|-----------|-----------|-------
150    | 120       | 110       | 80

GW803430 (mg/kg, p.o.)

Figure 5
Figure 6
Figure 7

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Figure 7

Immobility (s)

- Vehicle 0.3 mg/kg GW
- 1 mg/kg GW
- 0.3 mg/kg GW + imi 5
- 1 mg/kg GW + imi 5
- IMI 15

* * *

Figure 7

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Figure 7

Immobility (s)

- Vehicle 0.3 mg/kg GW
- 1 mg/kg GW
- 0.3 mg/kg GW + imi 5
- 1 mg/kg GW + imi 5
- IMI 15

* * *

Figure 7

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Figure 8
Figure 9

A

Dominance Level (sec.)

Week

0 1 2 3

B

Percent Weight Gain (vs. Day 0)

Treatment Days

0 5 10 15 20 25

Vehicle

GW803430

*
Figure 10

A

Marbles Buried

Dose (mg/kg)

GW803430

CDAP

B

Rotorod Failures (%)

Dose (mg/kg)

0 3 10 30

0 30

**

*
Figure 11

Ambulations per thirty minutes

GW803430 (mg/kg, po)
veh d- amph 3 10 30

1 mg/kg, ip

GW803430 (mg/kg, po)