Metabotropic Glutamate Receptor mGlu5 Is a Mediator of Appetite and Energy Balance in Rats and Mice

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ABSTRACT

The metabotropic glutamate receptor subtype mGlu5 modulates central reward pathways. Many transmitter systems within reward pathways affect feeding. We examined the potential role of mGlu5 in body weight regulation using genetic and pharmacological approaches. Adult mice lacking mGlu5, mGluR5/H11001, weighed significantly less than littermate controls, despite no difference in ad libitum food intake. After overnight food deprivation, mGluR5/H11001 mice ate significantly less than their mGluR5/H11001 controls when refeeding. When on a high fat diet, mGluR5/H11001 mice weighed less and had decreased plasma insulin and leptin concentrations. The selective mGlu5 antagonist MTEP [3-(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine; 15 mg/kg s.c.] reduced refeeding after over-night food deprivation in mGluR5/H11001 mice, whereas MTEP had no effect on food intake in mGluR5/H11001 mice, demonstrating that feeding suppression is mediated via a mGlu5 mechanism. MTEP (1–10 mg/kg) decreased night-time food intake in rats in a dose-related manner. At 10 mg/kg, MTEP injected at 8.5, 4.5, or 0.5 h before refeeding reduced overnight food intake by approximately ~30%. Diet-induced obese (DIO) and age-matched lean rats were treated for 12 days with MTEP (3 or 10 mg/kg/day s.c.), dexfenfluramine (3 mg/kg/day s.c.), or vehicle. Daily and cumulative food intakes were reduced in DIO rats by MTEP and dexfenfluramine. Weight gain was prevented with MTEP (3 mg/kg), and weight and adiposity loss was seen with MTEP (10 mg/kg) and dexfen-fluramine. Caloric efficiency was decreased, suggesting increased energy expenditure. In lean rats, similar, although smaller, effects were observed. In conclusion, using genetic and pharmacological approaches, we have shown that mGlu5 modulates food intake and energy balance in rodents.

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ABBREVIATIONS: mGluR, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; CB1, cannabinoid receptor 1; SR1711416, N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide; DIO, diet-induced obese; MTEP, 3-(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine; dexfen, dexfenfluramine; VEH, vehicle; ANOVA, analysis of variance; AUC, area under the curve; MTII: melanotan II; AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide.

Metabotropic glutamate (mGlu) receptors have received considerable attention within the neurobiological sciences as potential therapeutic targets for multiple diseases due to their ability to modulate excitatory glutamate transmission and postsynaptic signaling (Conn and Pin, 1997; Spooren et al., 2001). Eight mGlu receptor subtypes are distinguished by sequence homology and signal transduction pathways. Group II (mGlu2 and 3) and group III (mGlu4, 6, 7, and 8) mGlu receptors couple to Gq/11 (Conn and Pin, 1997). Of the mGlu receptors, mGlu5 is implicated in the potential treatment of neurological and psychiatric disorders, including anxiety, depression, pain, and Parkinson's disease (Spooren et al., 2001). Additionally, recent work implicates mGlu5 in central reward pathways. Glutamate release into the core of the nucleus accumbens accompanies the behavioral sequelae of cocaine administration in sensitized rats (Pierce et al., 1996). mGlu5 mRNA is expressed in areas implicated in reward pathways, including nucleus accumbens and the hypothalamus (van den Pol et al., 1995; Kerner...
et al., 1997); this expression is modulated by repeated exposure to, and withdrawal from, cocaine and amphetamines (Ghasemzadeh et al., 1999; Mao and Wang, 2001). Mice lacking mGlu5 do not demonstrate full locomotor responses to cocaine and do not self-administer cocaine (Chiamulera et al., 2001). Pharmacologically, mGlu5 antagonists appear to modulate addictive behaviors; the mGlu5 selective antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Gasparini et al., 1999) reduces cocaine administration in mice (Chiamulera et al., 2001), reduces ethanol consumption in rats (Kelley and Berridge, 2002; Bäckström et al., 2004), and blocks the rewarding associations of cocaine in rodents without producing aversion (Popik and Wrobel, 2002; McGeehan and Olive, 2003). In addition, MPEP decreases nicotine self-administration in rats and mice (Paterson et al., 2003) and reduces cocaine self-administration (Chiamulera et al., 2001). To date, an effect of mGlu5 antagonists on food intake has not been demonstrated.

Consistent with the commonalities between different rewards, both glutamate and dopamine receptors in the nucleus accumbens modulate drug-seeking behaviors and food consumption (Taber and Fibiger, 1997). Several transmitter systems interact with mGlu5-containing cellular signaling pathways, including dopaminergic, cannabinoid, and serotonergic systems, and they have the potential to modify reward behavior through interactions at the nucleus accumbens and accompanying ventral striatal and mesolimbic dopamine systems (Kelley and Berridge, 2002; Kelley et al., 2002). For instance, group I metabotropic receptors directly modulate rat dopaminergic neurons in ventral midbrain slices (Morikawa et al., 2003). MPEP blocks methamphetamine-induced decreases in striatal dopamine and dopamine metabolites (Golembiowska et al., 2003). These studies demonstrate that multiple reward systems utilize interactions of mGlu5 with dopamine. Additionally, we have recently demonstrated that mGlu5 antagonists may disinhibit serotonin release (Bradbury et al., 2003). As serotonin reuptake inhibitors and releasers acutely decrease appetite (Halford et al., 1998) and chronically decrease body weight, mGlu5-serotonin interactions may similarly modulate reward or feeding pathways. Finally, the cannabinoid receptor 1 (CB1) inverse agonist SR171416 reduces refeeding after food deprivation (Ravinet Trillou et al., 2003), alcohol consumption (Arnone et al., 1997), and neurochemical responses to nicotine (Cohen et al., 2002).

Given that appetite is influenced by dopamine-mediated reward systems (Salamone and Correa, 2002), the studies above suggest that mGlu5 has the potential to modulate appetite control through dopamine reward system interactions. Together, the studies described above support mGlu5 involvement in neurotransmitter systems linked to acute reward-based feeding and, in the case of serotonin interactions, with metabolic endpoints as well. Thus, this lends to the notion that mGlu5 antagonists may reduce food intake and decrease weight gain in human populations. Moreover, localization of mGlu5 in the ventromedial hypothalamus (Romano et al., 1995) suggests a potential role for mGlu5 in metabolic regulation of energy balance. These converging lines of evidence led us to test the hypothesis that mGlu5 is an important modulator of feeding and energy balance in rodents.

Materials and Methods

Animals

Procedures described below were approved by the Institutional Animal Care and Use Committee of Merck Research Laboratories, San Diego in accordance with The Guide for the Care and Use of Laboratory Animals. Male mice lacking mGlu5, mGluR5<sup>−/−</sup>, used in experiments 1 and 2 below were purchased from The Jackson Laboratory (B6.129-Gprc1<sup>tm1Rod</sup>), Bar Harbor, ME) developed from a line of mGluR5<sup>−/−</sup> mice originated at the University of Toronto (Lu et al., 1997). The genetic background of the mice in this study was a mixture between C57BL/6 and 129. Genotyping of mice was performed by standard polymerase chain reaction procedures. Littermate wild-type mice (mGluR5<sup>+/−</sup>) were used to control for the effects of a mixed genetic background; both mGluR5<sup>−/−</sup> and mGluR5<sup>+/−</sup> mice were obtained from crosses between mGluR5<sup>−/−</sup> mice. Mice were used between the ages of 8 and 12 weeks.

Mice were maintained on an ad libitum diet of standard chow of 3.82 total kcal/g (7001; Teklad, Madison, WI; 4% dietary fat, 24% protein, and 72% carbohydrates). For characterization of diet-induced obesity, mGluR5<sup>−/−</sup> and mGluR5<sup>+/−</sup> mice were placed on a medium high fat diet of 4.7 total kcal/g (D12451; Research Diets, New Brunswick, NJ) with a composition of 45% fat, 35% carbohydrate, and 20% protein.

Male Sprague-Dawley rats used in experiment 3 were purchased from Harlan (Indianapolis, IN) at 125 to 175 g. Male Crl:CD(SD)IghSBR rats used in experiment 4 were purchased from Charles River Laboratories, Inc. (Wilmington, MA) at 12 weeks of age. Lean rats were maintained on standard rat chow of 3.82 total kcal/g (7001; Teklad; 4% dietary fat, 24% protein, and 72% carbohydrates) from weaning. Diet-induced obese (DIO) rats were maintained on a moderately high fat, high sucrose diet of 4.41 total kcal/g (D12269B; Research Diets; 32% dietary fat, 16% protein, and 51% carbohydrates) from weaning.

In all experiments, rodents were housed in a light- and temperature-controlled facility with a 12-h light/dark cycle (lights on at 6:00 AM) and were provided with rodent chows and water ad libitum until subjected to the experimental procedures described below.

Compounds. For these studies, we used 3-[(2-methyl-1,3-thiazol-4-yethylidencyl)-pyridine (MTEP) (Cosford et al., 2003), a selective antagonist at the mGlu5 with a 5-fold greater potency and greater selectivity for mGlu5 than the prototype antagonist MPEP. MTEP was prepared for these experiments by the chemistry department at Merck Research Laboratories, San Diego, CA. Dexfenfluramine (dexfen) was purchased from Sigma-Aldrich (St. Louis, MO). Both compounds were dissolved by sonication into a vehicle consisting of 10% Tween 80 (Sigma-Aldrich) and 90% saline (VEH).

Experimental Procedures

Characterization of mGluR5<sup>−/−</sup> and mGluR5<sup>+/−</sup> Mice. Daily body weights to the nearest 0.1 g and ad libitum food intake to the nearest 0.01 g were measured for 3 days. To compensate for the variation in body weight, food intake was normalized to body weights. Additional mice were deprived of food for 14 h, from 1 h prior to lights off until 1 h after lights on. Preweighed food was placed onto the cage bottom, and intake was measured for each mouse 15, 30, 60, and 210 min later. At the end of the experiment, a retro-orbital blood sample was taken for glucose measurements (OneTouch Ultra; Lifescan, Milpitas, CA) 2 h after lights on in either ad libitum-fed mice or mice that had been food-deprived for 14 h.

Responsiveness of mGluR5<sup>−/−</sup> and mGluR5<sup>+/−</sup> Mice to a High Fat Diet. Mice were placed on a medium high fat diet at 5 to 6 weeks of age. Mice were maintained on this diet for 16 weeks. At that point, mice were weighed, euthanized, and blood taken for measurement of plasma insulin and leptin concentrations.

Effect of MTEP on Food Deprivation-Induced Food Intake in mGluR5<sup>−/−</sup> and mGluR5<sup>+/−</sup> Mice. Mice on standard chow were...
food-deprived as above. At the end of the deprivation period, mice were weighed and injected with either VEH or MTEP (7.5 or 15 mg/kg s.c.). Food was provided immediately afterward and intake was measured 15, 30, 60, and 180 min later. Intake was normalized to body weight to account for variability in body weights.

**Effect of MTEP on Night-Time Food Intake in Rats.** To accurately assess night-time feeding, Sprague-Dawley rats were food-deprived from 2 h after lights on until refeeding with preweighed food 30 min prior to lights off. Rats were weighed at the nearest 0.01 g, were injected with either VEH or MTEP (3 or 10 mg/kg s.c.) at either 8.5, 4.5, or 0.5 h prior to refeeding. Food intake, to the nearest 1.0 g and injected with VEH or MTEP (7.5 or 15 mg/kg s.c.). Food was provided immediately afterward and intake was measured 15, 30, 60, and 180 min later. In a separate portion of the experiment, additional rats were food-deprived from 2 h after lights on until refeeding with preweighed food 30 min prior to lights off. Rats were injected with either VEH or MTEP (1, 3, or 10 mg/kg s.c.) 4.5 h prior to refeeding. Intake was normalized to body weight to account for variability in body weights.

**Effect of 12-Day Treatment with MTEP on Food Intake and Weight Gain in Diet-Induced Obese and Lean Rats.** Twelve-week-old lean and DIO CD rats were maintained in the facility on their respective diets for 9 weeks. Rats were adapted to feeding from the cage floor and to daily s.c. injections of vehicle for 1 week. After this vehicle period, rats within each diet group were weight-matched into 5 groups (n = 9–9 per group): uninjected, VEH, dexfen (3 mg/kg), and MTEP (3 or 10 mg/kg). Food intake and body weights were measured for 12 days. Intake was normalized to body weight to account for variability in body weights. On day 13, rats were injected and blood samples were obtained for leptin and insulin measurements from the lateral tail vein at the time that final food intake and body weights were measured. In a separate cohort of lean and DIO rats, tail vein samples were taken periodically up to 4 h after a single s.c. injection with MTEP (3 and 10 mg/kg) for measurement of compound concentrations.

**Assays.** Blood samples from experiment 4 were collected into serum clot-activating tubes (BD Biosciences, Franklin Lakes, NJ). Serum was obtained by centrifugation and frozen at −80°C until analyzed. Serum leptin and insulin were measured by radioimmunoassay (Linco Research, Inc., St. Charles, MO) according to manufacturer’s instructions with the exception that volumes of all reagents were reduced by half, and serum was diluted to ensure that values obtained were within the linear portion of the assay standard curve. Plasma concentrations of MTEP were analyzed by the Medicinal Chemistry Department of Merck, San Diego, previously described (Anderson et al., 2002).

**Statistics.** Data are presented as mean ± S.E.M. Data were analyzed by t test, one-way ANOVA, or two-way ANOVA (time versus treatment) as appropriate and corrected for repeated measures as necessary. Data not normally distributed were rank-transformed. In the event of main effects within ANOVA, sources of significant differences compared with control conditions were determined with either Dunnett’s or, in the case of rank-transformed data, Dunn’s post hoc tests. In all cases, significance is defined as p < 0.05.

**Results**

**Characterization of mGluR5+/+ and mGluR5−/− Mice.** The following experiments were performed to characterize the effects of the mGlu5 gene mutation on weight gain, ad libitum food intake, and postfood-deprivation refeeding. The weights of adult male mGluR5−/− mice were significantly reduced versus age-matched mGluR5+/+ controls (mGluR5+/+ 26.5 ± 0.5 g; mGluR5−/− mice: 24.0 ± 0.4 g, n = 22–24; p = 0.00015). Although the effect is modest, it has been observed in three cohorts of mice (M. J. Bradbury, L. J. Bristow, and D. Girancello, unpublished observations). Over the course of a 3-day measurement period, weight gain tended to be less in mGluR5−/− mice, as expressed in grams (Fig. 1A) or as a percentage gain with respect to starting body weight (mGluR5+/+: 1.3% gain in 3 days, mGluR5−/−: 0.6% gain in 3 days). Cumulative ad libitum food intake over the 3-day period was not different between mGluR5+/+ or mGluR5−/− mice (Fig. 1B), suggesting that a metabolic parameter may contribute to the reduced weight gain of these mGluR5−/− mice. To assess the effects of the mGlu5 gene mutation on appetitive responses, age-matched male mGluR5+/+ and mGluR5−/− mice were deprived of food overnight and allowed to refeed for 3.5 h starting 1 h after lights on. After overnight food deprivation, the amount of food eaten after 1 and 3.5 h of refeeding was significantly reduced in mGluR5−/− mice. A repeated measures

![Fig. 1. Effects of mGlu5 receptor mutation on food intake and weight gain. A, weight gain in mGluR5+/+ and mGluR5−/− mice over 3 days (n = 22 and 24, respectively). B, cumulative ad libitum food intake normalized in mGluR5+/+ and mGluR5−/− mice over 3 days (n = 22 and 24, respectively). C, cumulative food intake normalized to body weight is greater in mGluR5+/+ (open symbols) than mGluR5−/− (open symbols) after overnight food deprivation (n = 4/group). *, significant effect genotype on cumulative food intake 1 and 3 h after refeeding.](image-url)
analysis of variance revealed a significant main effect of genotype \(F(1,6) = 13.04, p = 0.011\), a significant effect of hours after refeeding \(F(3,18) = 44.24, p < 0.001\), and a genotype \(\times\) hours after refeeding interaction \(F(3,18) = 14.02, p < 0.001\). Weight loss during the overnight food-deprivation period was similar in both genotypes \((\text{mGluR5}^{+/+} = 10.7 \pm 0.4; \text{mGluR5}^{-/-} = 10.3 \pm 0.6\% \text{ loss with respect to starting weight})\). Glucose levels in ad libitum-fed mice \((\text{mGluR5}^{+/+} = 120 \pm 6; \text{mGluR5}^{-/-} = 139 \pm 6 \text{ mg/dl})\) and food-deprived mice \((\text{mGluR5}^{+/+} = 77 \pm 2; \text{mGluR5}^{-/-} = 82 \pm 3 \text{ mg/dl}, n = 7–11)\) were not affected by genotype. Activity levels in \(\text{mGluR5}^{-/-}\) mice appeared to be similar to those observed in \(\text{mGluR5}^{+/+}\) mice, as has been observed previously (O’Meara et al., 2002).

### Responsiveness of \(\text{mGluR5}^{+/+}\) and \(\text{mGluR5}^{-/-}\) Mice to a High Fat Diet

Mice were placed on a medium high fat diet at 5 to 6 weeks of age. Body weights of \(\text{mGluR5}^{-/-}\) mice were significantly less than their wild-type counterparts from 6 through 16 weeks on a high fat diet. At 6 weeks, \(\text{mGluR5}^{-/-}\) mice weighed approximately 20.7% less than \(\text{mGluR5}^{+/+}\) mice, and at 16 weeks, \(\text{mGluR5}^{-/-}\) mice were 24.6% less (Fig. 2A). Decreased plasma levels of leptin and insulin demonstrate that the \(\text{mGluR5}^{-/-}\) mice had less adiposity [Fig. 2B; \(t(16) 5.2, p < 0.001\)] and lower insulin values [Fig. 2C; \(t(16) 2.5, p = 0.02\)], suggesting that the absence of \(\text{mGlu5}\) may render mice less susceptible to diet-induced insulin resistance.

#### Effect of MTEP on Food Deprivation-Induced Food Intake in \(\text{mGluR5}^{+/+}\) and \(\text{mGluR5}^{-/-}\) Mice

Age-matched male \(\text{mGluR5}^{+/+}\) and \(\text{mGluR5}^{-/-}\) mice were deprived of food overnight and allowed to refeed for 3 h starting 1 h after lights on, immediately after injection of MTEP \((7.5\text{ or }15\text{ mg/kg s.c.})\). At a dose of 15 mg/kg, but not 7.5 mg/kg, MTEP significantly reduced food intake in \(\text{mGluR5}^{+/+}\) mice compared with that in vehicle-injected mice (Fig. 3A) [significant main effect of MTEP in two-way ANOVA, \(F(2,27) = 10.72, p < 0.001\)]. By 24 h, food intake of the MTEP-treated mice was not different from their vehicle-treated controls (data not shown). Previous data from our group show that at 30 and 60 min postadministration, receptor occupancy of MTEP \((15\text{ mg/kg s.c.})\) is approximately 100 and 50%, respectively (J. Anderson and M. J. Bradbury, unpublished observations). By contrast, MTEP had no effect on food intake at any of the doses tested in \(\text{mGluR5}^{-/-}\) mice (Fig. 3B), suggesting that the attenuated response to deprivation-induced refeeding observed in \(\text{mGluR5}^{-/-}\) mice treated with MTEP was due to antagonism at \(\text{mGlu5}\).

![Fig. 2](image1.png)  
**Fig. 2.** Effects of 16 weeks of high fat diet feeding on \(\text{mGluR5}^{+/+}\) and \(\text{mGluR5}^{-/-}\) mice. **A**. body weight from 6 to 16 weeks on high fat. **B** and **C**, plasma leptin and plasma insulin levels, respectively, in \(\text{mGluR5}^{+/+}\) and \(\text{mGluR5}^{-/-}\) mice after 16 weeks on high fat diet. *, significant effect genotype. \(n = 10\) mice/group.

![Fig. 3](image2.png)  
**Fig. 3.** Effects of \(\text{mGlu5}\) receptor mutation on postdeprivation refeeding after MTEP injection. \(\text{mGluR5}^{+/+}\) (A) and \(\text{mGluR5}^{-/-}\) (B) mice were injected s.c. with VEH or MTEP at 7.5 or 15 mg/kg after an overnight period of food deprivation. Food intake was measured thereafter for 3 h. * significant main effect in two-way ANOVA of MTEP at 15 mg/kg compared with VEH on food intake. \(n = 9\) to 13 per group.
Effect of a Single MTEP Injection on Night-Time Food Intake in Rats. Adult male rats were deprived of food starting 2 h into the light cycle and until food was returned to the cage at 0.5 h prior to lights out. MTEP (10 mg/kg s.c.) was injected at one of three time points during the light cycle, corresponding to 8.5, 4.5, and 0.5 h prior to refeeding (Fig. 4A). MTEP significantly reduced overnight food intake after injection at all three times (significant main effect of MTEP compared with VEH on food intake at each time measured. B, rats were deprived and refed as above. MTEP at 1, 3, or 10 mg/kg s.c. was injected 4.5 h prior to refeeding. * Significant effect of MTEP compared with VEH on food intake. n = 12/group.

Effect of 12-Day Treatment with MTEP on Food Intake and Weight Gain in DIO and Lean Rats. At the start of the 12-day MTEP administration study, the weights of lean and DIO rats were 411 ± 5 and 581 ± 8 g, respectively. In both the lean and DIO rat groups, weight gain and food intake variables were not different between VEH-injected and -uninjected rats (data not shown). DIO and lean rats tolerated s.c. injection of MTEP well with no apparent signs of distress, malaise, or diarrhea during the course of the experiment. In DIO rats, MTEP (3 and 10 mg/kg s.c.) and the positive control, dexfen, each reduced the total cumulative food intake measured at the end of the experiment compared with that in vehicle-injected rats [F(3,31) = 33.15, p < 0.001] (Fig. 5, A and B). The reduction in food intake in rats evoked by dexfen or MTEP (10 mg/kg) was greater early in the 13-day experiment and tended to attenuate by day 13 (Fig. 5A). In contrast, the effects of low-dose MTEP (3 mg/kg) were modest and sustained. MTEP, at both doses tested, and dexfen reduced weight gain throughout the time course of the study [F(3,32) = 31.06, p < 0.001] (Fig. 5C). As an estimate of food intake-independent effects of the compounds on weight gain, caloric efficiency or the total weight gain normalized to the total food intake was calculated. Dexfen and MTEP (10 mg/kg) reduced caloric efficiency [F(3,31) = 17.45, p < 0.001], whereas the effects of low-dose MTEP (3 mg/kg) were not significant (Fig. 5D). In lean rats, dexfen and MTEP at 10 mg/kg, but not 3 mg/kg, reduced food intake over the 13-day experiment [F(3,20) = 8.84, p < 0.001] (Fig. 6, A and B). As was seen with DIO rats, the reduction in food intake was most evident within the first days of the study (Fig. 6A). Despite the near-normal food intake in lean rats over much of the study, weight gain at 13 days was reduced in rats receiving MTEP at 10 mg/kg or dexfen [F(3,20) = 10.20, p < 0.001] (Fig. 6C). Caloric efficiency was also reduced in these groups [F(3,20) = 5.54, p = 0.006] (Fig. 6D) although qualitatively, the effects were less than those measured in DIO rats.

MTEP Plasma Levels in Lean and DIO Rats. Plasma concentrations of MTEP were measured over 4 h after a single injection in a separate cohort of lean and DIO rats (Table 1). At the low dose of 3 mg/kg MTEP, parent drug concentrations were higher in DIO rats compared with lean rats when measured at the 240-min time point and by area under the curve (AUC). MTEP at 10 mg/kg was absorbed more quickly in the lean than in the DIO rat, but the AUC...
mice were resistant to diet-induced obesity, they gained less weight, and had lower plasma insulin and leptin concentrations than their wild-type counterparts. The selective mGlu5 antagonist MTEP dose-dependently decreased fasting-induced refeeding in wild-type, but not mGluR5−/− mice, suggesting that acute reduction in food intake by MTEP is mGlu5-dependent. MTEP decreased food intake in rats, and when chronically administered, it decreased body weight and adiposity in lean and DIO rats.

**Acute Appetite.** In both rats and mice, MTEP decreased food intake. In mouse, pharmacological effects of MTEP were corroborated by the lack of drug efficacy in mGluR5−/− mice. Both pharmacological and genetic evidence indicated that mGlu5 influenced food deprivation-induced refeeding. The glutamatergic transduction mechanisms via mGlu5 underlying the reduced feeding following food deprivation are unknown. Possible mechanisms include a decreased perception of metabolic need, decreased rewarding properties of food (Kelley and Berridge, 2002), and overt aversion to a stimulus. The decrease in body weight gain in mGluR5−/− mice exposed to a highly palatable, high fat diet support the notion that decreased reward systems and thus food intake may contribute to their slower weight gain. Possibly, MTEP reduced food intake by a mechanism- or compound-related stimulation of aversive behaviors. However, MPEP, an analog of MTEP, does not induce conditioned place aversion in mice or rats (Popik and Wrobel, 2002; McGeehan and Olive, 2003), reducing the likelihood that mGlu5 compounds as a class are aversive or that mechanism-based aversiveness is prevalent. We have observed similar reductions in food intake with MPEP in rats and mice and other mGlu5 antagonists with chemically distinct structures (M. J. Bradbury and D. Chapman, unpublished results). Additionally, we did not observe overt signs of discomfort after MTEP or MPEP injection.

**TABLE 1**

Plasma concentrations of MTEP (µM) in lean and DIO rats after a single s.c. injection

<table>
<thead>
<tr>
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<th>5 Min</th>
<th>15 Min</th>
<th>30 Min</th>
<th>60 Min</th>
<th>120 Min</th>
<th>240 Min</th>
<th>AUC (0–240 Min)</th>
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<tbody>
<tr>
<td>Lean</td>
<td></td>
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<tr>
<td>MTEP (3 mg/kg/day)</td>
<td>0.36 ± 0.12</td>
<td>0.80 ± 0.37</td>
<td>1.14 ± 0.22</td>
<td>1.21 ± 0.07</td>
<td>0.75 ± 0.05</td>
<td>0.02 ± 0.01</td>
<td>161 ± 12</td>
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<tr>
<td>MTEP (10 mg/kg/day)</td>
<td>4.52 ± 0.79</td>
<td>5.46 ± 0.68</td>
<td>5.19 ± 0.05</td>
<td>6.19 ± 0.64</td>
<td>4.59 ± 0.11</td>
<td>0.81 ± 0.40</td>
<td>948 ± 9</td>
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<tr>
<td>DIO</td>
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<td></td>
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<tr>
<td>MTEP (3 mg/kg/day)</td>
<td>0.69 ± 0.07</td>
<td>0.73 ± 0.02</td>
<td>1.22 ± 0.36</td>
<td>1.10 ± 0.27</td>
<td>1.36 ± 0.22</td>
<td>1.09 ± 0.11*</td>
<td>277 ± 32*</td>
</tr>
<tr>
<td>MTEP (10 mg/kg/day)</td>
<td>1.44 ± 0.81</td>
<td>2.10 ± 0.61*</td>
<td>1.66 ± 0.64*</td>
<td>2.47 ± 0.38*</td>
<td>4.54 ± 1.42</td>
<td>6.16 ± 1.42</td>
<td>960 ± 246</td>
</tr>
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</table>

* Statistically different from lean rats at same dose by two-tail t test, p < 0.05.
In rats, the time course for MTEP’s efficacy on food intake after a single administration far exceed the reported half-life of receptor occupancy in vivo (Anderson et al., 2003). For example, administration of MTEP in rats at 8.5 h prior to the dark cycle suppressed overnight food intake to an equal extent to MTEP given 30 min before the dark cycle. MTEP at 3 mg/kg i.p. results in 100% receptor occupancy within minutes and decreases to near zero by 4 h (Anderson et al., 2003). Mechanistically, MTEP’s ability to suppress feeding after receptor occupation has terminated is not understood. However, this extended efficacy in the absence of circulating compound is observed with other anorectic agents. For example, MTII, a nonselective melanocortin agonist, when injected i.v. has a half-life in rats of approximately 0.5 h (Mock et al., 2002), yet decreases food intake overnight (Trivedi et al., 2003). The consequence of receptor modulation may trigger a cascade of downstream events that block rebound feeding later in the night.

**Chronic Changes in Weight Gain.** mGlu5-mediated changes in body weight have not been previously demonstrated. Here, we have shown that mGlu5−/− mice on a high fat diet not only weighed less but had decreased body weight and adiposity. Furthermore, reduced weight gain after chronic MTEP administration in DIO rats recapitulates this observation. Plasma leptin decreases with MTEP treatment, demonstrating that body weight loss results, at least in part, from decreased adiposity. The suppression of weight gain is clearly initiated by the decreased feeding. However, in both lean and DIO rats, after repeated injection of MTEP, feeding suppression abated, whereas a reduction in the rate of weight gain persisted. This effect, quantitated by a decrease in caloric efficiency, argues for an additional metabolic component contributing to the net body weight change. These time-dependent changes in body weight and food intake, with an initial weight decrease with subsequent body weight gain paralleling that of vehicle-treated rats, are similar to that of several other mechanisms in which gene deletions of key components recapitulate ligand-induced changes in metabolism. Examples include CB1R and melanocortin 4 receptor, and several serotonergic receptors modulated by dexamphetamine and sibutramine (Kalra et al., 1999). It is postulated that the increase in food intake within a few days of repeated administration reflects a separate process from that which occurs during steady state of sustained weight loss.

Thus, it is unclear whether the discordance of the time-dependent observations in the 14-day study on body weight and food intake reflect receptor tolerance per se or equivalent receptor actions of MTEP with concomitant counterbalancing effects on food intake. There are at least two reports, however, that tolerance to some aspect of the MTEP or MPEP administration can occur. First, the anxiolytic actions of MTEP are reduced after 4 days of administration (Busse et al., 2004). Second, neuroendocrine responses to MPEP were nearly completely attenuated after repeated administration. This paradigm also reduced the sensitivity of the hypothalamic-pituitary adrenal axis to the stimulatory effects of bu- spirone (Bradbury et al., 2003). Repeated administration of MPEP did not alter mGlu5 or 5-hydroxytryptamine 1A, K1, Bmax, or serotonin-stimulated guanosine 5’-3’-O-(thio)triphosphate binding. Thus, there is precedence for downstream plasticity to modify responses to mGlu5 antagonists in the absence of receptor tolerance at the mGlu5 receptor. In addition, the level of receptor occupancy may play a role in the time course of reduced efficacy over time. MPEP at doses that generate only partial receptor occupancy does not appear to tolerate in anxiety and depression assays (Plic et al., 2002), however, MTEP at high doses (leading to complete receptor occupancy) does tolerate in a different anxiety assay of punished responding (Busse et al., 2004).

Based on the broad localization of mGlu5 and the network of sites that modulate energy homeostasis, several central nervous system regions could be involved in mGlu5-mediated regulation of energy balance. The lateral hypothalamic area has dense mGlu5 expression, is a major integrative site for converging reward and metabolic information, and has long been implicated in modulation of energy balance (Kalra et al., 1999). The lateral hypothalamic area also serves as the anatomical substrate by which the nucleus accumbens mediates reward-based feeding (Kelley and Berridge, 2002). The paraventricular nucleus of the hypothalamus is a second important integrative site for appetite and metabolic integration. Fewer mGlu5 are localized here, but in this region, metabotropic glutamatergic interactions with endocannabinoids have been demonstrated, thus suggesting this as a plausible site for mGlu5-containing neurons modulating energy balance. mGlu5 are also richly expressed in the subparaventricular nucleus of the hypothalamus zone, an area with extensive projections throughout the hypothalamus.

MTEP exerted greater acute and chronic efficacy when given to DIO rats compared with lean rats. Plasma concentrations of MTEP in the lean and DIO rats indicate that at the time ad libitum food intake began, sufficient circulating compound was present to fully occupy receptors. MTEP may be better absorbed and retained in the DIO animal. The longer time to absorption of the high dose of MTEP in the DIO rats may be due to increased adiposity (Cheymol, 2000), and thus, this may impact the time course of efficacy. With a low dose administration of 3 mg/kg MTEP, there was a small but significant increase in circulating MTEP in DIO rats compared with lean rats as measured by AUC. Although there was no significant change in MTEP levels between lean and DIO rats after 10 mg/kg MTEP administration, these data may underestimate group differences since plasma collection terminated at 240 min, a time at which plasma MTEP levels in the DIO rats were just reaching maximal plasma levels. In addition, lean rats may have greater resistance to weight loss due to their reduced adiposity. This has been demonstrated with other anorectic agents; the nonselective melanocortin receptor agonist MTII administered to low fat and high fat-fed rats with differences in adiposity results in a greater anorectic effect in the fatter rat (Clegg et al., 2003). In this case, the central administration of MTII obviates the pharmacokinetic differences that can occur with obesity (Cheymol, 2000). A similar increase in efficacy is seen with peripheral administration of cannabinoid inverse agonists. Zucker fatty rats have a greater suppression in food intake to the CB1 inverse agonist AM251 with less tolerance than their lean counterparts (Vickers et al., 2003). It is not known whether the AM251 effect is related to adiposity or pharmacokinetic differences.

In summary, the genetic and pharmacologic data presented demonstrate a role for mGlu5 in appetite and energy homeostasis. The acute and chronic appetite suppression and reductions in body weight and adiposity in obese rats suggest
a novel role for mGlur5 for intervention in the treatment of chronic obesity in humans.

References


