Behavioral/Systems/Cognitive

The Hypothalamic Neuropeptide Melanin-Concentrating Hormone Acts in the Nucleus Accumbens to Modulate Feeding Behavior and Forced-Swim Performance

Dan Georgescu,[1] Robert M. Sears,[1,2] Jonathan D. Hommel,[1] Michel Barrot,[1] Carlos A. Bolaños,[3] Donald J. Marsh,[4] Maria A. Bednarek,[5] James A. Bibb,[1] Eleftheria Maratos-Flier,[6] Eric J. Nestler,[1] and Ralph J. DiLeone[1,2]

[1]Department of Psychiatry, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390-9070, [2]Division of Molecular Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06508, [3]Departments of Psychology and Neuroscience, Florida State University, Tallahassee, Florida 32306, Departments of [4]Metabolic Disorders and [5]Medicinal Chemistry, Merck Research Laboratories, Rahway, New Jersey 07065, and [6]Division of Endocrinology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215

Melanin-concentrating hormone (MCH) is a hypothalamic neuropeptide with a prominent role in feeding and energy homeostasis. The rodent MCH receptor (MCH1R) is highly expressed in the nucleus accumbens shell (AcSh), a region that is important in the regulation of appetitive behavior. Here we establish a role for MCH and MCH1R in mediating a hypothalamic–limbic circuit that regulates feeding and related behaviors. Direct delivery of an MCH1R receptor antagonist to the AcSh blocked feeding and produced an antidepressant-like effect in the forced swim test, whereas intra-AcSh injection of MCH had the opposite effect. Expression studies demonstrated that MCH1R is present in both the enkephalin- and dynorphin-positive medium spiny neurons of the AcSh. Biochemical analysis in AcSh explants showed that MCH signaling blocks dopamine-induced phosphorylation of the AMPA glutamate receptor subunit GluR1 at Ser[845]. Finally, food deprivation, but not other stressors, stimulated cAMP response element-binding protein-dependent pathways selectively in MCH neurons of the hypothalamus, suggesting that these neurons are responsive to a specific set of physiologically relevant conditions. This work identifies a novel hypothalamic–AcSh circuit that influences appetitive behavior and mediates the antidepressant activity of MCH1R antagonists.

Key words: **feeding; MCH; accumbens; lateral hypothalamus; neuropeptide; obesity; depression; appetite**

Introduction

Feeding is a complex behavior under the control of both homeostatic and hedonic mechanisms (Ahima and Osei, 2001; Corbit et al., 2001; Saper et al., 2002; Flier, 2004). Early lesion studies identified the hypothalamus as a critical brain region controlling both feeding and metabolism. More recent work has confirmed the importance of the hypothalamus as the primary target for peripheral signals of metabolic state. For example, neurons located in the arcuate nucleus of the hypothalamus express receptors for leptin, a key protein hormone produced in adipocytes. Although current models suggest that the arcuate nucleus communicates with second-order feeding centers, such as the paraventricular nucleus or lateral hypothalamus (LH) (Friedman and Halaas, 1998; Cowley et al., 2001; Elmquist, 2001), little is known about the mechanisms and pathways that convey information to extrahypothalamic sites.

The LH in particular has long been regarded as an important brain structure in regulating feeding behavior, and recent molecular studies have identified the neuropeptide melanin-concentrating hormone (MCH), which is selectively expressed in a subset of LH neurons (Broberger et al., 1998), as a key orexigenic signal. Intracerebroventricular injection of MCH induces feeding in rodents, and fasting upregulates MCH mRNA expression in the LH (Qu et al., 1996). Moreover, prepro-MCH knockout (MCH-KO) mice are hypophagic and lean (Shimada et al., 1998), whereas mice that overexpress MCH are obese and hyperleptinemic (Ludwig et al., 2001).

Additional genetic and pharmacological evidence suggests that MCH has an important role in energy homeostasis, affecting both feeding and energy expenditure. Mice lacking both MCH and leptin show marked attenuation of the obesity seen with leptin deficiency alone (Segal-Lieberman et al., 2003). This effect is caused by both increased activity and increased basal energy expenditure, suggesting potential roles of MCH beyond feeding. In addition, mice missing the receptor for MCH (MCH1R-KO) are lean, although, in contrast to MCH-KO mice, they are hyperactive and hyperphagic (Chen et al., 2002; Marsh et al., 2002). Interestingly, systemic administration of an MCH1R antagonist to rodents attenuates feeding, reduces anxiety, and mimics antidepressant effects in the forced swim test (FST) (Borowsky et al., 2002; Takekawa et al., 2002).

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Correspondence should be addressed to Ralph J. DiLeone, Division of Molecular Psychiatry, Yale University School of Medicine, 34 Park Street-CMHC, New Haven, CT 06508. E-mail: ralph.dileone@yale.edu.

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Although these studies implicate MCH signaling in feeding and mood-related behavior, the site of action of MCH is not clear. Although work has suggested that MCH can drive feeding via medial hypothalamic nuclei (Abbott et al., 2003), MCH1R is expressed in several extrahypothalamic regions and is particularly enriched in the nucleus accumbens shell (AcSh) (Lembo et al., 1999; Saito et al., 1999). The nucleus accumbens is a region of the brain well known for its role in drug addiction (Hyman and Malenka, 2001; Koob and Le Moal, 2001; Nestler, 2001; Salamone et al., 2003) and may represent a forebrain target where hypothalamic signals could be converted into motivated behavior. Here we show that MCH signaling in the AcSh regulates feeding behavior and modulates performance in the forced swim test. This novel hypothalamic–limbic circuit begins to define brain regions and cellular mechanisms that mediate both the anorectic and the antidepressant action of MCH1R antagonism.

Materials and Methods

Subjects. Sprague Dawley rats (Charles River, Kingston, NC), C57BL/6 mice, cAMP response element (CRE)-LacZ reporter mice (line 37) (Barrot et al., 2002), and MCH-KO mice (Shimada et al., 1998) were used for experiments. Rats were housed alone, whereas mice were housed four to five per cage in a colony maintained at constant temperature (23°C) with a 12 h light/dark cycle (lights on from 7:00 A.M. to 7:00 P.M.) and *ad libitum* food and water. Rats weighed between 300 and 360 g at the beginning of the experiments, and mice were between 6–12 weeks of age. All animal protocols used were in compliance with University of Texas Southwestern Medical Center and Yale University animal regulations.

Drugs. MCH peptide was obtained from Bachem AG (Torrance, CA) and dissolved in artificial CSF (ACSF). The MCH1R peptide antagonist has been described previously [compound 30 from Bednarek et al. (2002)]. The reagent was resuspended in ACSF immediately before use.

Surgery. Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). Coordinates for placement of the guide cannula to end 1.0 mm above the injection point in the AcSh were as follows: anteroposterior (AP), +1.7 mm; mediolateral (ML), −0.75 mm; and dorsoventral (DV), −5.4 mm (Paxinos and Watson, 2004). The coordinates for nucleus accumbens core (AcCo) were as follows: AP, +1.7 mm; ML, +2.5 mm; and DV, −5.9 mm. After a midline incision was made over the skull, the skull was scraped clean and allowed to air dry for ~5 min. Small holes were drilled into the skull, and self-tapping stainless-steel screws were placed to secure the cranioplastic cement (Plastics One, Roanoke, VA) to the skull. Next, the sites for the cannula guides were marked, and holes were drilled through the skull for the bilateral placement of the 26 gauge stainless-steel guides. Once in place, the guides were attached to the skull using cranioplastic cement. When the cement was dry, the animal was removed from the stereotaxic instrument, and sterile obturators (33 gauge; 0.8 mm longer than the guide cannula) were inserted into the guides to prevent them from clogging and to reduce the potential for brain infection. The obturators were checked daily, cleaned with a sterile saline solution, and replaced as needed.

Microinjection procedure. The rats were gently hand-held while the obturators were removed and the injectors were inserted. The injectors were 0.8 mm longer than the guide cannulas. Once the injectors were inserted, the rats were placed into an open cage and allowed to move freely during the infusion. The infusion pumps were operated for 1 min, delivering 0.5 μl of drug or vehicle per side. The injectors were left in the brain for 1 additional minute after the end of the injection before being removed, and the sterile obturators were then replaced. After the injection, the rats were placed back in their home cage, where they remained until the start of the test sessions.

Feeding behavior assay. Sprague Dawley rats were allowed to acclimate for 1 week after arrival before a guide cannula (Plastics One) was implanted in AcSh bilaterally. After surgery, the rats were allowed to recover for 1 additional week, followed by daily manipulation and habituation to the injection procedure for 4 d. On the day of the test, food was removed at 6:30 P.M., and the rats were allowed to remain in their home cage until the drug or vehicle control was injected into the brain. After the injection, the rats were returned to their home cage, and 30 min later they were allowed full access to regular chow. Unless otherwise specified, the experiment started in the first 30 min after the beginning of the dark cycle (7:00 P.M.), and food intake was measured over 4 h.

FST paradigm. The FST is a 2 d procedure in which rats swim under conditions in which escape is not possible. In the FST paradigm for rats, on the first day, animals are forced to swim for 15 min. They initially struggle to escape from the water, but eventually they adopt a posture of immobility in which they make only the movements necessary to keep their heads above water. When the rats are retested 24 h later, latency to immobility is decreased and the total immobility time is increased. Treatment with standard antidepressant drugs within the 24 h period between the first exposure to forced swimming and retesting can block facilitated immobility, an effect that is highly correlated with antidepressant efficacy in humans (Porsolt et al., 1977a; Detke et al., 1995; Carlezon et al., 2002). On the first day of the FST, rats were placed in clear, 65-cm-tall × 25-cm-diameter cylinders filled to 25 cm with 25°C water. After 15 min of forced swimming, the rats were removed from the water, dried, and placed back in their home cage. The cylinders were emptied and cleaned between rats. Rats tested with MCH received three injections in the AcSh. The first injection was administered 15 min after the pretest, the second injection was given 6 h before the test, and the third injection was given 30 min before the test. At 24 h after the forced swim, rats were retested for 6 min under identical swim conditions. Retest sessions were videotaped from the top of the cylinders and scored using a behavioral sampling method (Porsolt et al., 1977a; Detke et al., 1995; Carlezon et al., 2002) by raters unaware of the treatment condition. Rats were rated at 2 s intervals throughout the duration of the retest session; at each 2 s interval, the predominant behavior was assigned to one of two categories: immobility or swimming. A rat was judged to be immobile if it was making only movements necessary to keep its head above water and judged to be swimming if it was actively making swimming movements that caused it to move within the center of the cylinder.

The FST protocol used for the MCH-KO mice was similar to the one used for rats, with following exceptions: the containers used were 500 ml beakers filled three-quarters with 25°C water, and central injections were not performed. The mice were videotaped from the side, and scoring was done using the same procedure described above. Finally, FST analysis of mice was a 1 d, one trial procedure, as originally described (Porsolt et al., 1977b).

Locomotor activity assay. Sprague Dawley rats implanted with bilateral cannulas in the AcSh were habituated to circular locomotor chambers (MED Associates, St. Albans, VT) and to the microinjection procedure for 3–4 d starting at 7:00 P.M. The animals were then split into two groups based on the average performance on the training days. On the test day, the drug tested or ACSF was delivered 30 min before the animals were placed in the boxes, and locomotor activity was measured over 2 h.

Preparation and treatment of AcSh slices. Male C57BL/6 mice (8–12 weeks of age) were killed by decapitation, and brains were rapidly blocked and fixed to the cutting surface of a Vibratome (Ted Pella, Redding, CA) maintained at 4°C. Brains were placed in cold, oxygenated (95% O_2 and 5% CO_2) bicarbonate buffer (in mM: 125 NaCl, 5 KCl, 26 $NaHCO_3$, 1.5 $CaCl_2$, 1.5 $MgSO_4$, and 10 glucose, pH 7.4). Coronal slices of mouse brain (400 μm in thickness) were cut and pooled in 10 ml of cold buffer. AcSh slices were cut from the coronal sections under a dissecting microscope. The slices were pooled and then transferred individually to 4 ml polypropylene tubes containing 2 ml of fresh, cold, oxygenated buffer. The tissue was incubated for 30 min at 30°C, the buffer was replaced, and tissue sections were incubated for an additional 30 min. At the end of this second incubation period, the buffer was replaced with fresh buffer or buffer containing MCH (10 μM) or MCH1R antagonist (10 μM) for 30 min. Finally, designated slices were incubated with SKF 81297 for an additional 5 min. All of the pretreatment and treatment solutions also contained 10 μg/ml adenosine deaminase (Sigma-Aldrich, St. Louis, MO). After each treatment, the slices were immediately frozen on dry ice and stored at −80°C until they were assayed.

Immunoblotting for phospho-glutamate receptor 1. Frozen tissue sam-

ples were sonicated in 1% SDS. Small aliquots of the homogenate were retained for protein determination by the BCA protein assay method (Pierce, Rockford, IL) using bovine serum albumin as a standard. Equal amounts of protein (40 μg) were loaded onto 8% acrylamide gels. The proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes (0.2 μm; Schleicher & Schuell, Keane, NH). Membranes were blocked for 30–60 min in PBS (in mM: 124 NaCl, 4 KCl, 10 Na_2HPO_4, and 10 KH_2PO_4, pH 7.2) containing 5% nonfat dry milk and 0.2% Tween 20 (Blotto). The membranes were immunoblotted using an antiserum that selectively detects the Ser^{845}-phosphorylated form of glutamate receptor 1 (GluR1) (1:5000; Upstate Biotechnology, Lake Placid, NY) or an antiserum (1:5000; Chemicon, Temecula, CA) that detects the C-terminal region of GluR1, regardless of phosphorylation state. Antibody binding was revealed by incubation with a goat anti-rabbit horseradish peroxidase (HRP)-linked IgG (1:6000–8000 dilution; Pierce) and the SuperSignal West Dura immunoblotting detection system (Pierce). Chemiluminescence was detected by autoradiography using Kodak MS (Eastman Kodak, Rochester, NY) autoradiography films and the bands were quantified by analysis of scanned images using NIH Image 1.52 software. Because the linear range for quantitation of ECL signals by densitometry is limited, several film exposures were obtained for each set of samples to ensure that signals were within a density range that allowed accurate quantitation. In all of the experiments for this study, nitrocellulose membranes were sequentially analyzed for phospho-Ser^{845} GluR1 and then for total levels of C-terminal GluR1. After probing a membrane for phospho-GluR1, the filter was washed three times for 5 min each in TBS plus Tween 20 to remove any remaining chemiluminescent reagent. The membrane was then stripped of antibody by incubation at room temperature for 15 min in Restore Western Blot Stripping Buffer (Pierce). The filter was washed several times in large volumes of PBS and blocked in Blotto for 30–60 min before immunoblotting with the C-terminal GluR1 antibody.

Fluorescent in situ *hybridization.* Probes for double *in situ* hybridization with MCH1R were prepared using an *in vitro* transcription kit with digoxigenin-labeled (Roche, Basel, Switzerland) or fluorescein-labeled (Molecular Probes, Eugene, OR) UTP for making the dynorphin (Dyn) and enkephalin (Enk) probes. For the *in situ* analysis, fresh frozen brains were cryosectioned at 14 μm thickness and dried onto slides. The sections were then fixed in ice-cold 4% paraformaldehyde for 20 min, dehydrated in an ethanol series, and allowed to air dry. The sections were rehydrated, acetylated for 5 min, dehydrated, and air dried again. The hybridization mix (50% formamide, 5× SSC, 5× Denhardt's solution, 250 μg/ml yeast RNA, 0.5 mg/ml salmon testes DNA, and 200–300 ng/ml RNA probe) was then added to the slides, which were incubated in humidified chambers at 60°C overnight. After washing and blocking with 5% normal rabbit IgG and 1% blocking reagent (Roche), the MCH1R probe was first detected by using of a 1:200 anti-digoxigenin antibody coupled to HRP (Dako, Carpinteria, CA). The digoxigenin signal was amplified and detected using tyramide signal amplification (TSA)-direct coupled to cyanine 3 (PerkinElmer, Wellesley, MA). Hydrogen peroxide treatment (3%, 15 min) was used to eliminate HRP activity. The fluorescein-labeled probe was detected with 1:500 rabbit anti-fluorescein coupled to HRP (Molecular Probes), followed by amplification with TSA-direct coupled to fluorescein (PerkinElmer). The sections were then dehydrated and mounted in DPX (Fluka, Neu-Ulm, Germany).

Immunohistochemical studies. Immunohistochemistry was performed as described previously (Georgescu et al., 2003) using goat polyclonal anti-β-galactosidase (β-Gal) antibody (1:5000; Biogenesis, Poole, UK), rabbit polyclonal anti-MCH antibody (1:5000; a gift from W. Vale, Salk Institute, La Jolla, CA), and rabbit polyclonal anti-orexin antibody (1:400; Chemicon). Quantification and colocalization of β-Gal with MCH or orexin expression was performed using fluorescent light microscopy and confocal microscopy by an investigator blind to treatment conditions.

Stress paradigms. CRE-LacZ mice were subjected to different stress conditions as described previously (Barrot et al., 2002) and perfused 4 h after the start of the stress. The unpredictable footshock procedure consisted of 120 shocks (0.3 mA, 5 s duration) delivered through a metallic rod floor at random intervals over 1 h. The restraint stress lasted 1 h, during which mice were placed in a cylinder (2.8 cm diameter and 11 cm long, with a 0.6 cm hole at the end). The social stress involved placing the mice for 4 h in a cage of five C57BL/6J mice that had been housed together for 2 weeks. A group of mice was also subjected to 5 d of repeated unpredictable stress similar to that described previously (Ortiz et al., 1996); they were perfused 4 h after the start of the last stress (1 h of restraint).

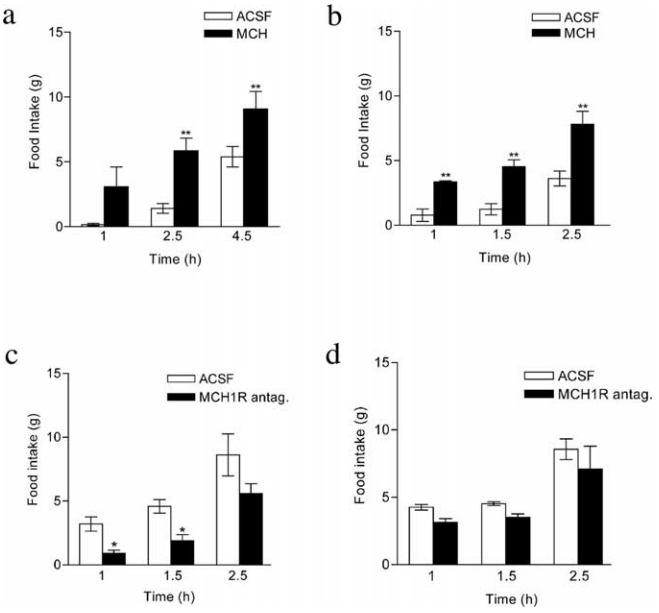

Figure 1. MCH signaling in the AcSh modulates feeding behavior. MCH (1 μg/side) was injected into the AcSh of rats at the beginning of the dark cycle (***a***) or 4 h after the start of the dark cycle (***b***). The MCH1R antagonist (antag.) [1 μg/side (***c***) or 200 ng/side (***d***)] was injected into the AcSh at the beginning of the dark cycle. *$p < 0.05$; **$p < 0.01$; $n = 6$–10 per group. Error bars indicate SEM.

Statistical analysis. Statistical significance was assessed using one-way and factorial design (two- and three-way) ANOVA, followed by *post hoc* tests (Newman–Keuls and Scheffé's) to further analyze significant differences between treatment groups. When appropriate, a Student's *t* test was used to assess statistical significance of preplanned comparisons. Statistical significance was defined as $p < 0.05$.

Results

MCH signaling in AcSh regulates feeding behavior

Based on the high level of expression of MCH1R in the AcSh, we hypothesized that the AcSh is involved in mediating the behavioral effects of MCH. To test this hypothesis, 1 μg (419 pmol) of MCH was delivered bilaterally into the AcSh or the AcCo of *ad libitum*-fed rats at the beginning of the dark cycle, and feeding was measured over 4 h. Two-way ANOVA indicated a main effect for treatment ($F_{(1,71)} = 11.27$; $p < 0.01$) and subregion ($F_{(1,71)} = 7.32$; $p < 0.01$), with a significant treatment by subregion interaction ($F_{(1,71)} = 12.62$; $p < 0.001$). *Post hoc* analysis confirmed a significant effect of MCH ($p < 0.001$) and subregion ($p < 0.001$) on feeding. Effects of MCH were evident at 1 h after injection ($p = 0.056$) and highly significant at 2.5 h ($p < 0.001$) and 4.5 h ($p < 0.01$), as shown in Figure 1*a*. In addition, administration of 1 μg of MCH to the AcSh produced a hyperphagic effect when injected 4 h after the start of the dark cycle, when the rats have already consumed food and are likely to be sated ($F_{(1,6)} = 21.42$; $p < 0.05$) (Fig. 1*b*).

To evaluate the role of endogenous AcSh MCH1R signaling in feeding, a peptide antagonist shown previously to specifically block MCH1R function was administered to rats (Bednarek et al., 2002). Feeding was measured after delivery of 1 μg (856 pmol) of

the MCH1R antagonist bilaterally in the AcSh. Analysis showed significant main effects for treatment ($F_{(1,67)} = 12.41$; $p < 0.001$) and subregion ($F_{(1,67)} = 4.43$; $p < 0.05$), with a nearly significant interaction seen between treatment and subregion ($p = 0.09$). These statistical differences were also confirmed for subregion ($p < 0.01$) as well as drug treatment ($p < 0.01$) using *post hoc* analysis. *Post hoc* analysis also confirmed the effects of 1 μg treatments ($p < 0.0001$), with significant effects observed at 1 h ($p < 0.05$) and 1.5 h ($p < 0.05$) and a strong trend toward significance seen at 2.5 h ($p = 0.07$) (Fig. 1*c*). An effect of treatment was seen between 200 ng and 1 μg ($F_{(3,45)} = 26.38$; $p < 0.0001$). *Post hoc* analysis confirmed the effects of 200 ng ($p < 0.05$), with trends at all time points and a near significant effect ($p = 0.053$) at 1.5 h (Fig. 1*d*). This dose-dependent hypophagic effect of the MCH1R antagonist further supports a role for the nucleus accumbens in mediating the actions of MCH on feeding.

To evaluate its specificity *in vivo*, the MCH1R antagonist was chronically administered intracerebroventricularly to both wild-type and MCH1R knock-out mice. Although the antagonist treatment significantly attenuated body weight gain in wild-type mice, no effects were seen in MCH1R-KO mice (D. J. Marsh, unpublished observations). These data strongly suggest that the compound specifically acts via interactions with MCH1R.

MCH1R antagonist delivered to the AcSh shows antidepressant-like activity

Systemic delivery of the MCH1R antagonist has been shown recently to have antidepressant-like activity in the FST (Borowsky et al., 2002). Therefore, we evaluated the MCH-KO mice in this behavioral paradigm and found that they have increased performance in the FST ($p = 0.10$ for latency; $p < 0.05$ for total swim time) (Fig. 2*a*,*b*). This is consistent with the previously reported antidepressant-like effects of systemic MCH1R antagonist delivery and further substantiates a potential role for MCH in modulating mood-related behavior.

To investigate the role played by the AcSh in this phenomenon, rats were tested in the FST after injection of MCH1R antagonist into the AcSh or AcCo. Multiple-factors ANOVA yielded significant main effects for accumbens subregion ($F_{(1,21)} = 122.05$; $p < 0.0001$) and treatment ($F_{(1,21)} = 10.72$; $p < 0.01$) on total swimming time, with confirmation via *post hoc* analysis ($p < 0.001$). In addition, a subregion by treatment interaction was observed ($F_{(1,21)} = 13.03$; $p < 0.0001$; *post hoc* analysis, $p < 0.05$). Comparison of 0.5 μg/side and 1 μg/side doses revealed significant differences in total swimming time ($F_{(3,22)} = 8.08$; $p < 0.001$), with the 1 μg dose significantly different from ACSF ($p < 0.01$) (Fig. 2*d*,*f*). Similar results were seen for latency to immobility, for which a trend was seen with 0.5 μg and a significant difference was observed at the 1 μg dose ($F_{(1,10)} = 6.3$; $p < 0.05$) (Fig. 2*e*).

We also investigated the performance of rats in the FST after MCH administration into the AcSh. Analysis of total activity revealed a significant main effect of subregion ($F_{(1,19)} = 13.37$; $p < 0.01$) and near significant main effects of treatment ($F_{(1,19)} = 3.78$; $p = 0.067$). *Post hoc* analysis revealed significant effects of 1 μg of MCH in the AcSh ($p < 0.05$) (Fig. 3*d*) and no effects of 0.5 μg (Fig. 3*b*). As with total swim time, analysis of latency revealed main effects of subregion ($F_{(1,30)} = 8.58$; $p < 0.05$), with this effect specific to the AcSh ($p < 0.05$). Significant differences were observed between doses ($F_{(3,31)} = 3.25$; $p < 0.05$), and *post hoc* tests showed a significant effect of 1 μg ($p < 0.05$) on latency, as seen in Figure 3*c*.

It is important to note that the effects of MCH and the MCH1R antagonist in the FST are not attributable to changes in locomotor activity. Rats receiving MCH (1 μg/side) or the MCH1R antagonist (1 μg/side) in the AcSh did not differ from control rats receiving ACSF in terms of their locomotor response to either a familiar or novel environment (Fig. 3*e*,*f*; data not shown). These findings localize the most potent antidepressant-like effect of the MCH1R antagonist to action in the AcSh.

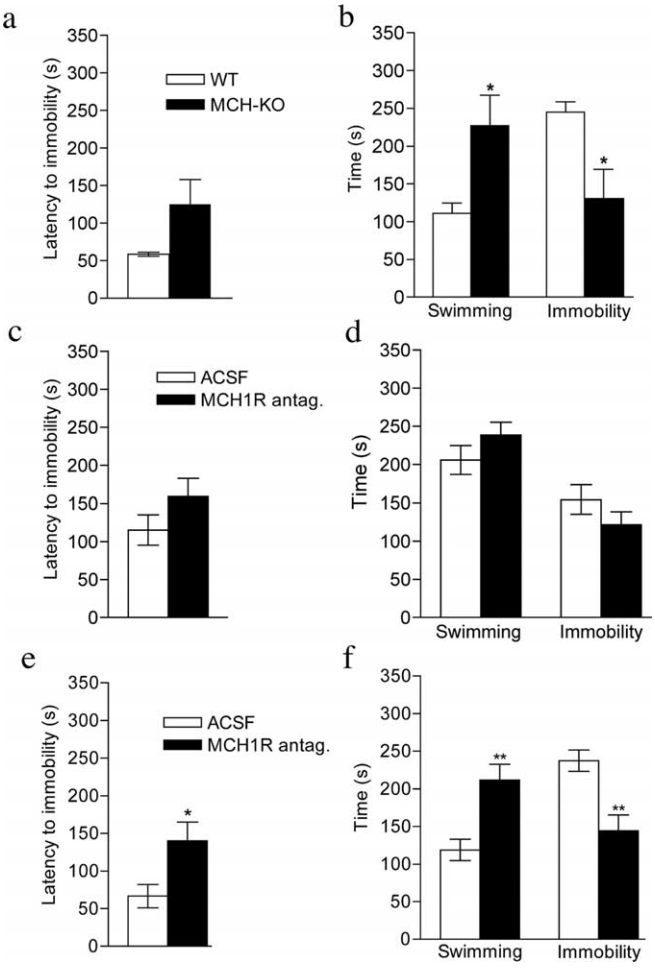

Figure 2. Antidepressant-like effect of MCH1R antagonism. The effect on latency to immobility (***a***) and total swimming time (***b***) on day 1 of the FST was investigated in MCH-KO mice versus wild-type (WT) mice. The same analysis was performed in rats (day 2, FST) that received injections of MCH1R antagonist (antag.) [0.5 μg/side (***c***, ***d***) or 1.0 μg/side (***e***, ***f***)] directly into the AcSh. *$p < 0.05$; **$p < 0.01$; $n = 5$–9 per group. Error bars indicate SEM.

Rats were killed after completion of the behavior experiments, and all brains were analyzed. The targeting events and our assignments of AcCo or AcSh are shown in Figure 4.

MCH1R colocalizes with both dynorphin- and enkephalin-positive neurons in the AcSh

Medium spiny projection neurons are the primary neuronal type in the AcSh and can be classified based on expression of the endogenous opioid peptides Enk or Dyn (Angulo and McEwen, 1994; Curran and Watson, 1995). To identify the neuronal populations that express MCH1R in the AcSh, double *in situ* hybridization was performed with riboprobes to detect colocalization of MCH1R with either Enk or Dyn (Fig. 5*a*). Confocal analysis revealed that the majority of both Enk neurons (Fig. 5*b*) and Dyn neurons (Fig. 5*c*) express MCH1R, confirming that most medium spiny neurons in the nucleus accumbens express MCH1R.

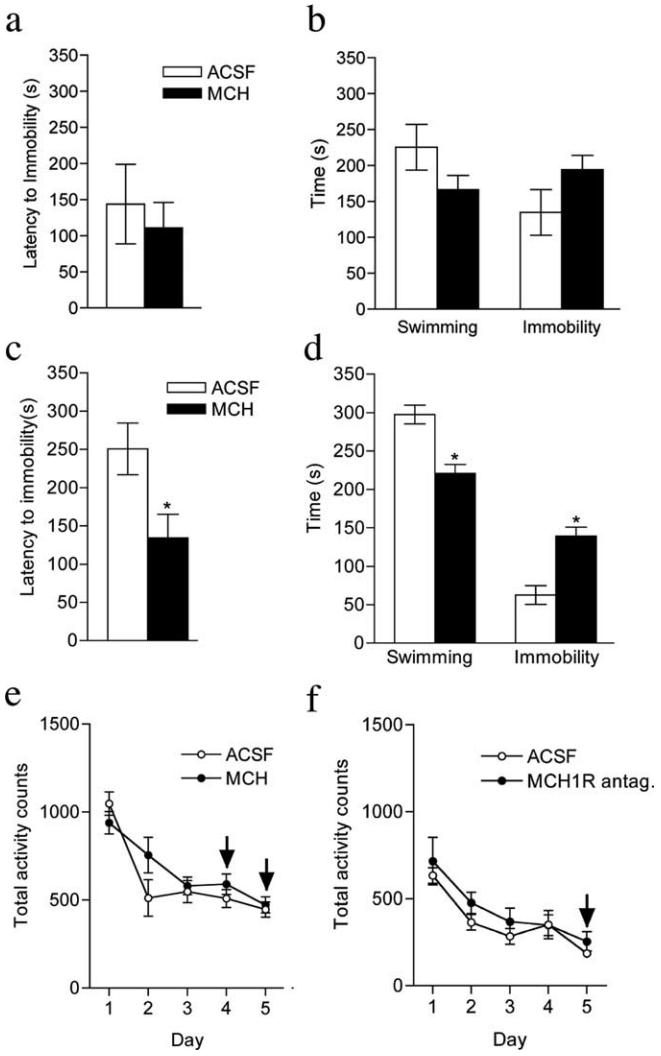

Figure 3. MCH reduces performance in the FST. The effect of MCH [0.5 μg/side (***a***, ***b***) or 1.0 μg/side (***c***, ***d***)] injected into the AcSh in rats was tested in the FST. *$p < 0.05$; $n = 7$–10 per group. The effect of MCH (1 μg/side; ***e***) or MCH1R antagonist (antag.) (1 μg/side; ***f***) on locomotor activity was tested in separate groups of rats. Arrows indicate the days of MCH or MCH1R antagonist administration after habituation. Error bars indicate SEM.

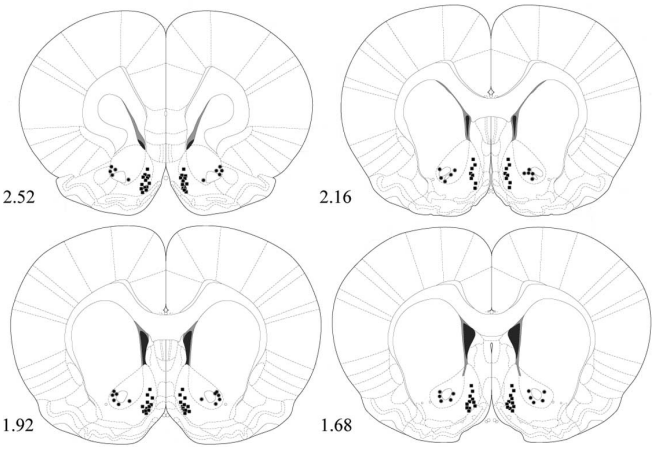

Figure 4. Location of microinjection sites within the AcSh (■) and AcCo (●). Numbers are distances (in centimeters) anterior to bregma (Paxinos and Watson, 2004). Overlapping sites are eliminated for clarity. This figure was adapted from Paxinos and Watson (2004).

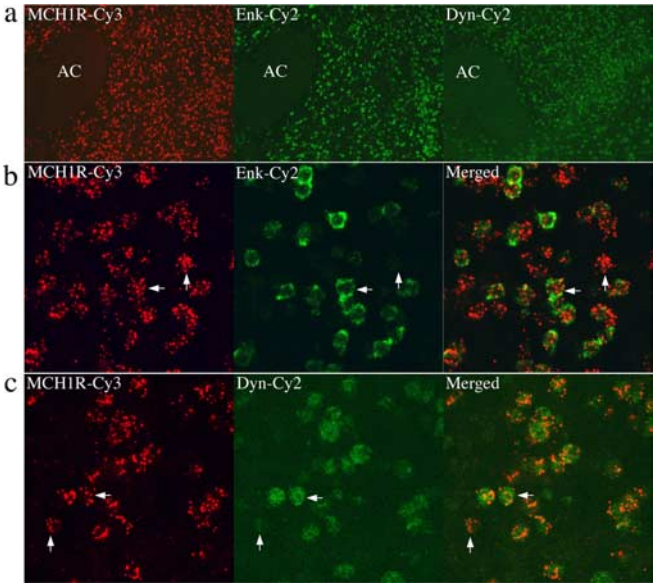

Figure 5. MCH1R is expressed in both Dyn and Enk neurons within the AcSh. ***a***, Fluorescent microscopy shows the expression pattern of MCH1R, Enk mRNA, and Dyn mRNA in the AcSh. Confocal microscopy shows the colocalization of MCH1R with Enk (***b***) or Dyn (***c***). Horizontal arrows indicate examples of colocalization; vertical arrows indicate examples of cells that express MCH1R but not the neuropeptide. AC, Anterior commissure; Cy, cyanine.

MCH signaling modulates phosphorylation of the AMPA receptor subunit GluR1 in AcSh explants

To identify the signaling pathways and molecules responsible for the behavioral effects of MCH in the AcSh, phosphoprotein changes were evaluated after administration of MCH to mouse AcSh explants. Because MCH1R couples to $G_{i/o/q}$ (Chambers et al., 1999; Saito et al., 1999; Hawes et al., 2000) and activates extracellular signal-regulated kinase pathways in the forebrain (Pissios et al., 2003), we hypothesized that MCH would modulate additional phosphoproteins via protein kinase and phosphatase cascades that have been described for striatal neurons (Greengard, 2001). Specifically, the phosphorylation status of Ser^{845} of the AMPA glutamate receptor subunit GluR1, known to be an important target of protein kinase A (PKA) in the dorsal striatum, was investigated using acute slice cultures (Snyder et al., 2000). Using phosphoisoform-specific antibodies, we found that 1 μM SKF 81297, a selective D_1 receptor agonist, significantly increased the phosphorylation of GluR1 at this site in AcSh slices, as reported previously for the striatum (Snyder et al., 2000). Although MCH (10 μM) by itself had no effect on the phosphorylation of GluR1 at Ser^{845}, it completely blocked the induction of phosphorylation caused by SKF 81297 (Fig. 6*a*,*b*). The MCH1R antagonist had no effect on the phosphorylation of GluR1 at Ser^{845} when administered alone, nor did it affect the SKF85991-induced phosphorylation of this site ($p = 0.37$) (Fig. 6*a*, column 2 vs column 6).

The effect of food deprivation or stress on cAMP-mediated transcription in LH neurons

To understand the physiological states that result in activation of MCH neurons, we evaluated changes in CRE reporter mice (CRE-LacZ) (Impey et al., 1998) in the LH. These mice serve to help identify neurons that are responsive to environmental stimuli (Thome et al., 2000; Barrot et al., 2002; Shaw-Lutchman et al., 2002). Based on our evidence that MCH regulates feeding and depression-related behavior, the following behavioral paradigms

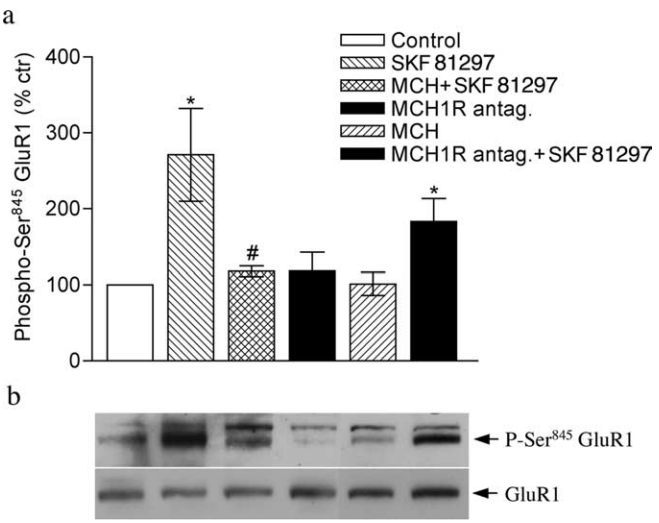

Figure 6. MCH blocks the SKF 81297-induced, Ser[845]-phosphorylated form of GluR1 in AcSh explants. ***a***, Summary densitometric analysis of phospho-Ser[845]/total GluR1 after incubations with MCH (10 μM), MCH1R antagonist (antag.) (10 μM), and the D_1 dopamine receptor agonist SKF 81297 (1 μM) as specified. $^{*}p < 0.05$ for the control group; $^{\#}p < 0.05$ for the SKF 81297 group; data were pooled from five experiments with five animals per experiment. ***b***, Representative Western blots showing the phospho-Ser[845] (P-Ser[845]) and total GluR1 signal in lanes that correspond to the columns above in ***a***. Error bars indicate SEM.

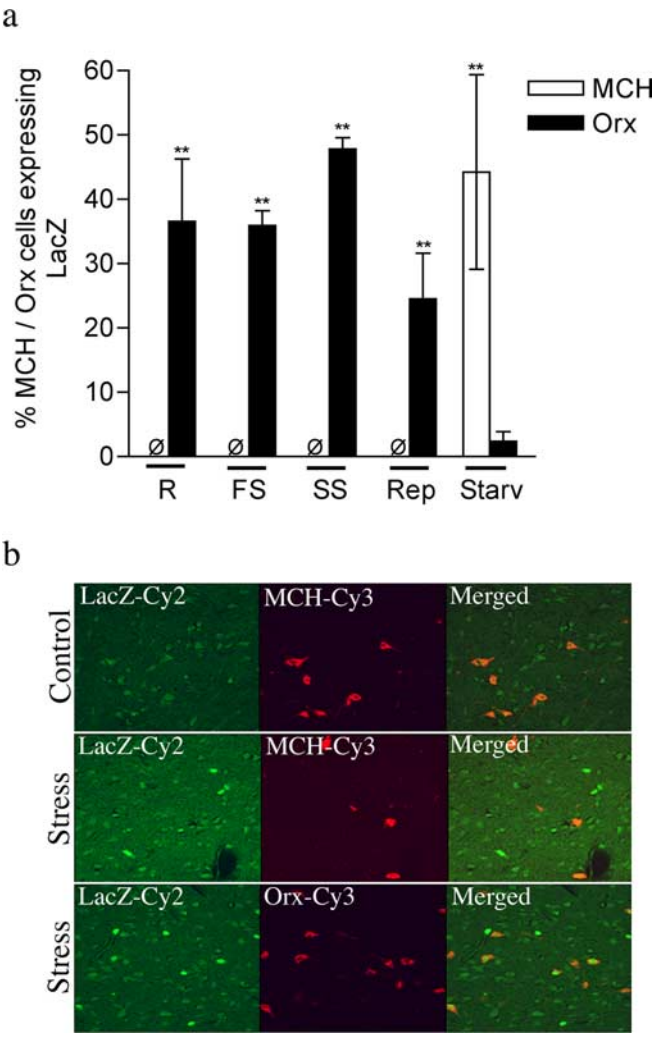

Figure 7. Starvation activates CRE-mediated transcription selectively in MCH neurons. CRE-LacZ reporter mice were subjected to restraint stress for 1 h (R), footshock stress (FS), social stress (SS), repeated unpredictable stress (Rep), or starvation for 24 h (Starv). ***a***, Summary of the percentage of β-Gal colocalization with MCH or orexin (Orx) neurons after the stressors. $^{**}p < 0.01$. ***b***, Representative immunostaining for β-Gal, MCH, and orexin in a CRE-LacZ mouse exposed to restraint stress. Cy, Cyanine. Error bars indicate SEM.

were used: 24 h of food deprivation, restraint stress, footshock, social stress, and repeated unpredictable stress (Barrot et al., 2002). We assessed the activation of the cAMP pathway by performing double fluorescent immunostaining for β-Gal and MCH or orexin/hypocretin (another neuropeptide with expression restricted to the LH). After 24 h of food deprivation, CRE-mediated transcription was robustly upregulated in MCH neurons but not in the adjacent orexin neurons (Fig. 7*a*). In contrast, none of the stress behaviors led to an induction of CRE-mediated transcription in MCH neurons, but there was a significant induction of CRE activity in orexin neurons in the mice exposed to the stress paradigms (Fig. 7*a*,*b*).

Discussion

Results from the present study identify an important hypothalamic–limbic circuit that regulates appetitive behavior. Blockade of MCH activity in the AcSh reduced feeding and produced an antidepressant-like effect in the FST, whereas MCH administration produced the opposite effect. We also determined that both dynorphin and enkephalin AcSh neurons express MCH1R and found that MCH antagonizes the dopamine-mediated phosphorylation of the AMPA receptor subunit GluR1 at Ser[845]. Finally, we demonstrated that MCH neurons respond to a state of food deprivation, but not to several other stress paradigms, suggesting MCH neuronal activation specifically in response to starvation.

The hypothalamus has long been regarded as important in mediating homeostasis and control of feeding (Anand and Brobeck, 1951; Bruce and Kennedy, 1951; Schwartz et al., 2000; Ahima and Osei, 2001; Flier, 2004). In the last 10 years, a series of genetic and pharmacological experiments have indicated that circulating factors, such as leptin and insulin, act via medial and ventral hypothalamic nuclei to modulate feeding and metabolism (Friedman and Halaas, 1998; Cowley et al., 2001; Elmquist, 2001). However, it is not clear how information (orexigenic or anorexigenic) travels outside of the hypothalamus to other brain nuclei. Although hypothalamic projections to the brainstem autonomic nuclei are likely to be important in regulating metabolic aspects of leptin action, our data are the first to establish the AcSh as a site of action for the hypothalamic-derived MCH signal that controls feeding.

Our work thereby extends previous studies, which have shown the importance of MCH in feeding, by establishing a circuit that mediates these effects. Although it is likely that MCH1R signaling in other brain structures, such as the medial hypothalamus, is also relevant for overall MCH function, the AcSh represents a novel, extrahypothalamic target for MCH action. Moreover, the nucleus accumbens is well established as a critical brain region for the actions of drugs of abuse (Koob and Le Moal, 2001; Nestler, 2001). It is notable that the feeding status of an animal, and leptin signaling itself, is known to influence the rewarding effects of drugs of abuse and intracranial self-stimulation (Wise and Hoffman, 1992; Carr et al., 2000; Fulton et al., 2000; Carr, 2002). A hypothalamic–nucleus accumbens circuit mediated by MCH and MCH1R is a strong candidate for mediating these well described interactions between feeding and addiction (DiLeone et al., 2003).

Although a role for the nucleus accumbens in feeding is likely, the relevant neural and molecular mechanisms are still unclear. Manipulations of glutamate, GABA, and opioid pathways result in clear alterations in *ad libitum* feeding behavior (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997, 1999; Zhang and Kelley, 1997) and could all play a role in mediating the effects of MCH1R. In particular, it is possible that MCH modulation of AMPA phosphorylation may account in part for its ability to induce feeding. Phosphorylation of the AMPA subunit GluR1 at Ser^{845} is known to increase AMPA channel currents by increasing their probability of opening (Roche et al., 1996; Banke et al., 2000). Reduced phosphorylation of GluR1 at Ser^{845}, as caused by MCH in AcSh explants, would be predicted to lead to a corresponding decrease in AMPA currents. A reduction in AMPA current might then mimic the effect of AMPA antagonist administration to the AcSh and induce feeding (Maldonado-Irizarry et al., 1995).

Our results also raise the possibility that MCH1R signaling interfaces with nucleus accumbens dopamine signals to produce effects on feeding. Interestingly, MCH did not alter the phosphorylation status of GluR1 when added alone but did block D_1-agonist induction of Ser^{845} phosphorylation. This suggests that MCH1R signaling may be modulatory and that MCH action in the AcSh may require a certain dopamine "tone" that is lost in acute slice cultures but is replenished with administration of a D_1 agonist. Moreover, expression of MCH1R in enkephalin and dynorphin cells suggests interactions with both D_1 and D_2 dopamine receptor circuits.

The relationship between AcSh dopamine and feeding, however, has proven difficult to define. Although genetic elimination of dopamine results in severely hypophagic mice (Zhou and Palmiter, 1995; Szczypka et al., 2001), it is difficult to distinguish between the role of dopamine in locomotor activity and movement control versus feeding. In fact, pharmacological and genetic experiments in the AcSh have suggested that dopamine is not absolutely necessary for feeding but may have a primary role in modifying the incentive drive for food (Nowend et al., 2001; Baldo et al., 2002; Cannon and Palmiter, 2003). It also is possible that MCH, through its action in the AcSh, is a starvation-induced hypothalamic signal that modulates the AcSh reward pathway to increase the drive to feed (Kelley and Berridge, 2002; Saper et al., 2002). However, more extensive tests are needed to clarify the molecular and neural mechanisms whereby MCH1R mediates feeding through the AcSh.

Our data further establish the MCH pathway as a promising target for antidepressant development and support a role for mesolimbic structures in the regulation of mood (Nestler et al., 2002). The antidepressant-like effects of the MCH1R antagonist in the FST could also be the result of AMPA current modulation. Previous studies have shown that drugs that potentiate AMPA currents have antidepressant effects (Li et al., 2001; Knapp et al., 2002). Interestingly, it has also been demonstrated that the antidepressant fluoxetine increases the phosphorylation of GluR1 at Ser^{845} in the striatum (Svenningsson et al., 2002), consistent with the possibility that PKA–GluR1 molecular circuits may mediate the antidepressant effects of MCH1R activity in the AcSh.

The ability of MCH activity in the AcSh to modulate both feeding and performance in the FST suggests that this hypothalamic–nucleus accumbens circuit is an important neural substrate that can influence these interrelated behaviors. These studies may help to establish common molecular and neural circuits to help explain clinical associations between depression and eating disorders (Walsh et al., 1985). Moreover, the MCH neuropeptide may be a critical link between homeostatic roles of the hypothalamus and the motivating or hedonic role of the nucleus accumbens in feeding.

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