Melanin-concentrating hormone activates signaling pathways in 3T3-L1 adipocytes

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Received 17 April 2002; accepted in final form 19 May 2002

Melanin-concentrating hormone activates signaling pathways in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 283: E584–E592, 2002—Energy homeostasis is regulated by peripheral signals, such as leptin, and by several orexigenic and anorectic neuropeptides. Recently, we reported that the orexigenic neuropeptide melanin-concentrating hormone (MCH) stimulates leptin production by rat adipocytes and that the MCH receptor (MCH-R1) is present on these cells. Here, we show that MCH-R1 is present on murine 3T3-L1 adipocytes. Treatment of 3T3-L1 adipocytes with 1 μM MCH for up to 2 h acutely downregulated MCH-R1, indicating a mechanism of ligand-induced receptor downregulation. Potential signaling pathways mediating MCH-R1 action in adipocytes were investigated. Treatment of 3T3-L1 adipocytes with 1 μM MCH rapidly induced a threefold and a fivefold increase in p44/42 MAPK and pp70 S6 kinase activities, respectively. In addition, 3T3-L1 adipocytes transiently transfected with a murine leptin-luciferase promoter construct showed a fourfold and a sixfold increase in leptin promoter-reporter gene expression at 1 h and 4 h, respectively, in response to MCH. Activity decreased to basal levels at 8 h. Furthermore, MCH-stimulated leptin promoter-driven luciferase activity was diminished in the presence of the MAP/ERK kinase inhibitor PD-98059 and in the presence of rapamycin, an inhibitor of pp70 S6 kinase activation. These results provide further evidence for a functional MCH signaling pathway in adipocytes.

Melanin-concentrating hormone receptor; adipose tissue; leptin promoter

ADIPOCYTES, in addition to being the primary site for energy storage (as triglycerides) and release, also act as endocrine cells and have an active role in regulating energy balance. Energy homeostasis is regulated by a complex neuroendocrine network involving peripheral signals such as the adipocyte-derived hormone leptin, as well as by a number of orexigenic and anorectic neuropeptides. Obesity in both humans and rodents can result from dysregulation of either the peripheral or central signals. In humans, obesity is an increasingly common problem, particularly in western society, and contributes to the pathophysiology and sequelae of numerous cardiovascular and metabolic diseases. Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide with distinct physiological roles (32, 48). In mammals, MCH has been localized primarily to the lateral hypothalamus and zona incerta regions of the brain (5, 32, 33, 43). MCH is an important regulator of energy homeostasis, on the basis of both its pharmacological effect to induce an immediate and rapid increase in feeding (34, 35) and the finding that mice lacking MCH are thin (41). Furthermore, mice ectopically overexpressing MCH are mildly obese and demonstrate an increased susceptibility to diet-induced obesity (29).

Initial efforts to identify a specific MCH receptor by use of radioligand binding assays were largely unsuccessful, due to a lack of suitable radioligands and high nonspecific binding (18, 21). However, using function-based assays, several groups recently reported identification of an MCH receptor, designated MCH-R1 (4, 9, 26, 38, 42). MCH-R1 is a previously orphan G protein-coupled receptor, originally termed SLC-1/GPR24 (23). It is a seven-transmembrane domain G protein-coupled receptor that comprises 353 amino acids and is highly conserved in rats, mice, and humans (23). Furthermore, it binds MCH with nanomolar affinity and couples to Gi, Go, and Gq proteins to activate multiple intracellular signaling pathways (16). The receptor is primarily expressed in the brain, specifically in the hippocampal formation, olfactory regions, and the medial nucleus accumbens (17, 23, 37). MCH-R1 localization in these areas suggests a role for MCH in olfactory learning and reinforcement mechanisms, which are fundamental processes in the regulation of feeding. Of particular interest is MCH-R1 expression in both the ventromedial hypothalamic nucleus and the lateral parabrachial nucleus, which are known for their role in feeding behavior (26). This localization of the receptor indicates that MCH-R1 may specifically mediate the effects of MCH on appetite. There are also some reports that the receptor is expressed peripherally at lower levels compared with those found in brain (8, 14, 23, 37).

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Recently, we reported that, in addition to its central effects, MCH-R1 is present on primary rat adipocytes, and MCH can act on these cells to stimulate both synthesis and secretion of leptin (8). Leptin is an important satiety signal in the central nervous system, where it acts to stimulate proopiomelanocortin/cocaine- and amphetamine-regulated transcript neurons and inhibit neuropeptide Y (NPY)/agouti-related peptide neurons in the arcuate nucleus (1, 14, 24, 39, 47). Our data suggest not only that the actions of MCH in adipocytes are mediated by MCH-R1 but also that adipocytes contain an intact MCH-signaling pathway. In addition, this report identified the presence of MCH, or an MCH-like peptide, in rat plasma. A very recent study also reported the presence of MCH in plasma from both lean and obese Zucker rats, with increased circulating levels of MCH being present in obese Zucker rats compared with lean rats (45). Taken together, these findings support a functional role for MCH-R1 in the periphery. To further define the biological role(s) of MCH-R1 in adipocytes, we have utilized the 3T3-L1 cell line. These cells represent a well-characterized and routinely used immortalized murine preadipocyte cell line that can be induced to differentiate into adipocytes, and they have been shown to be a good model for studying the adipocyte in vitro (10). Thus they represent a potentially ideal culture model for studying MCH-R1-mediated effects in adipocytes. In the present study, we have used the 3T3-L1 cell line to examine regulation of MCH-R1 expression, as well as to begin to characterize MCH-activated signaling pathways in adipocytes.

MATERIALS AND METHODS

Materials. DMEM-high glucose, FBS, IBMX, dexamethasone, human recombinant leptin, and BSA were purchased from Sigma (St. Louis, MO). FCS was acquired from HyClone (Logan, UT). Human insulin was obtained from Boehringer Mannheim (Indianapolis, IN), and LY-294002, rapamycin, and PD-98059 were purchased from CalBiochem (La Jolla, CA). HEK 293 cells were kindly provided by Dr. C. Ronald Kahn, Joslin Diabetes Center (Boston, MA). Mammalian MCH was obtained from Bachem (King of Prussia, PA), and the β3-adrenergic receptor agonist BRL-37344 was from Research Biochemicals International (Natick, MA). Phosphospecific and horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Cell Signaling Technology (Beverly, MA). Total rat brain RNA was acquired from Clontech (Palo Alto, CA). Nylon and nitrocellulose membranes were purchased from Schleicher and Schuell (Needham, NH). A RadPrime DNA-labeling kit was purchased from GibCO-BRL (Gaithersburg, MD), and α-[32P]dCTP was from Perkin-Elmer Life Sciences (Boston, MA).

3T3-L1 cell cultures. 3T3-L1 preadipocytes were maintained in DMEM containing 10% FCS in a 10% CO2, humidified environment at 37°C. Cells were differentiated over an 8-day period, with day 8 representing fully differentiated adipocytes. Differentiation of confluent preadipocyte cultures (day 0) was induced using DMEM containing 10% FBS, 0.5 mM IBMX, 5 μg/ml insulin, and 0.4 μg/ml dexamethasone. On day 2, the medium was replaced with DMEM containing only 10% FBS and 5 μg/ml insulin. Insulin was removed on day 4 by changing the medium to DMEM containing only 10% FBS, and cells were maintained thereafter in this medium for the duration of the experiment. Cells were harvested every 2 days, and total RNA was isolated and processed as follows.

RNA isolation and Northern blotting. Total RNA was prepared using the Ultraspec RNA Isolation System (Biotec, Houston, TX). As described previously (8), 10-μg samples of total RNA per lane were run in 1.2% formaldehyde-agarose denaturing gels and transferred to nylon membranes. Northern blotting was performed using a 1.1-kb [α-32P]dCTP-labeled mouse MCH-R1 cDNA described previously (8). Hybridizations were carried out for 2 h at 55°C using QuikHyb (Stratagene). Membranes were washed twice with 2× SSC + 0.1% SDS for 15 min at room temperature and once with 0.1× SSC + 0.1% SDS for 15 min at 42°C. Signals were visualized by autoradiography. RNA integrity and loading were verified by ethidium bromide staining of 28S ribosomal RNA.

Regulation of MCH-R1 gene expression and MCH-activated signaling pathways. To evaluate potential regulators of MCH-R1 gene expression in fully differentiated 3T3-L1 adipocytes were incubated in serum-free medium (DMEM + 0.1% fatty-acid free, insulin-free BSA) overnight and then incubated for ≤ 2 h or for 24 h in the absence and presence of each of the following: 1 μM MCH, 100 nM leptin, 100 nM insulin, and 1 μM BRL-37344. Total RNA was isolated from these cells and processed, as described in RNA isolation and Northern blotting. To examine MCH-activated signaling pathways, lysates were prepared from cells treated with MCH for ≤ 1 h and processed for immunoblotting as follows.

Immunoblotting. 3T3-L1 adipocytes were lysed in cold buffer containing 50 mM HEPES (pH 7.6), 1% Triton, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM PMSF, 100 mM sodium fluoride, 10 mM sodium pyrophosphate, and 2 mM sodium orthovanadate. Protein concentrations were determined by Bradford analysis, samples were solubilized in Laemmli sample buffer, and equal amounts of protein were separated by SDS-PAGE. Proteins were transferred to nitrocellulose membranes and probed with a polyclonal α-MCH-R1 antibody (8) or with α-phospho-p44/42 MAPK and -pp70 S6 kinase antibodies. Proteins were detected using [125I]-labeled protein A (ICN Biomedicals, Costa Mesa, CA) and autoradiography for MCH-R1 and with HRP-conjugated secondary antibodies and an enhanced chemiluminescence detection kit (Perkin-Elmer Life Sciences) for the phosphospecific antibodies.

Transfection assays. Transient transfections were performed in six-well plates using the FuGENE 6 Transfection Reagent (Roche Diagnostics), as described by the manufacturer. 3T3-L1 adipocytes were transfected for 40 h with a murine leptin promoter (− 454 to +9)-luciferase construct. This leptin promoter construct has previously been successfully transfected into both cultured cell lines and primary rat adipocytes to analyze CCAAT/enhancer-binding protein-α and peroxisome proliferator-activated receptor-γ actions on the leptin promoter (19). 3T3-L1 adipocytes were simultaneously co-transfected with a Renilla luciferase control reporter from the constitutive pRL-TK plasmid generously provided by Dr. Martin G. Myers (Joslin Diabetes Center). After being transfected for 40 h, cells were incubated in serum-free medium for 4 h and then treated with 1 μM MCH for ≤ 8 h. Luciferase activity was determined using the Dual-Luciferase Reporter Assay System (Promega) in accordance with the manufacturer’s instructions. To further characterize signaling pathways mediating MCH action, cells were transfected as described above, incubated in serum-free medium for 3.5 h, and then preincubated for 30 min with or without...
RESULTS

MCH-R1 expression in 3T3-L1 cells. To begin to characterize expression of MCH-R1 in 3T3-L1 preadipocytes and adipocytes, the pattern of MCH-R1 mRNA expression was examined over the course of 8 days of differentiation was analyzed. Total RNA was isolated from preadipocytes and from cells collected every 2 days during differentiation (day 0: preadipocyte stage) and ending at the completion of differentiation (day 8). These RNA samples from preadipocytes and differentiated cells were subjected to Northern blot analysis. RNA from rat brain was included as a positive control, and as a negative control, total RNA was used from the human embryonic kidney cell line HEK 293, which does not express MCH-R1 (8). As shown in Fig. 1, the probe hybridized with a 2.4-kb transcript from both 3T3-L1 RNA and rat brain RNA sample. This transcript size corresponds to that previously described for both rat and human MCH-R1 mRNA (23). No hybridization signal was seen with HEK 293 RNA. Thus MCH-R1 is expressed in both 3T3-L1 preadipocytes and adipocytes, albeit at substantially lower levels than in brain. In addition, MCH-R1 expression does not change significantly during differentiation.

Regulation of MCH-R1. To evaluate potential regulators of MCH-R1 transcription in 3T3-L1 adipocytes, fully differentiated 3T3-L1 cells were incubated in serum-free medium overnight and then in the absence or presence of 1 µM MCH, 100 nM leptin, 100 nM insulin, and 1 µM BRL-37344 (a selective β3-adrenergic agonist) for 30, 60, and 120 min to assess potential acute effects. Control cells were incubated for 2 h in the absence of any agent. Total RNA from each culture was isolated and subjected to Northern blot, as described for Fig. 1. As depicted in Fig. 2, MCH acutely downregulated MCH-R1 mRNA. This effect manifested itself within the first 30 min of treatment and persisted throughout the 2-h incubation period. Thus MCH is able to downregulate the expression of MCH-R1 in 3T3-L1 adipocytes. By comparison, no acute effect of insulin on MCH-R1 expression was observed (Fig. 2). Similarly, treatment of 3T3-L1 adipocytes with 100 nM leptin or 1 µM BRL-37344 had no effect on MCH-R1 expression (data not shown). Effects of all of the aforementioned agents on MCH receptor expression were also assessed by Northern analysis during a prolonged incubation period (24 h). In contrast to its ability to acutely decrease MCH-R1 expression during the 2-h incubation, MCH treatment for 24 h had no effect on MCH-R1 expression. Similarly, none of the other agents tested had any effect on receptor expression at this time point (data not shown).

To determine the ability of MCH-R1 to undergo acute ligand-induced downregulation, adipocytes were treated with MCH for ≥2 h, whole cell lysates were prepared, and aliquots containing equal protein were subjected to immunoblotting by use of a polyclonal MCH-R1 peptide antibody described previously (8). Consistent with its effect on MCH-R1 gene expression, treatment with 1 µM MCH acutely downregulated MCH-R1 protein within 30 min of treatment (Fig. 3).

Analysis of MCH-regulated signaling pathways. We also examined potential signaling pathways mediating MCH action in adipocytes. To begin to define these pathways, we examined the ability of MCH to activate intracellular kinases. Specifically, the ability of MCH to activate MAPK and pp70 S6 kinase was assessed by immunoblotting by use of phosphospecific MAPK and pp70 S6 kinase antibodies. As shown in Fig. 4, during a 1-h incubation, treatment of 3T3-L1 adipocytes with 1 µM MCH induced a threefold increase in the phos-
phosphorylation of the extracellular signal-regulated kinases (ERKs) ERK1 and ERK2 (also termed p44MAPK and p42MAPK, respectively), and also induced a sixfold increase in phosphorylation of pp70 S6 kinase. These respective increases occurred acutely (within 5 min) and gradually declined over the course of the 1-h incubation. In conjunction with these experiments, we also examined the ability of MCH to activate several other intracellular kinases, including SAPK/JNK, p38MAPK, and Akt (protein kinase B). However, none of these other kinases were phosphorylated in the MCH-responsive element on the leptin promoter. Furthermore, the time course of MCH-activated leptin-luciferase in 3T3-L1 adipocytes correlates with that which we previously reported for an MCH-induced increase in ob gene expression in primary rat adipocytes (8).

As just described, MCH induces a rapid transient increase in phosphorylation of p44/42 MAPK (ERKs 1/2) and pp70 S6 kinase. To evaluate a role(s) for these enzymes in MCH-stimulated leptin promoter-driven luciferase activity, the effects of the MEK inhibitor PD-98059 (2) and rapamycin, a well documented inhibitor of growth factor-stimulated activation of pp70 S6 kinase (15) on leptin promoter-reporter gene expression, were determined. As shown in Fig. 6 (top), treatment with both of these inhibitors had no effect on basal leptin promoter-driven luciferase activity but markedly decreased MCH-stimulated leptin promoter luciferase activity. Treatment with PD-98059 induced a 50 ± 4% decrease in luciferase activity, and treatment with rapamycin resulted in a 35 ± 4% decrease in luciferase activity. These findings indicate that the ability of MCH to enhance leptin promoter activity involves a complex set of pathways, including steps that are MAPK dependent, as well as steps that are dependent on pp70 S6 kinase activation. In parallel with these experiments, the effect of the PI 3-kinase inhibitor LY-294002 (50) on MCH-stimulated leptin promoter-reporter gene expression was also examined.
However, in contrast to the other two inhibitors, LY-294002 did not inhibit MCH-stimulated leptin promoter-reporter activity, suggesting that MCH-activated leptin promoter-driven luciferase activity may occur via a PI 3-kinase-independent mechanism. As a positive control index of the action of this inhibitor, we measured its ability to block insulin-stimulated pp70 S6 kinase activation, which is mediated via a PI 3-kinase-dependent pathway. As shown in Fig. 6 (bottom), during a 1-h time-course assay, insulin stimulated a marked increase in pp70 S6 kinase activity at 15-, 30-, and 60-min intervals. Consistent with previous reports (15), treatment with 50 μM LY-294002 completely abolished the insulin-stimulated increases in pp70 S6 kinase phosphorylation.

**DISCUSSION**

The current study extends our earlier findings that MCH-R1 is expressed and functional on primary rat adipocytes. Here, we demonstrate its presence on both murine 3T3-L1 preadipocytes and adipocytes. Steady-state levels of MCH-R1 mRNA were unaltered during differentiation of the 3T3-L1 cells. Given that MCH-R1 appears to have a specific role in regulating leptin production, this finding was somewhat surprising, as leptin is not normally expressed in 3T3-L1 preadipocytes and is induced only during differentiation of 3T3-L1 preadipocytes to adipocytes (30). The expression of MCH-R1 in 3T3-L1 preadipocytes at levels similar to those seen in mature adipocytes suggests a potential functional role for MCH-R1 in preadipocytes. Transforming G protein-coupled receptors have been shown to modulate adipocytic differentiation in 3T3-L1 cells (11). Further studies are warranted to determine whether MCH-R1 may have a potential role in this regard. The presence of MCH-R1 on 3T3-L1 adipocytes also indicates a potential role for MCH in this cell line. A very recent study reported that MCH-R1 is expressed in insulinoma cell lines and that MCH stimulates insulin secretion from these cells (46). Taken together, these data support the hypothesis of a peripheral role for MCH in adipocytes in addition to its centrally mediated effects.

Very recently, a second MCH receptor subtype (MCH-R2) has been described in humans (3, 36). MCH-R2 shares ~38% amino acid identity with MCH-R1 (36). MCH-R2 is also peripherally expressed on human adipocytes (3). However, analysis of the genomic databases revealed no homologous sequences in mice (P. Pissios, Joslin Diabetes Center, personal communication), indicating that the MCH-R2 subtype is not present in mice.
Highly specific manner (26). Here, we demonstrate that 3T3-L1 adipocytes also are capable of responding to MCH insofar as exposure to MCH leads to downregulation of MCH-R1 expression. This effect is acute, occurring within 30 min of treatment, and is sustained, as it persisted to 2 h. Furthermore, this effect is specific to MCH, because no other agent tested produced a similar response. Receptor downregulation has been described for other ligands, such as insulin, and a decrease in plasma membrane insulin receptor number has been linked to several insulin-resistant states, including obesity and type 2 diabetes (13). MCH levels are elevated in genetically obese mice (34). Thus it is possible that MCH-induced decreases in its cell surface receptor number may have important implications in the pathophysiology of obesity. The observation that MCH-R1 protein decreased with approximately the same time course as the decline in MCH-R1 mRNA was somewhat surprising, and it suggests that MCH-R1 protein turns over very rapidly. Alternatively, the MCH receptor is known to contain three consensus N-glycosylation sites and several potential phosphorylation sites in the intracellular loops (25). Thus it is also possible that the receptor may have been modified to a form not recognized by the antibody.

As previously mentioned, the use of radioligand binding assays to detect the MCHR receptor and measure receptor number has been confounded by the lack of suitable radioligands and high nonspecific binding. Indeed, binding assays performed have in retrospect proved artifactual because of the nature of nonspecific binding of these ligands (21). To date, these binding assays have only been successful in transfected cell lines that overexpress the MCHR receptor. The development of more sensitive MCHR radioligand binding assays may facilitate measurement of MCHR receptor number in the 3T3-L1 adipocyte cell line and provide further insight into MCH-induced internalization of MCHR-R1.

Although MCH did not appear to affect MCHR-R1 expression during a more prolonged incubation (24 h), a potential effect may have escaped detection for a number of reasons. First, MCH may have degraded or been metabolized over the 24-h time period and thus would not have induced an effect. Alternatively, the sensitivity of the Northern blotting technique may not be adequate to detect small changes in receptor expression stimulated by MCH or any of the potential regulatory agents tested at this more extended time point. Interestingly, leptin had no detectable effect on MCHR-R1 mRNA expression during either acute or prolonged incubations. Recent findings suggest that M CHR-R1 expression in the central nervous system is regulated by leptin. In mice, MCHR-R1 expression in brain is increased by fasting or genetic leptin deficiency; however, this effect is blunted by leptin administration (22). Therefore, leptin may act as a negative regulator of MCHR-R1 expression in the mammalian brain. The apparent difference in expression patterns of MCHR-R1 in the brain compared with the adipocyte suggests that central and peripheral expression of the

As is the case for all membrane proteins, MCHR-R1 is in a constant state of turnover. Furthermore, G protein-coupled receptors possess an intrinsic capacity for agonist-promoted internalization (12, 52). Indeed, MCHR has been shown to promote internalization of its receptor in HEK 293 cells stably expressing a MCHR-R1-green fluorescent protein (GFP) construct in a
receptor may be differentially regulated or that alternative forms of the MCH receptor may exist in these tissues. It is also possible that the effect of leptin on MCH-R1 in brain may be indirect and reflect a complex interaction of leptin on a number of neuropeptides.

G protein-coupled receptors are capable of inducing a wide variety of responses, including G<sub>i</sub> and G<sub>q</sub>-mediated activation of several intracellular kinases. Numerous studies have documented the activation of several kinase cascades, including ERK/MAPK, JNK/p38, PI 3-kinase, and pp70 S6 kinase, by agonists that signal via G protein-coupled receptors (6, 27, 28, 51). These kinases in turn modulate several biological processes, including regulation of numerous transcription factors, regulation of cell growth and proliferation, and regulation of protein synthesis. MCH-R1 is known to couple to G<sub>i</sub>, G<sub>q</sub>, and G<sub>q</sub> proteins, but essentially nothing is known regarding the downstream signaling events through MCH-R1. Here, we show that MCH activates both MAPK and pp70 S6 kinase in 3T3-L1 cells, which supports a role(s) for these enzymes in mediating MCH action in adipocytes. Consistent with our observations, a very recent study reported an MCH-induced increase in MAPK activity in Chinese hamster ovary (CHO) cells stably transfected with the MCH receptor; it demonstrated that MCH-stimulated MAPK activity in these CHO cells was mediated by (a) G<sub>i</sub>/G<sub>qi</sub>-type protein(s) (16).

Our observation of an MCH-induced increase in leptin promoter-driven luciferase activity in 3T3-L1 cells indicates the presence of a potential MCH-responsive element in the leptin promoter. Fully differentiated 3T3-L1 adipocytes have been shown to express ob mRNA, albeit at extremely low levels (≈1% relative to that in mouse adipose tissue) that make its detection difficult in this cell line (30). Nonetheless, our finding of an MCH-stimulated increase in leptin promoter-driven luciferase activity in 3T3-L1 adipocytes is consistent with an MCH-stimulated increase in ob gene expression that we reported previously in rat adipocytes; it suggests that MCH effects on leptin gene expression may result from a direct action of MCH on putative leptin promoter-associated transcription factors. Future studies will focus on identification of an MCH-responsive element(s) and subsequent identification of potential DNA binding proteins. It is especially noteworthy that MCH-activated luciferase activity was markedly reduced by inhibition of MAPK and pp70 S6 kinase, but not by inhibition of PI 3-kinase. PI 3-kinase is a lipid kinase that phosphorylates inositol lipids, and several reports support the view that it is a primary signaling molecule linking growth factor receptors to activation of pp70 S6 kinase, which in turn plays an important role in growth factor signaling (15). However, other studies have indicated that activation of pp70 S6 kinase can also occur via mechanisms that are independent of PI 3-kinase activation (20, 44, 49), and in some cases, are dependent on protein kinase C activation (49). It has been proposed that there are both PI 3-kinase-dependent and -independent signaling pathways that lead to activation of pp70 S6 kinase. These can be distinguished on the basis of their sensitivity to inhibitors of PI 3-kinase (20). Our results support the notion that MCH-induced increases in pp70 S6 kinase activity may occur via a PI 3-kinase-independent pathway. Alternatively, it is possible that the MCH-stimulated increase in pp70 S6 kinase activity is mediated by PI 3-kinases that are less sensitive to inhibition by LY-294002, such as class 2 PI 3-kinases (40). These possibilities are currently being investigated.

Finally, these results indicate that MCH, along with other neuropeptides such as NPY (31) and α-melanocyte-stimulating hormone (α-MSH) (7), may have direct effects on adipocytes. Considerations of such effects will be of particular importance when potential agonist or antagonist action that may be considered for therapeutic use is developed.

In summary, this study documents the presence of MCH-R1 in 3T3-L1 cells and begins to define the signaling mechanisms that mediate MCH action in adipocytes. This cell line represents a potentially ideal culture model for further analyzing MCH action and for examining endogenous MCH-R1 signaling pathways in the adipocyte. Continued dissection of the MCH-MCH-R1 system may ultimately provide valuable insight into the dysregulation underlying obesity.

We are grateful to Dr. Bradford B. Lowell for providing leptin-luciferase constructs. We also thank Drs. Markus Pfister and Efi G. Kokotou for helpful discussions.

These studies were supported by a Joslin Diabetes Center Institution Research Support Grant and National Institute of Diabetes and Digestive and Kidney Diseases Grants RO1 DK-51668 (B. Cheatham), RO1 DK-53978 (E. Maratos-Flier), and National Research Service Award DK-09745 (R. L. Bradley).

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