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Melanin-concentrating hormone facilitates migration of preadipocytes

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ABSTRACT

Adipose tissue develops from differentiating preadipocytes that expand and migrate. 3T3-L1 preadipocytes respond to melanin-concentrating hormone (MCH) by increasing leptin production. Here, we investigate whether MCH elicits remodeling of the actin cytoskeleton and whether this translates into altered migratory capacity of these cells. Incubation with MCH resulted in a loss of actin stress fibers accompanied by a change in morphology from a stretched-out fibroblast to a rounded cell. PMC-3881-PI, a MCH receptor 1 antagonist blocked the effect, confirming this receptor is solely responsible for MCH-mediated actin rearrangements. Both a pharmacological activator and inhibitor of phospholipase C were used to demonstrate this molecule's importance to the signaling pathway. Finally, MCH was shown to facilitate preadipocyte migration into a scratch wound, revealing a previously unknown role for MCH in the regulation of cellular migration. We conclude that MCH could influence the expansion of adipose tissue through its ability to enhance preadipocyte migration.

1. Introduction

Melanin-concentrating hormone (MCH) is a peptide hormone which functions to regulate appetite and energy expenditure in the lateral hypothalamus (Pissios et al., 2006), however functional MCH receptor (MCHR) signaling pathways have also been identified in neuronal cells, pancreatic beta cells, intestinal epithelia and adipose tissue. In higher order mammals, MCH couples to two independent G protein-coupled receptors (GPCRs). When MCHR1 was deleted from mice in two key experiments, the mice became lean, hyperactive and less susceptible to diet-induced obesity (Chen et al., 2002; Marsh et al., 2002). In addition, MCHR1 antagonists were able to limit the weight gain observed in leptin-deficient mice (Segal-Lieberman et al., 2003), which are models for obesity. These pieces of evidence strongly suggest that pharmacological activators and/or inhibitors of the MCHR1 signaling pathway might be useful in the fight against anorexia and obesity, and this has been supported by recent animal studies (Ito et al., 2003; McBriar et al., 2006; Kowalski et al., 2004). A slew of recent experiments have revealed additional physiological processes regulated by MCH. For instance, Hassani et al. (2009) recently discovered that MCH, together with orexin, contributes to the regulation of sleep/wake cycles. Gehlert et al. (2009) reported anti-depressive effects of an MCHR1 antagonist, and Hegde et al. (2009) demonstrated the effectiveness of MCHR1 antagonists in inhibiting the voiding-reflex in rats. Additionally, enhanced expression of MCH and its receptor in colon tissue samples from Irritable Bowel Syndrome patients implicate them in the manifestation of this condition (Kokkotou et al., 2008). The physiological role of MCHR2 is still unclear, although a genetic mutation in this receptor has been correlated to cases of extreme childhood obesity (Ghoussaini et al., 2007). Thus, the global importance of MCH in maintaining physiological homeostasis is becoming increasingly evident.

Adipose tissue actively participates in the regulation of appetite. Hormones secreted from this tissue include insulin, adiponectin and leptin which, in concert with gut-derived hormones like ghrelin and cholecystokinin, provide feedback to the brain regarding the metabolic state of the periphery (Wynne et al., 2005). The expansion and differentiation of preadipocytes into adipose tissue precludes the obese condition, however the mechanisms which dictate such a transformation are largely unknown. 3T3-L1 cells are a well-known tissue-culture model for adipose cell development (Green and Kehinde, 1975). Incubation with 1-methyl-3-isobutylxanthine and dexamethasone hastens differentiation (Rubin et al., 1978) resulting in a transformation from fibroblast to a rounded cell accompanied by a tremendous rearrangement of the actin cytoskeleton towards a cortical placement (Kawaguchi et al., 2003). Interestingly, MCHR1 is expressed at low levels in both undifferentiated and differentiated 3T3-L1 cells, where it has been shown to signal to the ERK/MAPK pathway (Bradley et al., 2002).

We know very little about the downstream participants in MCH-signaling pathways, however when we examine what is known, an interesting theme emerges. MCHR1 participates in potentially important interactions with (1) MCHR1-interacting zinc-finger protein (MIZIP), a tubulin-binding protein, (2) periplakin, which

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is an actin- and intermediate filament-binding protein, and (3) neurochondrin, a cytoplasmic protein involved in neurite growth (Francke et al., 2005, 2006; Murdoch et al., 2005). All three of these MCHR1-interacting proteins could potentially act as signaling mediators between MCH and the cytoskeleton, though the possibility that MCH triggers cytoskeletal changes has not yet been tested. In this study, we utilized the 3T3-L1 preadipose cell line to ask whether MCH can signal actin rearrangements, and whether MCH can influence preadipocyte migration.

2. Materials and methods

2.1. Reagents

3T3-L1 cells were obtained from ATCC (Manassas, VA) and subcutaneous human preadipocytes and specialized media were from ZenBio, Inc. (Research Triangle Park, NC). Melanin-concentrating hormone was from American Peptide Company, Inc. (Vista, CA). PMC-3881-PI was from Peptides, International (Louisville, KY). ProLong Gold, Alexa-Fluor 488 anti-rabbit secondary antibody, and Alexa-Fluor 568 Phalloidin were from Molecular Probes (Eugene, OR). m-3M3FBS and U-73122 were from Calbiochem (La Jolla, CA). DMEM was from Sigma–Aldrich (St. Louis, MO). MCH-1R antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and fetal calf serum and goat serum were from Atlanta Biologicals (Lawrenceville, GA).

2.2. Cell culture

3T3-L1 preadipocytes were cultured in high glucose DMEM containing 10% bovine calf serum and passaged when 50–70% confluent. Subcutaneous human preadipocytes were cultured in specially formulated preadipocyte medium from ZenBio. Cells were maintained as monolayer cultures at 37 °C in a 95% air, 5% CO_2 humidified environment.

2.3. Visualization of actin filaments

3T3-L1 preadipocytes were grown to 50–60% confluency on coverslips in 35-mm dishes. Where indicated, it became necessary to coat slides with polylysine prior to plating cells. Cells were serum starved for 1 h prior to all treatments. Following treatments, cells were rinsed twice with PBS, and then fixed with 4% paraformaldehyde in PBS for 10 min. After three PBS washes, and one PBS-0.1%Tween wash, actin filaments were stained with Alexa-Fluor 546-labeled Phalloidin (1:50 dilution). ProLong Gold was added to mount coverslips. Cells were viewed using a Zeiss Axiocam MRm fluorescence microscope with AxioView imaging software.

2.4. Statistical analysis

Statistical significance was determined using a Student's *t*-test as compared to untreated cells in the same category. Data scoring in the 95th percentile or higher were considered statistically significant. Averages ± SEM are reported.

2.5. Cell migration assay

3T3-L1 preadipocytes were cultured to near confluency in 35 mm dishes. Monolayers were wounded by scratching with a 20–200 μl pipet tip. Cells were incubated at 37 °C and 5% CO_2 for up to 12 h following wounding. Images were taken at each time point using an inverted Nikon camera and Spot Advanced software with a 10× objective. Images were taken at three separate points along each scratch. Tscratch, a software tool developed to automatically analyze scratch-wound assays, was used to determine the size of the wound in each image, as well as compare each time point to the starting images (Gebäck et al., 2009). Wound closure was determined as a percentage of the initial wound size.

2.6. Immunocytochemistry and fluorescence microscopy

Cells grown on 25 mm coverslips were rinsed twice with PBS, then fixed with 1 ml 4% (w/v) paraformaldehyde in PBS for 10 min. Following three PBS rinses, cells were blocked with PBS containing 5% goat serum and 0.1% Triton X-100 for 20 min. Cells were incubated with anti-MCHR1 primary antibody (1:25) overnight and after three 5 min washes in PBS-T, anti-rabbit Alexa-Fluor 488 secondary antibody was added for 20 min. After another set of washes, coverslips were mounted onto slides using ProLong Gold. Images at were taken using a 100× oil immersion objective on a Zeiss Axioskop fluorescence microscope with an AxioCam MRm and Axiovision software.

3. Results

3.1. MCH initiates a transient change in actin morphology in 3T3-L1 cells

3T3-L1 preadipocytes harbor a functional MCH-signaling pathway resulting in detectable ERK activation (Bradley et al., 2002). In order to determine if MCH receptors in these cells can also signal changes in actin morphology, we treated 3T3-L1

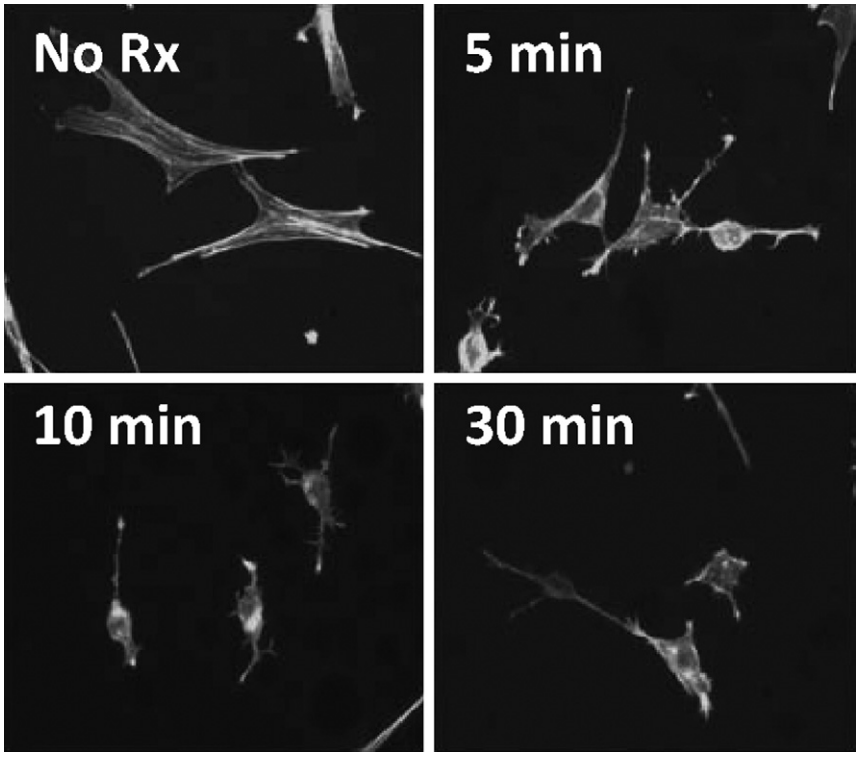

Fig. 1. 3T3-L1 preadipocyte morphology following MCH addition. 3T3-L1 preadipocytes were serum starved overnight, then treated with 1 μM MCH for the indicated times. Cells were fixed with 4% paraformaldehyde and actin filaments were stained with Alexa-Fluor Phalloidin.