α-MSH 1-12

Product of PREP activity on the substrate α-MSH (1-13)

In vivo multiplex quantitative analysis of 3 forms of alpha melanocyte stimulating hormone in pituitary of prolyl endopeptidase deficient mice.

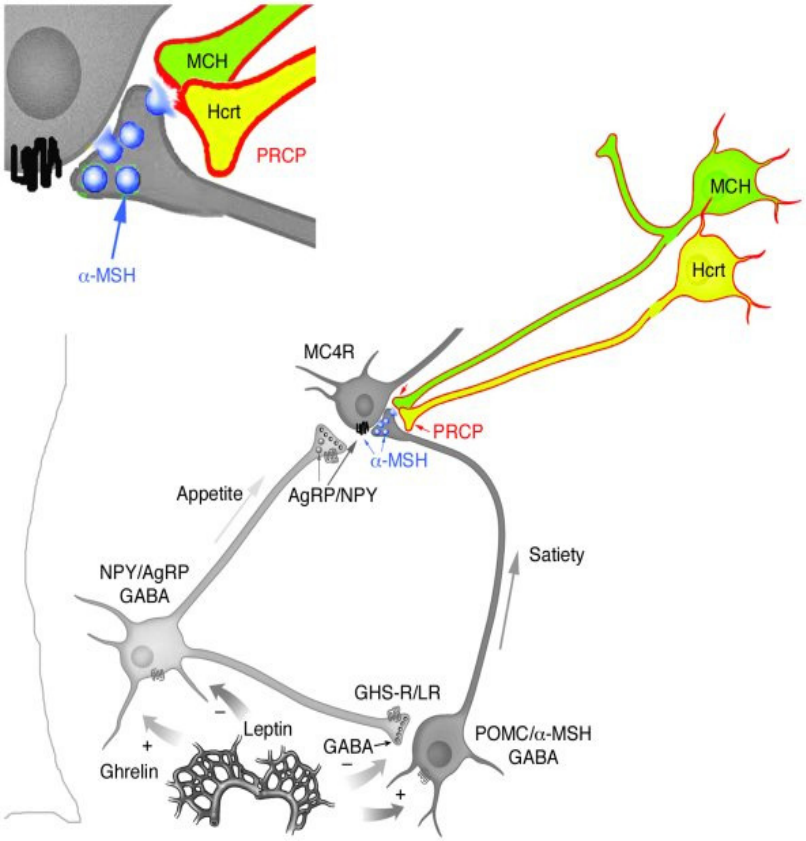
BACKGROUND: In vitro reactions are useful to identify putative enzyme substrates, but in vivo validation is required to identify actual enzyme substrates that have biological meaning. To investigate in vivo effects of prolyl endopeptidase (PREP), a serine protease, on alpha melanocyte stimulating hormone (alpha-MSH), we developed a new mass spectrometry based technique to quantitate, in multiplex, the various forms of alpha-MSH. METHODS: Using Multiple Reaction Monitoring (MRM), we analyzed peptide transitions to quantify three different forms of alpha-MSH. Transitions were first confirmed using standard peptides. Samples were then analyzed by mass spectrometry using a triple quadrupole mass spectrometer, after elution from a reverse phase C18 column by a gradient of acetonitrile. RESULTS: We first demonstrate in vitro that PREP digests biological active alpha melanocyte stimulating hormone (alpha-MSH1-13), by cleaving the terminal amidated valine and releasing a truncated alpha melanocyte stimulating hormone (alpha-MSH1-12) product - the 12 residues alpha-MSH form. We then use the technique in vivo to analyze the MRM transitions of the three different forms of alpha-MSH: the deacetylated alpha-MSH1-13, the acetylated alpha-MSH1-13 and the truncated form alpha-MSH1-12. For this experiment, we used a mouse model (PREP-GT) in which the serine protease, prolyl endopeptidase, is deficient due to a genetrap insertion. Here we report that the ratio between acetylated alpha-MSH1-13 and alpha-MSH1-12 is significantly increased (P-value = 0.015, N = 6) in the pituitaries of PREP-GT mice when compared to wild type littermates. In addition no significant changes were revealed in the relative level of alpha-MSH1-13 versus the deacetylated alpha-MSH1-13. These results combined with the demonstration that PREP digests alpha-MSH1-13 in vitro, strongly suggest that alpha-MSH1-13 is an in vivo substrate of PREP. CONCLUSION: The multiplex targeted quantitative peptidomics technique we present in this study will be decidedly useful to monitor several neuropeptide enzymatic reactions in vivo under varying conditions.

Perroud et al. Mol Brain. 2009 Jun 2;2(1):14.

PHOENIX PHARMACEUTICALS, INC.
330 BEACH ROAD, BURLINGAME CA, 94010, USA
PHONE: (650) 558-8898 EMAIL: info@phoenixpeptide.com
WWW.PHOENIXPEPTIDE.COM

PHOENIX EUROPE GMBH
VIKTORIASTRASSE 3-5, D-76133 KARLSRUHE, GERMANY
PHONE: +49-721-1611950 EMAIL: germany@phoenixpeptide.com
WWW.PHOENIXPEPTIDE.COM

Schematic illustration showing that hypothalamic PRCP is in an anatomical position to determine the efficacy of released -MSH1–13, thereby controlling the output of the melanocortin system.



We found that PRCP is mainly expressed in the lateral hypothalamic Hcrt and MCH neurons. These neurons project to various areas of the hypothalamus, such as the PVN, where α-MSH1–13 terminals strongly innervate MC4R-expressing neurons. It is our hypothesis that PRCP, once released from the Hcrt and/or MCH terminals, will degrade α-MSH, thus increasing the antagonist effect of agouti-related protein (AgRP) and enhancing the orexigenic tone of the system. In support of this, congenic mice and PRCPgt/gt mice are leaner than the wild-type controls. GHS-R/LR, growth hormone secretagogue receptor/leptin receptor; NPY, neuropeptide Y.

Wallingford et al. J Clin Invest. 2009 Aug 3;119(8):2291-2303.

Prolylcarboxypeptidase regulates food intake by inactivating alpha-MSH in rodents.

The anorexigenic neuromodulator alpha-melanocyte-stimulating hormone (alpha-MSH; referred to here as alpha-MSH1-13) undergoes extensive posttranslational processing, and its in vivo activity is short lived due to rapid inactivation. The enzymatic control of alpha-MSH1-13 maturation and inactivation is incompletely understood. Here we have provided insight into alpha-MSH1-13 inactivation through the generation and analysis of a subcongenic mouse strain with reduced body fat compared with controls. Using positional cloning, we identified a maximum of 6 coding genes, including that encoding prolyl-carboxypeptidase (PRCP), in the donor region. Real-time PCR revealed a marked genotype effect on Prcp mRNA expression in brain tissue. Biochemical studies using recombinant PRCP demonstrated that PRCP removes the C-terminal amino acid of alpha-MSH1-13, producing alpha-MSH1-12, which is not neuroactive. We found that Prcp was expressed in the hypothalamus in neuronal populations that send efferents to areas where alpha-MSH1-13 is released from axon terminals. The inhibition of PRCP activity by small molecule protease inhibitors administered peripherally or centrally decreased food intake in both wild-type and obese mice. Furthermore, Prcp-null mice had elevated levels of alpha-MSH1-13 in the hypothalamus and were leaner and shorter than the wild-type controls on a regular chow diet; they were also resistant to high-fat diet-induced obesity. Our results suggest that PRCP is an important component of melanocortin signaling and weight maintenance via control of active alpha-MSH1-13 levels.

Wallingford et al. J Clin Invest. 2009 Aug 3;119(8):2291-2303.

Related Products

Catalog Number	Product Name	Standard Size
043-03	MSH [Des-Acetyl] Alpha	500 µg
043-05	MSH, Alpha (1-12) (Human, Rat, Mouse)	500 µg
043-01	MSH, Alpha (Human, Rat, Mouse)	500 µg
H-043-01	MSH, Alpha (Human, Rat, Mouse) - Antibody for Immunohistochemistry	50 µl
FC3--G-043-01	MSH, Alpha (Human, Rat, Mouse) - Cy3 Labeled Purified IgG	100 µl
EK-043-01	MSH, Alpha (Human, Rat, Mouse) - EIA Kit	1 kit
FG-043-01A	MSH, Alpha (Human, Rat, Mouse) - FAM Labeled	1 nmol
FEK-043-01	MSH, Alpha (Human, Rat, Mouse) - Fluorescent EIA Kit	1 kit
T-043-01	MSH, Alpha (Human, Rat, Mouse) - I-125 Labeled	10 µCi
T-G-043-01	MSH, Alpha (Human, Rat, Mouse) - I-125 Labeled Purified IgG	10 µCi
MRK-043-01	MSH, Alpha (Human, Rat, Mouse) - Magnetic Bead RIA kit	1 kit
G-043-01	MSH, Alpha (Human, Rat, Mouse) - Purified IgG Antibody	400 µg
FR-043-01	MSH, Alpha (Human, Rat, Mouse) - Rhodamine Labeled	1 nmol
RK-043-01	MSH, Alpha (Human, Rat, Mouse) - RIA Kit	1 kit