

Network: Obesity and Related Disorders

Project: Altered Melanocortin-4 and Melanin-concentrating Hormone Receptor Signalling in Obesity

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Introduction

Melanocortin receptors (MCR), which belong to the superfamily of G protein-coupled receptors (GPCR), are believed to play a major role in the regulation of energy homeostasis in humans. In line with this hypothesis, mutations in the gene encoding the MC4R subtype are the most frequent monogenic cause of severe obesity in human beings. In a mutation screen of 808 extremely obese candidates in a case-controlled study a total of 16 different missense, nonsense or frameshift mutations of the MC4R gene were found within the group of obese individuals. *In vitro* assays revealed that nine of the 16 mutations led to impaired MC4R signalling after recombinant expression of the gene product in Cos-7 cells. In contrast, only one missense mutation which did not alter receptor function was detected in the control cohort. These results supported the hypothesis that MC4R are key players in the regulation of energy homeostasis and point to the importance of the MC4R gene product as a target for body weight regulation.

Results/Project Status

A total of 16 different nonsilent MC4R gene variants were detected in 43 of the 808 obese children and adolescents in whom the coding region of the MC4R was screened for mutations (1). PCR fragments were amplified using specific MC4R primers, subcloned into a mammalian expression vector and identified by DNA sequence analysis. cDNA-s of the MC4R wild type (wt) or various MC4R variants were introduced into Cos-7 cells and the ligand binding profile as well as the signalling properties of the receptors were studied. Regarding the classical signalling pathway of MC4R (Gs/adenylyl cyclase/cAMP), the identified mutations were graded into three groups: 1) mutations with an unchanged ligand binding profile but blunted Gs signalling (MC4R-D90N), 2) mutations with a decreased potency to activate Gs signalling compared to the MC4R-wt (MC4R-I121T) and 3) mutations with increased basal, agonist-independent receptor activity (MC4R-P230L).

	K_D	Basal ^a	E_{max} ^b	EC_{50}
MC4R-wt	7.6 nM	1.0	9.8	51.0 nM
MC4R-D90N	8.7 nM	1.0	1.3	-
MC4R-I121T	-	0.4	7.7	518 nM
MC4R-P230L	-	3.1	27.0	107 nM

Tab 1: Functional characterization of various MC4R based on α -MSH-promoted cAMP accumulation; ^a and ^b fold over wt-basal.

This observed impaired receptor function of MC4R mutants found with a higher likelihood in extremely obese candidates, further raised the importance of MC4R signalling in the regulation of body weight.

Interestingly, all MC4R mutants isolated from obese candidates were found to be heterozygous for these mutations, indicative either of gene-dose effects or dominant-negative effects of the mutated receptors on the function of the wild-type receptor. Receptor oligomerization is a prerequisite for the occurrence of such a dominant-negative effect. Hence, we took advantage of the bioluminescence resonance energy transfer (BRET) technique (2) and studied the propensity of the MC4R-wt and the MC4R-D90N mutant to homo or hetero oligomerize.

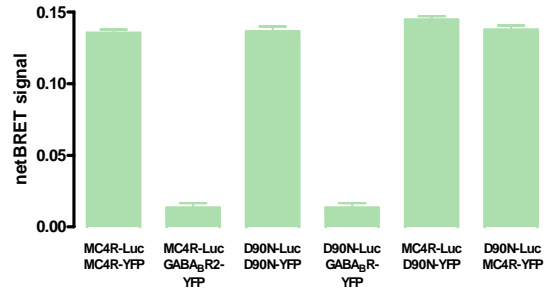


Fig 1: Ligand-independent receptor oligomerization of MC4R-wt and the MC4R-D90N mutant monitored by BRET in living HEK-293 cells.

As indicated in figure 1, both the wild-type and the MC4R-D90N mutant form homooligomers when expressed in HEK-293 cells. Further, co-expression of the MC4R-wt and the MC4R-D90N mutant led to the formation of heterooligomers, while co-expression of both receptor variants with an unrelated receptor (GABA_BR2) did not lead to the formation of any receptor oligomers. To analyze the functional consequences of the oligomerization between the MCR-wt and the Gs-deficient MCR-D90N variant on the ability of the wild-type to activate the Gs signalling pathway, we next measured the α -MSH-promoted cAMP accumulation mediated by the MC4R-wt in the presence of increasing amounts of the co-expressed MC4R-D90N mutant (3).

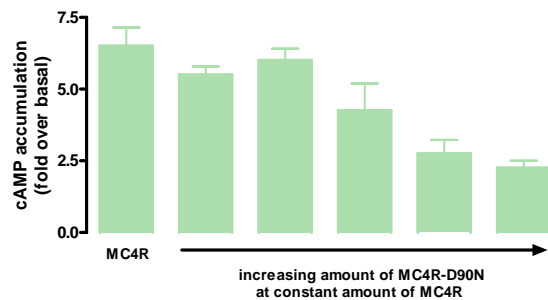


Fig 2: Co-expression of the MC4R-D90N mutant blocks α -MSH-promoted cAMP accumulation induced by the MC4R-wt.

Co-expression of the MC4R-D90N mutant clearly inhibited the cAMP accumulation mediated by the MC4R-wt, indicating a dominant-negative effect of the MC4R-D90N mutant on the signalling of the wild-type. Therefore, we conclude that obese candidates not only suffer from a reduced number of functional MC4R binding sites caused by loss of function mutations within one allele, but also from an inhibition of MC4R-wt function resulting from oligomerization with signalling deficient mutants.

However, the opposite effects of the MC4R-D90N mutation (loss of function) in contrast to the MC4R-P230L variant (gain of function) on MC4R signalling (Tab. 1) raised the intriguing possibility that apart from the classical Gs pathway other signalling cascades might contribute to the MC4R-mediated regulation of energy homeostasis. So far, no

evidence has been provided for MC4R-mediated activation of a G Protein subunit beside Gs. Therefore, we tried to identify putative interactions of the MC4R with signalling molecules distinct from the Gs subunit.

Members of the arrestin family have been established as adaptor proteins responsible for the termination or desensitization of GPCR signalling in general and particularly for the desensitization of Gs signalling promoted by MC4R specific agonists. However, recently, several reports indicated that arrestins not only act as signalling inhibitors but can also simultaneously act as signalling activators by enhancing signalling cascades (e.g. mitogen-activated protein kinase cascade) on their own independent of G protein activity. Assuming that such a G protein-independent but arrestin-dependent signalling pathway might be activated by MC4R, it would be predicted that the Gs-deficient MC4R-D90N variant interacts with arrestin in an agonist-dependent manner. Therefore we used the BRET technique and studied the agonist-promoted recruitment of arrestin by the MC4R-D90N variant in HEK-293 cells.

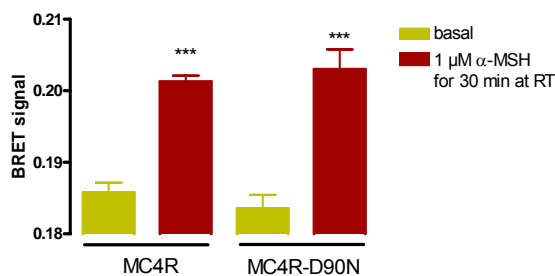


Fig 3: Agonist-promoted arrestin-receptor interactions monitored in living HEK-293 cells by BRET. Asterisks indicate a significant ($p < 0.001$) difference of agonist-treated vs. non-treated cells.

As shown in figure 3, the specific MC4R agonist α -MSH promoted the formation of MC4R-wt or MC4R-D90N complexes with arrestin to a similar extent. This result indicates firstly that no Gs protein activity is required for the recruitment of arrestin to the MC4R and secondly that the MC4R-D90N variant has the potential to promote arrestin-dependent signalling pathways.

Outlook

Although it is well established that MC4R signalling contributes to the regulation of body weight, the exact molecular mechanism of this regulatory process remains unknown. MC4R mutants isolated from obese candidates exhibit impaired receptor function with respect to the classical Gs signalling pathway, but no clear correlation between the alterations induced by the mutation and the dysregulation of energy homeostasis could be drawn. Therefore, we hypothesize that other signalling pathways apart from Gs signalling might be involved in the regulation of body weight. To test this hypothesis and to identify such a putative signalling pathway, we will investigate the ability of MC4R mutants with impaired Gs signalling to interact with arrestin and to regulate arrestin-dependent, G protein-independent signalling pathways. These results will shed new light on physiological and pathophysiological signalling via the MC4R and help identify novel pharmacological targets.

Lit.: 1. Hinney A. et al. Melanocortin-4 Receptor Gene: Case-Control Study and Transmission Disequilibrium Test Confirm that Functionally Relevant Mutations Are Compatible with a major Gene Effect for Extreme Obesity. J Clin Endocrinol Metab. 2003 September, 88, (9):4258-4267. 2. Breit A. et al. Hetero-oligomerization between β_2 - and β_3 -adrenergic receptors generates a β -adrenergic Signalling Unit with Distinct Functional Properties. J Biol Chem. 2004. April. (279):28756-765. 3. Biebermann H. et al. Autosomal-Dominant Mode of Inheritance of a Melanocortin-4 Receptor Mutation in a Patient with severe Early-Onset Obesity Is Due to a Dominant-Negative Effect Caused by Receptor Dimerization. Diabetes. 2003 December, (52):2984-2988.