



Akzeptierte Version

Received Date : 14-Sep-2015  
Revised Date : 17-Dec-2015  
Accepted Date : 22-Dec-2015  
Article type : Research Report

Title page

Proposed journal section: Neurosystems

**Title:** Amylin receptor components and the leptin receptor are co-expressed in single rat area postrema neurons.

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**Running title:** Amylin receptor components in the rat AP

**Keywords:** CTR, RAMP1, RAMP2, RAMP3, laser capture microdissection

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ejn.13163  
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Accepted Article

Publizierte Version

EJN European Journal of Neuroscience

European Journal of Neuroscience, Vol. 43, pp. 653-661, 2016

FENS

doi:10.1111/ejn.13163

MOLECULAR AND SYNAPTIC MECHANISMS

Amylin receptor components and the leptin receptor are co-expressed in single rat area postrema neurons

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**Keywords:** calcitonin receptor, laser capture microdissection, receptor activity-modifying protein 1, receptor activity-modifying protein 2, receptor activity-modifying protein 3

Edited by Masahiko Watanabe

Received 14 September 2015, revised 17 December 2015, accepted 22 December 2015

Abstract

Amylin is a pancreatic  $\beta$ -cell hormone that acts as a satiety signal to inhibit food intake by binding to amylin receptors (AMYs) and activating a specific neuronal population in the area postrema (AP). AMYs are heterodimers that include a calcitonin receptor (CTR) subunit (CTR isoform a or b (CTR<sub>a</sub> or CTR<sub>b</sub>)) and a member of the receptor activity-modifying proteins (RAMPs). Here, we used single-cell quantitative polymerase chain reaction to assess co-expression of AMY subunits in AP neurons from rats that were injected with amylin or vehicle. Because amylin interacts synergistically with the adipokine leptin to reduce body weight, we also assessed the co-expression of AMY and the leptin receptor isoform b (LepR<sub>b</sub>) in amylin-activated AP neurons. Single cells were collected from Wistar rats and from transgenic *Fos-GFP* rats that express green fluorescent protein (GFP) under the control of the *Fos* promoter. We found that the mRNAs of CTR<sub>a</sub>, RAMP1, RAMP2 and RAMP3 were all co-expressed in single AP neurons. Moreover, most of the CTR<sub>a</sub><sup>+</sup> cells co-expressed more than one of the RAMPs. Amylin down-regulated RAMP1 and RAMP3 but not CTR mRNAs in AMY<sup>+</sup> neurons, suggesting a possible negative feedback mechanism of amylin at its own primary receptors. Interestingly, amylin up-regulated RAMP2 mRNA. We also found that a high percentage of single cells that co-expressed all components of a functional AMY expressed LepR<sub>b</sub> mRNA. Thus, single AP cells expressed both AMY and LepR<sub>b</sub>, which formed a population of first-order neurons that presumably can be directly activated by amylin and, at least in part, also by leptin.

Introduction

Amylin, also known as islet amyloid polypeptide, is co-secreted with insulin by pancreatic  $\beta$ -cells in response to nutrient stimuli (Lutz, 2010). Amylin reduces food intake and body weight (Lutz *et al.*, 2001; Roth *et al.*, 2012) and may also act as an adiposity signal to control energy expenditure (Wieling *et al.*, 2007; Zhang *et al.*, 2011). Circulating amylin acts centrally to control the energy balance by primarily activating neurons of the area postrema (AP), a circumventricular organ located in the hindbrain (Riediger *et al.*, 2001, 2004; Lutz, 2009; Potes & Lutz, 2010; Potes *et al.*, 2012).

A functional amylin receptor (AMY) results from a heterodimer of the calcitonin receptor (CTR) with one member of the receptor activity-modifying proteins (RAMPs) (Christopoulos *et al.*, 1999). The rat CTR exists in two different isoforms, CTR<sub>a</sub> and CTR<sub>b</sub>, but the exact functional relevance of action mediated by either isoform

is not yet fully understood. *In situ* hybridization studies that mapped the localization of CTR<sub>a</sub> and RAMP3 suggested that only CTR<sub>a</sub> is present in the AP of rodents (Ueda *et al.*, 2001; Barth *et al.*, 2004). Three members of the RAMP family have been identified (McLatchie *et al.*, 1998; Sexton *et al.*, 2000): RAMP1, RAMP2 and RAMP3. They are associated in the endoplasmic reticulum and are co-trafficked to the cell surface in order to form stable complexes that act as chaperones to form different receptors with selective ligand specificity. The dimerization of RAMP1, RAMP2 and RAMP3 with CTR<sub>a</sub> generates AMY<sub>1</sub>, AMY<sub>2</sub>, and AMY<sub>3</sub>, respectively (Bailey *et al.*, 2012; Alexander *et al.*, 2013).

The presence of CTR and RAMPs has been shown in different brain areas (Sexton *et al.*, 1994; Christopoulos *et al.*, 1995; Skofitsch *et al.*, 1995; Beckel *et al.*, 2004; Miatlicki-Bease *et al.*, 2013). However, none of these studies tested the co-localization of the AMY components at the single-cell level, which is necessary to study the physiological relevance of CTR and RAMPs *in vivo*.

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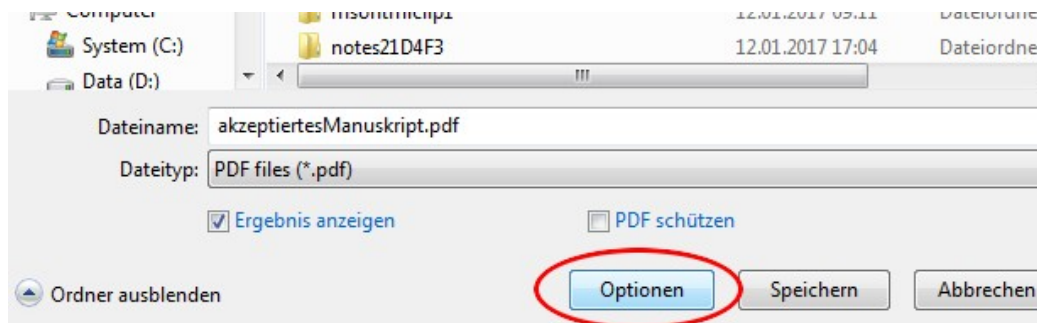
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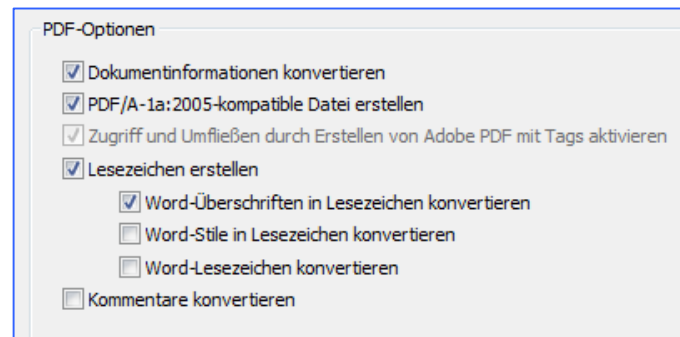
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