Accepted 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.

Authors: Claudia G. Liberini[1,2,3], Christina Neuner Boyle[1], Carlo Cifani[4,5], Marco Venniro[5], Bruce T. Hope[5], and Thomas A. Lutz[1,2].

Addresses: [1]Institute of Veterinary Physiology, Vetsuisse Faculty University of Zurich (UZH), Zurich, Switzerland; [2]Zurich Centre for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland; [3]Zurich Centre for Clinical Studies, Vetsuisse Faculty University of Zurich, Zurich, Switzerland [4]School of Pharmacy, Pharmacology Unit, University of Camerino, Italy; [5]Intramural Research Program, National Institutes of Health/National Institute on Drug Abuse, Baltimore, Maryland 21224

Running title: Amylin receptor components in the rat AP

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

Published Version

EJN European Journal of Neuroscience FENS

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

Claudia G. Liberini,[1,2,3] Christina N. Boyle,[1] Carlo Cifani,[4,5] Marco Venniro,[5] Bruce T. Hope[5] and Thomas A. Lutz[1,2]
[1]Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zürich (UZH), Winterthurerstrasse 260, CH-8057 Zürich, Switzerland
[2]Zürich Centre for Integrative Human Physiology (ZIHP), University of Zürich, Zürich, Switzerland
[3]Zürich Centre for Clinical Studies, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland
[4]School of Pharmacy, Pharmacology Unit, University of Camerino, Camerino, Italy
[5]Intramural Research Program, National Institutes of Health/National Institute on Drug Abuse, Baltimore, MD, USA

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 β-cell hormone that acts as a satiating 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 (CTRa or CTRb)] 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 (LepRb) 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 CTRa, RAMP1, RAMP2 and RAMP3 were all co-expressed in single AP neurons. Moreover, most of the CTRa+ cells co-expressed more than one of the RAMPs. Amylin down-regulated RAMP1 and RAMP3 but not CTR mRNAs in AMY+ 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 LepRb mRNA. Thus, single AP cells expressed both AMY and LepRb, 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

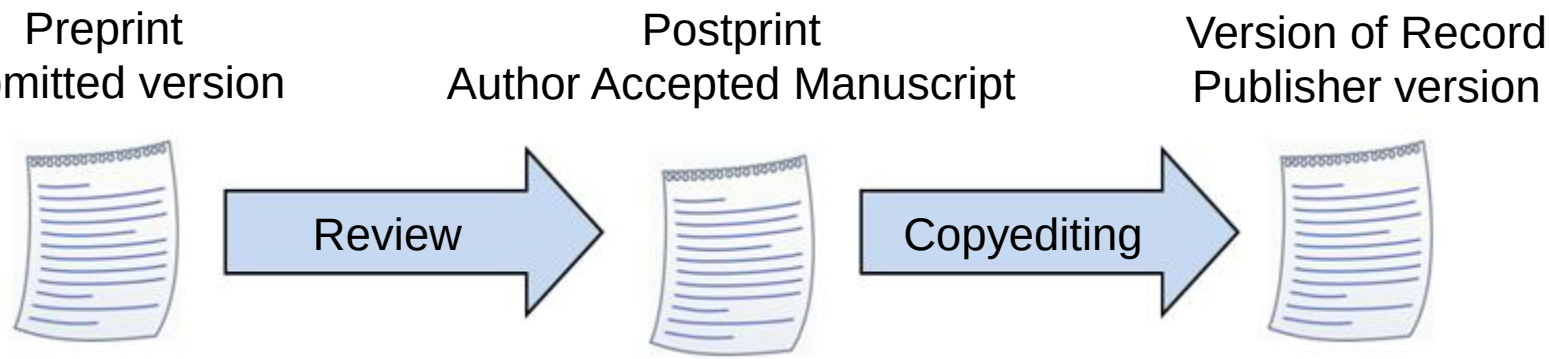
Amylin, also known as islet amyloid polypeptide, is co-secreted with insulin by pancreatic β-cells in response to nutrient stimuli (Lutz, 2010). Amylin reduces food intake and body weight (Lutz *et al.*, 2001; Roth *et al.*, 2012) and may also act as an adiposity signal to control energy expenditure (Wielinga *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, CTRa and CTRb, 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 CTRa/b and RAMPs suggested that only CTRa 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.*, 2001): 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 CTRa generates AMY_1, AMY_2 and AMY_3, 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; Becskei *et al.*, 2004; Mietlicki-Baase *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*.

Correspondence: Christina Neuner Boyle, as above.
E-mail: boyle@vetphysuzh.ch

According to the UZH Open Science Policy, all researchers are obliged to make a full text of their work freely available on ZORA, provided this is legally permitted. So always supplement your submission with a full text.



First version submitted to a publisher

Peer-reviewed and revised final version, accepted by the editor. No publisher layout and logos.

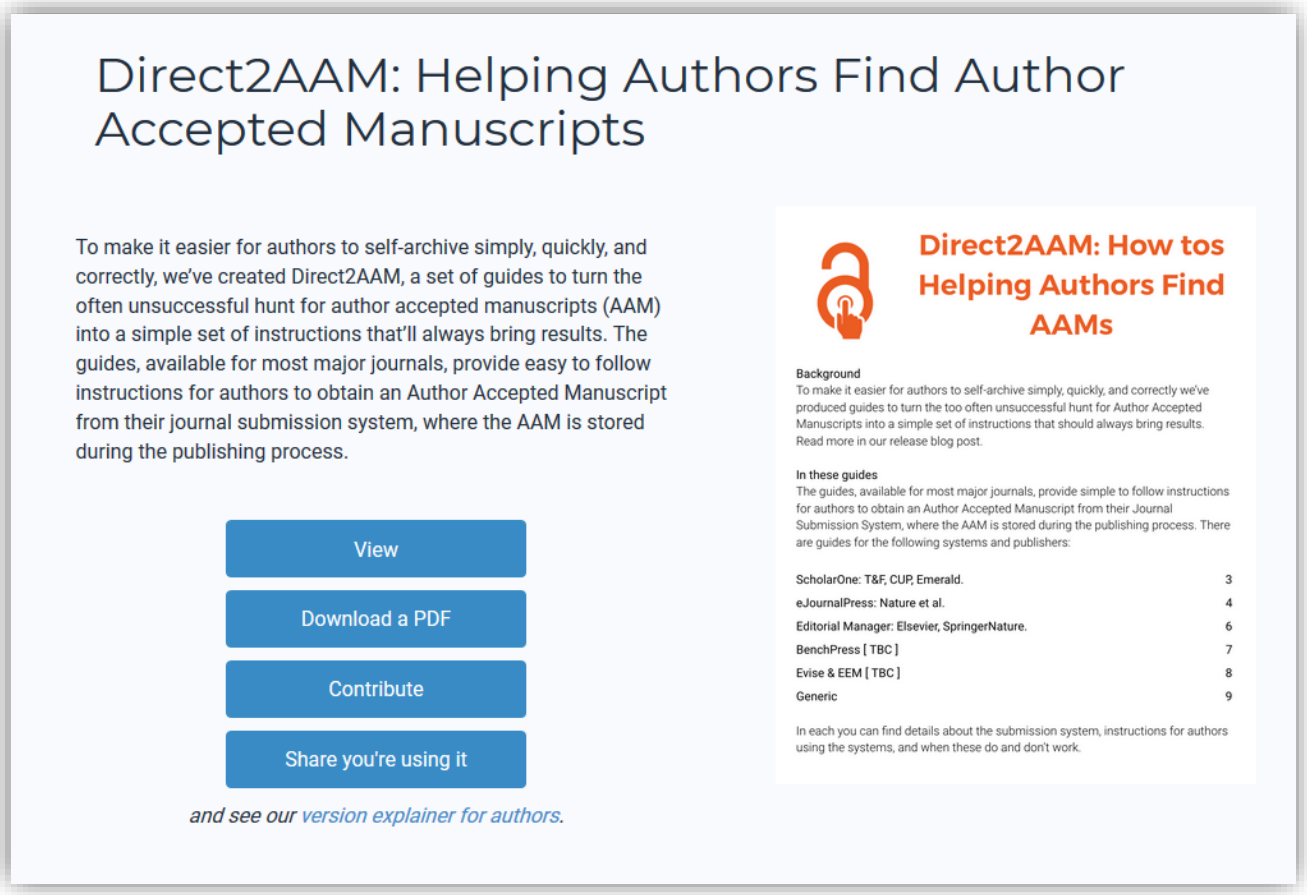
Copyedited version with layout and logos

The ZORA editorial office will clarify any embargo periods and post this in ZORA accordingly.
Please send the accepted version of the publication to: oa@ub.uzh.ch

How do I get accepted manuscripts?

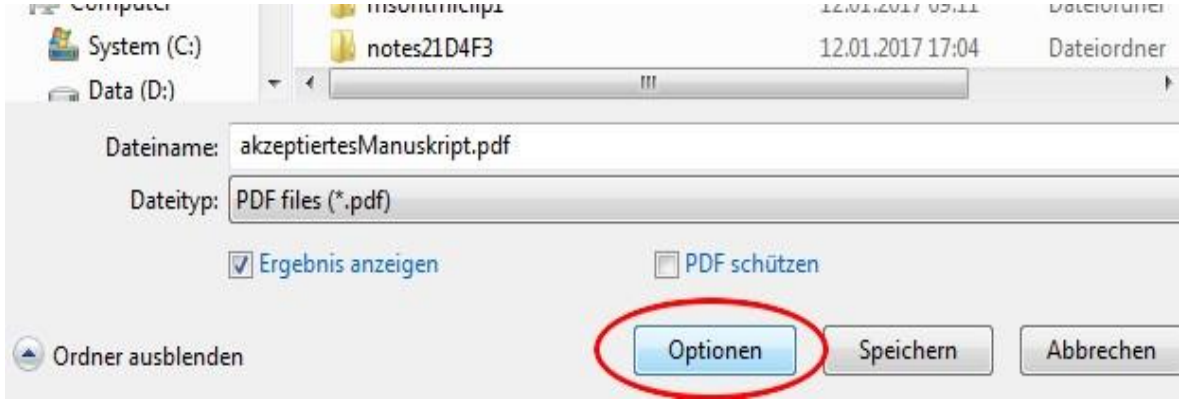
In many submission systems at publishers (Ex: Elsevier, SpringerNature, Taylor & Francis, Wiley, etc.) the accepted manuscript is stored.

Find out how to download the accepted manuscript yourself with the Direct2AAM (Open Access Button) guide.

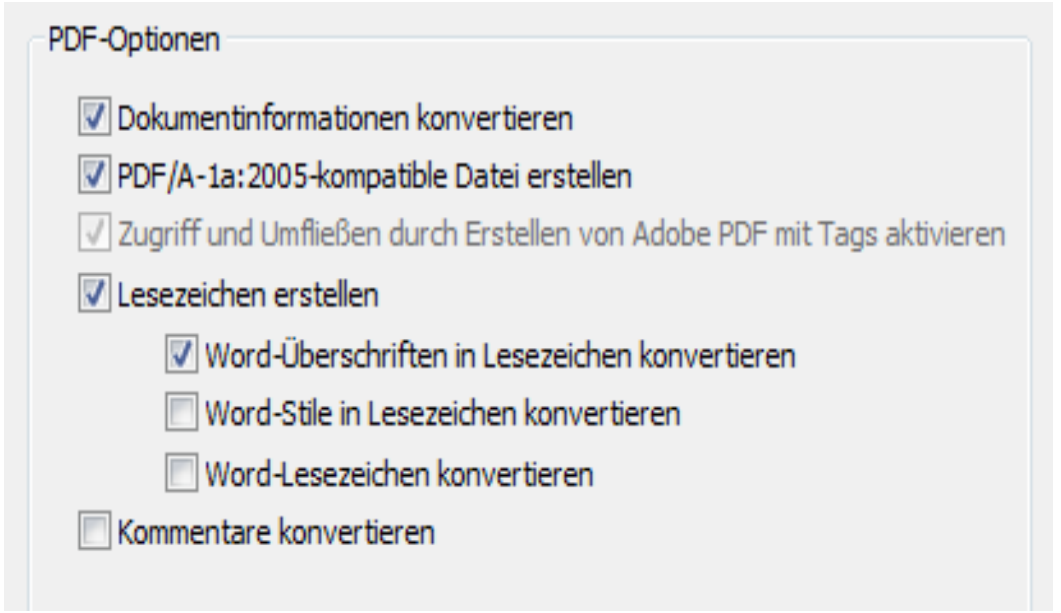


Instructions for creating PDF/A files for long-term archiving from a Microsoft file (Word, PowerPoint)

1. Save your document and select "*.pdf" for "File type".
2. Under "Options" activate the **checkbox PDF/A-1a:2005**



3 . Confirm with "OK" and save your document.



Notes on creating PDF/A files for long-term archiving with an Open Office file

1. Open the "Export" dialog via the "File"/"Export as PDF" menu. In the "General" tab, select the "PDF/A-1a" option.
2. Select the "Export Bookmarks" option.
3. In the "Initial Display" tab select the option "Bookmark window and page". Click on "Export" to close the "Options" dialog. Save the file.