



Accepted Version Published Version FENS EIN European Journal of Neuroscience European Journal of Neuroscience, Vol. 43, pp. 653-661, 2016 doi:10.1111/ejn.13163 Received Date : 14-Sep-2015 MOLECULAR AND SYNAPTIC MECHANISMS Revised Date : 17-Dec-2015 Accepted Date : 22-Dec-2015 Article type : Research Report Amylin receptor components and the leptin receptor are Title page co-expressed in single rat area postrema neurons ρ Proposed journal section: Neurosystems 0 Claudia G. Liberini,^{1,2,3} Christina N. Boyle,¹ Carlo Cifani,^{4,5} Marco Venniro,⁵ Bruce T. Hope⁵ and Thomas A. Lutz^{1,2} Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zürich (UZH), Winterthurerstrasse 260, CH-8057 Zürich, Switzerland Title: Amylin receptor components and the leptin receptor are co-expressed in single Zürich Centre for Integrative Human Physiology (ZIHP), University of Zürich, Zürich, Switzerland Zürich Centre for Clinical Studies, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland ⁴School of Pharmacy, Pharmacology Unit, University of Camerino, Camerino, Italy ⁵Intramural Research Program, National Institutes of Health/National Institute on Drug Abuse, Baltimore, MD, USA rat area postrema neurons. Keywords: calcitonin receptor, laser capture microdissection, receptor activity-modifying protein 1, receptor activity-modifying protein 2, receptor activity-modifying protein 3 Authors: Claudia G. Liberini^{1,2,3}, Christina Neuner Boyle¹, Carlo Cifani^{4,5}, Marco Venniro⁵, Edited to Marshike Watershe Received 14 September 2015, revised 17 December 2015, accepted 22 December 2015 Bruce T. Hope5, and Thomas A. Lutz1,2. Abstract Amylin is a pancreatic 6-cell hormone that acts as a satiating signal to inhibit food intake by binding to amylin receptors (AMYs) Addresses: ¹Institute of Veterinary Physiology, Vetsuisse Faculty University of Zurich 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 (UZH), Zurich, Switzerland; ²Zurich Centre for Integrative Human Physiology (ZIHP), 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 University of Zurich, Zurich, Switzerland; ³Zurich Centre for Clinical Studies, Vetsuisse 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 Faculty University of Zurich, Zurich, Switzerland ⁴School of Pharmacy, Pharmacology Unit, 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-University of Camerino, Italy; ⁵Intramural Research Program, National Institutes of 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 Health/National Institute on Drug Abuse, Baltimore, Maryland 21224 leptin Introduction Amylin, also known as islet amyloid polypeptide, is co-secreted is not yet fully understood. In situ hybridization studies that mapped the localization of CTRab and RAMPs suggested that only CTRa is with insulin by pancreatic B-cells in response to nutrient stimuli Running title: Amylin receptor components in the rat AP (Lutz 2010). Amylin reduces food intake and body weight (Lutz present in the AP of rodents (Ueda et al., 2001; Barth et al., 2004). Three members of the RAMP family have been identified et al., 2001; Roth et al., 2012) and may also act as an adiposity signal to control energy expenditure (Wielinga et al., 2007; Zhang (McLatchie et al., 1998; Sexton et al., 2001): RAMP1, RAMP2 and et al., 2011). Circulating amylin acts centrally to control the energy RAMP3. They are associated in the endoplasmic reticulum and are balance by primarily activating neurons of the area postrema (AP), a co-trafficked to the cell surface in order to form stable complexes Keywords: CTR, RAMP1, RAMP2, RAMP3, laser capture microdissection circumventricular organ located in the hindbrain (Riediger et al, that act as chaperones to form different receptors with selective 2001, 2004; Lutz, 2009; Potes & Lutz, 2010; Potes et al., 2012). ligand specificity. The dimerization of RAMP1, RAMP2 and A functional amylin receptor (AMY) results from a heterodimer RAMP3 with CTRa generates AMY1, AMY2 and AMY3, respecof the calcitonin receptor (CTR) with one member of the receptor tively (Bailey et al., 2012; Alexander et al., 2013). activity-modifying proteins (RAMPs) (Christopoulos et al., 1999). The presence of CTR and RAMPs has been shown in different The rat CTR exists in two different isoforms, CTRa and CTRb, but brain areas (Sexton et al., 1994; Christopoulos et al., 1995; Skothe exact functional relevance of action mediated by either isoform fitsch et al., 1995; Becskei et al., 2004; Mietlicki-Baase et al., This article has been accepted for publication and undergone full peer review but has not 2013). However, none of these studies tested the co-localization of been through the copyediting, typesetting, pagination and proofreading process, which may the AMY components at the single-cell level, which is necessary to Correspondence: Christina Neurer Boyle, as above. E-mail: boyle@vetphysuzh.ch lead to differences between this version and the Version of Record. Please cite this article as study the physiological relevance of CTR and RAMPs in vivo. doi: 10.1111/ejn.13163 This article is protected by copyright. All rights reserved. © 2016 Federation of European Neuroscience Societies and John Wiley & Sons Ltd





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