FEBS Letters 588 (2014) 3233–3239

FEBS Letters

journal homepage: www.FEBSLetters.org

Pancreatic polypeptide inhibits somatostatin secretion

Wook Kim [a], Jennifer L. Fiori [b], Yu-Kyong Shin [b], Eitan Okun [c], Jung Seok Kim [a], Peter R. Rapp [d], Josephine M. Egan [b,*]

[a] Department of Molecular Science and Technology, Ajou University, Suwon 443-749, South Korea
[b] Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA
[c] The Mina and Everard Goodman Faculty of Life Sciences, The Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat Gan 52900, Israel
[d] Laboratory of Behavioral Neuroscience, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA

ARTICLE INFO

Article history:
Received 10 March 2014
Revised 14 May 2014
Accepted 3 July 2014
Available online 11 July 2014

Edited by Laszlo Nagy

Keywords:
Pancreatic polypeptide
NPY4 receptor
Somatostatin secretion

ABSTRACT

Pancreatic polypeptide (PP) is a major agonist for neuropeptide Y4 receptors (NPY4R). While NPY4R has been identified in various tissues, the cells on which it is expressed and its function in those cells has not been clearly delineated. Here we report that NPY4R is present in all somatostatin-containing cells of tissues that we tested, including pancreatic islets, duodenum, hippocampus, and hypothalamus. Its agonism by PP decreases somatostatin secretion from human islets. Mouse embryonic hippocampal (mHippo E18) cells expressed NPY4Rs and their activation by PP consistently decreased somatostatin secretion. Furthermore, central injection of PP in mice induced c-Fos immunoreactivity in somatostatin-containing cells in the hippocampus compared with PBS-injected mice. In sum, our results identify PP as a pivotal modulator of somatostatin secretion.

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1. Introduction

Pancreatic polypeptide (PP), gut derived peptide YY (PYY) and the neuronal derived peptide neuropeptide Y (NPY) belong to a family of structurally related peptides which have functions in both neural and endocrine signaling [1,2]. Relatively little is known about the functional significance of PP, a 36-amino acid peptide secreted by PP (or F) cells of the islets of Langerhans in the pancreas and released into the circulation. PP is also expressed in endocrine cells of both small and large intestine [3,4]. It shares considerable homology with the peptide sequences of PYY and NPY, and is reported to have effects on several gastrointestinal functions such as gastric motility, gallbladder contraction, and pancreatic exocrine secretion [5]. Previous studies have shown that PP appears to be involved in regulating food intake and energy balance [6]. Transgenic mice overexpressing PP gained less weight because of decreased food intake and this was accompanied by decreased fat mass [7]. Peripheral administration of PP to genetically obese ob/ob mice induced a state of negative energy balance because of decreased food intake and increased energy expenditure [8]. In humans, intravenous infusions of PP reduced food intake [9], and low circulating levels of PP have been observed in obese people [10–12]. PP secretion was thought to be primarily under vagal control [13], although other factors have also been shown to alter circulating PP concentrations [14]. Increasing plasma concentrations are seen after the ingestion of food and remain elevated for up to 6 h [1,2,15]. In type 2 diabetic subjects, PP cells secrete excess PP and plasma PP levels are significantly elevated in the postprandial state, compared to non-diabetic subjects [16].

The biological effects of the NPY family peptides are exerted through the NPY receptor family, which consists of at least five distinct members (Y1, Y2, Y4, Y5, and Y6) [17]. As PP has the highest affinity for the NPY4 receptor (NPY4R), it is thought to be the major endogenous ligand for this receptor [18]. PP dose-dependently reduced food intake in fed and fasted mice, and this effect was completely abolished in NPY4R-null mice, indicating that the effect is entirely mediated by NPY4Rs [19]. NPY4R is a G protein-coupled receptor and its activation by PP inhibits forskolin-stimulated cAMP synthesis in several NPY4R-containing cell lines [18,20,21]. NPY4Rs are present in the peripheral organs, including the gastrointestinal tract, liver, pancreas, and heart [18,20,22–24]. Additionally, significant amounts of NPY4R mRNA and specific binding sites have been found in key areas of the brain including hypothalamus and hippocampus [20,23,24], underscoring the importance of the receptor to food intake. Because circulating PP levels are highest after eating, we investigated if PP, besides modulating food intake, is involved in locally regulating secretion from any of the islet cell

* Corresponding author. Address: National Institute on Aging, 251 Bayview Boulevard, Suite 100, Baltimore, MD 21224, USA. Fax: +1 410 558 8381.
E-mail address: eganj@grc.nia.nih.gov (J.M. Egan).

http://dx.doi.org/10.1016/j.febslet.2014.07.005
0014-5793/Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.

types. We found that NPY4Rs are expressed in somatostatin-containing delta (δ) cells in islets of Langerhans, but not the other islet cell types. We then investigated if somatostatin-expressing cells in other organs, such as in duodenum and brain, also express NPY4R.

2. Materials and methods

2.1. Materials and reagents

mHippo E18 cells were from CELLutions Biosystems Inc. The somatostatin EIA kit was from Phoenix Pharmaceuticals, human insulin ELISA from Mercodia, and pancreatic polypeptide (PP) was from BACHEM. Human islets were provided by the NIDDK-funded Integrated Islet Distribution Program (IIDP) at City of Hope. Frozen brain sections (40 μm) were from a 10 year old male rhesus macaque. Dr. Frederic B. Askin from the Department of Pathology at The Johns Hopkins University School of Medicine provided anonymous human tissues.

2.2. Cell culture and somatostatin and insulin secretion

mHippo E18 cells and insulin-secreting MIN6 cells were cultured in DMEM (Invitrogen) with 10% fetal bovine serum (FBS), 25 mM glucose, and 1% penicillin/streptomycin and maintained at 37 °C with 5% CO_2. Cells were seeded into 35 mm dishes, grown to confluency, washed 1X with PBS, and treated with 0.1 μM of PP or PBS as vehicle in triplicate for 48 h. Media was collected and somatostatin levels were measured using a somatostatin EIA kit. For human islet experiments, we picked 100 size-matched islets per tube in Krebs buffer followed by 30 min incubation at 37 °C. We pelleted the islets and treated with Krebs buffer containing 1 μM of PP or PBS. After 20 min incubation at 37 °C, we collected the supernatant and somatostatin levels were measured using a somatostatin EIA kit and insulin was measured by ELISA according to the manufacturers' instructions. Total protein concentration was measured by BSA assay (Pierce) for normalization.

2.3. Western blotting and densitometry

Cells and homogenized mouse liver were lysed in RIPA buffer (25 mM HEPES, 134 mM NaCl, 1% NP-40, 0.1% SDS, 1 mM sodium orthovanadate, 0.5% sodium deoxycholate, 100 mM NaF) supplemented with protease inhibitor cocktail set I and phosphatase inhibitor cocktail set II (Calbiochem) for 20 min ice with occasional vortexing, and then pelleted in a microcentrifuge at maximum speed for 20 min to remove insoluble material. Following a BCA protein assay, 50 μg of lysate was resolved on 4–12% Tris–glycine gels (Invitrogen) and blotted onto polyvinylidene difluoride (PVDF) membranes using the iBlot (Invitrogen). The blots were blocked in 5% milk/TBS-T for 1 h at room temperature and probed with anti-NPY4R (1:1000, Santa Cruz) or anti-GAPDH (1:500, Santa Cruz) antibodies in blocking buffer overnight at 4 °C. Membranes were washed with TBS-T and the appropriate secondary antibody (1:5000, GE Heathcare) was added in 5% milk/TBS-T for 1 h at room temperature. Membranes were washed again with TBS-T and developed using ECL Plus (GE Heathcare). Western blot images were scanned, saved as Tiff files, inverted, and integrated density was analyzed using ImageJ software (National Institutes of Health). Values were normalized to GAPDH.

2.4. Transcranial stereotaxic injection of PP to the hippocampus

Two-month-old male B6C3F1/J mice were initially anesthetized with 4% isoflurane and then maintained on 1.5% isoflurane anesthesia for the duration of the surgery. During stereotaxic surgeries, mice were maintained on a heating pad to ensure constant body temperature. Mouse heads were secured in a stereotaxic apparatus and a longitudinal midsagittal cut was made with a sterile scalpel to expose the skull. Injections were performed through a small hole in the skull drilled along the anterior-posterior and mediolateral coordinates using a 1.0 mm drill head. Injections to the dorsal hippocampus were performed at the following coordinates, representing distances in mm from skull bregma: anterior–posterior −2.1, mediolateral ±1.35 and dorsoventral −2.1. PP (1 μM) was injected in a constant flow of 0.2 μl/min followed by 3 min to allow the fluid to be absorbed by the tissue. PP was stereotaxically injected into right side of the hippocampus. An equal volume of PBS was injected into the left hippocampus to serve as a control. Injections were performed using a CMA-400 automatic pump pushing two Hamilton syringes simultaneously. The syringes were connected via PE10 tubing to a 27 gauge needles. Following the injections, the skin was sutured and mice were placed in a separate cage. Exactly at 15, 30 and 60 min after PP injection, mice were perfused with 4% paraformaldehyde and the brains were rapidly dissected.

2.5. Immunostaining

Paraffin-embedded human islets and tissues were prepared as previously described [25]. For frozen sections, tissues were rapidly dissected, fixed in 4% paraformaldehyde, immersed in 30% sucrose, embedded in O.C.T compound (Tissue-Tek) before freezing, and then sectioned at a thickness of 7 μm. After antigen unmasking (BioGenex), the tissues were blocked with 5% BSA/PBS for 1 h at room temperature and incubated overnight at 4 °C with anti-insulin (1:500; Millipore), anti-glucagon (1:1000; Sigma), anti-PP (1:1000; Millipore), anti-NPY4R (1:100; Santa Cruz), anti-somatostatin (1:200; Santa Cruz) and anti-c-Fos (1:100; Santa Cruz) antibodies. After washing, tissues were incubated with the appropriate secondary antibodies (1:500, Jackson ImmunoResearch) along with TO-PRO-3 (1:5000, Molecular Probes), in some cases, for nuclear staining. Slides were viewed using a LSM-710 confocal microscope (Carl Zeiss MicroImaging).

2.6. Statistical analysis

Quantitative data are presented as the mean ± S.E.M. Differences between mean values were compared statistically by Student's *t*-test. Comparisons were performed by using Graphpad Prism (GraphPad Software). A P value of <0.05 was considered statistically significant.

3. Results

3.1. NPY4R is expressed in somatostatin-containing cells

Somatostatin-containing cells are dispersed throughout the gut, pancreas, and brain [26–28]. We first looked for evidence of the presence of NPY4Rs in both human and mouse islets. By immunostaining, NPY4Rs were colocalized with somatostatin-containing cells (Fig. 1A), but were absent from PP (F), insulin-containing (β), and glucagon-containing (α) cells in human islets (Fig. 1A and B). Consistent with this result, NPY4Rs were only present in somatostatin-containing cells in mouse islets (Fig. 1C and D). We also confirmed their presence in the somatostatin-containing cells in both human and mouse duodena by immunostaining (Fig. 2A and B).

As somatostatin is also found in neural tissues including hypothalamus and hippocampus [29,30], we evaluated the presence of NPY4R in somatostatin-containing cells in brain. NPY4Rs were expressed in somatostatin-containing cells in the hippocampus