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Corticotropin releasing hormone can selectively stimulate glucose uptake in corticotropinoma via glucose transporter 1

Jie Lu [a], Blake K. Montgomery [b, c, 1], Grégoire P. Chatain [a, 1], Alejandro Bugarini [b, 1], Qi Zhang [b], Xiang Wang [b], Nancy A. Edwards [b], Abhik Ray-Chaudhury [b], Marsha J. Merrill [b], Russell R. Lonser [d], Prashant Chittiboina [a, b, *]

[a] Neurosurgery Unit for Pituitary and Inheritable Diseases, National Institute of Neurological Diseases and Stroke, Bethesda, MD, United States
[b] Surgical Neurology Branch, National Institute of Neurological Diseases and Stroke, Bethesda, MD, United States
[c] Department of Orthopedic Surgery, Stanford Medicine, Stanford, CA, United States
[d] Department of Neurological Surgery, Wexner Medical Center, The Ohio State University, Columbus, OH, United States

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ABSTRACT

Background: Pre-operative detection of corticotropin (ACTH) secreting microadenomas causing Cushing's disease (CD) improves surgical outcomes. Current best magnetic resonance imaging fails to detect up to 40% of these microadenomas. ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) is specific, but not sensitive in detecting corticotropinomas. Theoretically, secretagogue stimulation with corticotropin releasing hormone (CRH) could improve detection of adenomas with ^{18}F-FDG PET. Previous attempts with simultaneous CRH stimulation have failed to demonstrate increased ^{18}F-FDG uptake in corticotropinomas. We hypothesized that CRH stimulation leads to a delayed elevation in glucose uptake in corticotropinomas.

Methods: Clinical data was analyzed for efficacy of CRH in improving ^{18}FDG-PET detection of corticotropinomas in CD. Glucose transporter 1 (GLUT1) immunoreactivity was performed on surgical specimens. Ex-vivo, viable cells from these tumors were tested for secretagogue effects (colorimetric glucose uptake), and for fate of intracellular glucose (glycolysis stress analysis). Validation of ex-vivo findings was performed with AtT-20 cells.

Results: CRH increased glucose uptake in human-derived corticotroph tumor cells and AtT-20, but not in normal murine or human corticotrophs ($p < 0.0001$). Continuous and intermittent (1 h) CRH exposure increased glucose uptake in AtT-20 with maximal effect at 4 h ($p = 0.001$). Similarly, CRH and 8-Br-cAMP led to robust GLUT1 upregulation and increased membrane translocation at 2 h, while fasentin suppressed baseline ($p < 0.0001$) and CRH-mediated glucose uptake. Expectedly, intra-operatively collected corticotropinomas demonstrated GLUT1 overexpression. Lastly, human derived corticotroph tumor cells demonstrated increased glycolysis and low glucose oxidation.

Conclusion: Increased and delayed CRH-mediated glucose uptake differentially occurs in adenomatous corticotrophs. Delayed secretagogue-stimulated ^{18}F-FDG PET could improve microadenoma detection.

1. Introduction

Improved remission rates and decreased adverse events following transsphenoidal surgery in Cushing's disease (CD) rely on accurate pre-operative localization of microadenomas (Bochicchio et al., 1995; Moshang, 2003). Magnetic resonance imaging (MRI) remains the gold standard for detection of pituitary adenomas, although modern MRI modalities such as dynamic or volumetric sequences fail to detect an adenoma in 40% of CD cases (Chowdhury et al., 2010; Kasaliwal et al., 2013; Lonser et al., 2013). Incidental sellar uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) is highly specific but not sensitive for pituitary adenomas (Ju et al., 2017; Hyun et al., 2011; Jeong et al., 2010).

* Corresponding author. Neurosurgery Unit for Pituitary and Inheritable Diseases, National Institute of Neurological Diseases and Stroke, National Institutes of Health, 10 Center Drive, Room 3D20, Bethesda, MD 20892-1414, United States.
E-mail address: prashant.chittiboina@nih.gov (P. Chittiboina).
[1] These authors contributed equally to this work.

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Abbreviations used

^{18}F-FDG	^{18}F-fluorodeoxyglucose
ACTH	adrenocorticotropic hormone or corticotropin
AtT-20	murine derived ACTH secreting tumor cells
BPTES	bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl) ethyl sulfide
CD	Cushing's disease
DEX	dexamethasone
CRH	corticotropin releasing hormone
ECAR	extracellular acidification rate
ETO	etomoxir
FCCP	carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone
GLUT1	glucose transporter 1
GLUT2	glucose transporter 2
GLUT3	glucose transporter 3
GLUT4	glucose transporter 4
GLUT8	glucose transporter 8
MRI	magnetic resonance imaging
OCR	oxygen consumption rate
PET	positron emission tomography
RT-qPCR	real-time quantitative polymerase chain reaction
SUV	standardized uptake value

Corticotropin releasing hormone (CRH) has secretagogue effects on adrenocorticotropic hormone or corticotropin (ACTH) secreting pituitary adenomas (Takano et al., 1996), but not on suppressed adjacent normal gland. This effect could theoretically improve the imaging detection of adenomas with ^{18}F-FDG PET imaging following secretagogue stimulation. However, our group (unpublished data) and others (Patt et al., 2014), have failed to demonstrate increased ^{18}F-FDG uptake in corticotropinomas by simultaneous CRH administration. To date, it remains unclear whether corticotropinomas are resistant to CRH-mediated glucose uptake, or if the effects are delayed.

We hypothesized that CRH stimulation leads to a delayed elevation in glucose uptake in corticotropinomas. In this study, we investigated the kinetics of CRH-modulated glucose uptake. A time-course *in-vitro* study revealed that maximum glucose uptake occurs approximately 4 h post CRH administration. Moreover, we demonstrate for the first time that CRH stimulation results in a differential glucose uptake in adenomatous, but not in normal corticotrophs. Mechanistically, this was associated with a robust increase in glucose transporter 1 (GLUT1) expression. Taken together, these novel findings support the potential use of delayed ^{18}F-FDG PET imaging following CRH stimulation to improve microadenoma detection in CD.

2. Materials and methods

2.1. Tissue sample collection

A total of 10 patients with CD were enrolled in a clinical trial (NIH 12-N-0067, NCT01459237) conducted at the National Institute of Neurological Diseases and Stroke (NINDS) to evaluate the utility of CRH-stimulated ^{18}F-FDG PET. Study was approved by the Combined Neuroscience Institutional Review Board (IRB). Pituitary adenoma tissues were obtained from these patients at the time of transsphenoidal adenomectomy at the National Institutes of Health Clinical Center (NIHCC) under a clinical trial (NIH 03-N-0164, NCT00060541) for subsequent immunohistochemical and metabolic analyses. Written informed consent was obtained from each patient for research study participation. The study was conducted in accordance to the standards and guidelines established by the IRB.

2.2. CRH-stimulated ^{18}F-FDG PET study

PET imaging was performed using a high-resolution research tomography scanner (Siemens AG). Each subject underwent two randomly ordered ^{18}F-FDG high resolution PET (hrPET) studies with and without CRH stimulation separated by at least 24 h. For CRH stimulated studies, ovine CRH (oCRH or Acthrel®) 1 mcg/kg (up to a maximum dose of 100 mcg) was administered immediately preceding ^{18}F-FDG administration (10 mCi). Pituitary metabolic activity was quantified using the standardized uptake values (SUV). Maximum SUV (SUV-Max) and averaged SUV (SUV-Avg) were calculated as previously described (Chittiboina et al., 2014).

2.3. Cell culture

AtT-20/D16:16 (a generous gift from Dr. Steven L. Sabol at the National Heart Lung and Blood Institute) murine corticotroph tumor cell lines were cultured in DMEM (Thermo Fisher Scientific, USA), 10% FCS (Thermo Fisher Scientific) following comparison with commercially available AtT-20 cells (Supplementary Fig. 1). Cells were incubated for 2 h prior to all experiments in serum-free DMEM supplemented with 0.25% BSA (hereafter DMEM-BSA) and 90 mg/dL glucose, to simulate physiologic glucose levels. Pooled corticotroph cells were harvested (<30 min post-euthanasia) from female, BALB/c mice (aged 6–8 weeks; Taconic Biosciences, USA), digested and homogenized in 1 mg/mL collagenase (Sigma-Aldrich, USA) for 30 min, and cultured as above. All animals were euthanized in accordance with the standards and guidelines of the Institutional Animal Care and Use Committee of the National Institutes of Health (NIH). Western blot analysis was performed with anti glucocortidoid receptor antibody (Santa Cruz Biotechnology), anti Serine 211 phophorylated glucocorticoid receptor antibody (antibodies-online.com), and anti CRH receptor 1 antibody (Thermo Fisher Scientific).

2.4. Fluorescence activating sorting of murine corticotrophs

After dissociation of murine hypophyseal cells, corticotrophs were isolated by flow cytometry. Briefly, pooled cells were incubated with anti-CRH receptor 1 antibody (CRH-R1) (Thermo Fisher Scientific) for 1 h, followed by Alexa Fluor-555 conjugated antibody (Invitrogen, USA) and finally stained with DAPI (Thermo Fisher Scientific) for 30 min. Cell sorting analysis was carried out using MoFlo Astrios Cell Sorter and Summit Acquisition and Analysis software (Beckman Coulter, USA).

2.5. ACTH release studies

Following several washes in PBS, cells were incubated in serum-free DMEM-BSA and stimulated with 50 nM CRH (Sigma-Aldrich, USA), 100 nM arginine vasopressin (Sigma-Aldrich), 5 mM 8-bromoadenosine 3′,5′-cyclic monophosphate (8-Br-cAMP; Sigma-Aldrich), and/or 10 nM dexamethasone (DEX; Sigma-Aldrich) for the time points indicated. Media was then diluted and assessed via enzyme linked immunosorbant assay (MD Bioproducts, USA) according to manufacturer instructions.

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