Transcellular activation of gene expression in co-cultured neurons and glial cells

Master's thesis

Student: Helena Tull

Supervisors: Florencia Cabrera-Cabrera, PhD

Postdoctoral researcher, Department of Chemistry and Biotechnology,

Indrek Koppel, PhD

Assistant Professor, Department of Chemistry and Biotechnology,

Study program: Applied Chemistry and Biotechnology

Tallinn 2023

Ko-kultuuris kasvatatud neuronite ja gliiarakkude transtsellulaarselt aktiveeritud geenieksressioon

Magistritöö

Üliõpilane: Helena Tull

Juhendajad: Florencia Cabrera-Cabrera, PhD

Järeldoktor-teadur, Keemia ja biotehnoloogia instituut,

Indrek Koppel, PhD

Nooremprofessor, Keemia ja biotehnoloogia instituut

Õppeprogramm: Rakenduskeemia ja biotehnoloogia

Tallinn 2023

Declaration

Hereby I declare that I have compiled the paper independently and all works, important standpoints and data by other authors have been properly referenced and the same paper has not been previously been presented for grading.

Author: Helena Tull

[Signature, date]

The paper conforms to requirements in force.

Supervisor: Florencia Cabrera-Cabrera

[Signature, date]

Permitted to the defence.

Chairman of the Defence Committee:

[Signature, date]

Table of contents:

Abstract

The brain consists of multiple cell types and specialized brain cells are divided into neurons and glial cells. These cells communicate with each other, and in this communication Ca^{2+} signaling and exocytotic release of neurotransmitters have important roles.

We are interested in studying communication of neurons and astrocytes, one major type of glial cells. We hypothesized that activation of Ca^{2+} release in astrocytes can trans-activate expression of activity-dependent genes in neurons. As a test system we used neuron-glia co-cultures prepared from prenatal rat brain cortex.

In this thesis, I validated hM3Dq-DREADD for stimulating astrocytes and RiboTag to measure changes in activity-dependent neuronal genes. The astrocytic hM3Dq-DREADD is validated by astrocytic markers and neuronal RiboTag with neuronal markers with immunocytochemistry. Here, we show that astrocytic stimulation is expressed as a significant change in astrocytic pCREB induction. When astrocytic hM3Dq-DREADD was stimulated, the changes were measured with activity-dependent neuronal genes with RiboTag. The candidate neuronal activity-dependent genes showed in qPCR analyses tendencies of activation.

Annotatsioon

Aju koosneb erinevatest rakutüüpidest ja spetsialiseerunud ajurakud jaotatakse neuroniteks ning gliiarakkudeks. Neuronid ja gliiarakud on omavahelises suhtluses, kus Ca^{2+} signaalimine ja neurotransmitterite vabanemine rakust mängivad olulist rolli. Selles töös keskendusime gliiarakkudest astrotsüütidele ja oletasime, et rakusisese Ca^{2+} vabastamine astrotsüütides võib trans-aktiveerida aktiivsus-sõltuvate geenide avaldumist neuronites. Katsesüsteemina kasutasime sünnieelse roti ajukoorest eraldatud primaarsete neuronite ja gliiarakkude ko-kultuuri.

Antud töös kasutasin astrotsüüdi-spetsiifiliseks Ca^{2+} vabastamiseks kemogeneetilist tööriista hM3Dq-DREADD'i, ning ribosoomide afiinsuspuhastamise metoodikat (RiboTag) rakuspetsiifiliseks mRNA eraldamiseks neuronitest. Nende tööriistade rakuspetsiifilise avaldumise valideerimiseks kasutasin immunotsütokeemia metoodikat. Kasutasin astrotsüütide markereid, et valideerida astrotsüütiline hM3Dq-DREADD ja neuronaalseid markereid, et valideerida neuronaalne RiboTag. Lisaks näitan selles töös, et hM3Dq-DREADD stimulatsioon astrotsüüdis tõstab CREB transkriptsioonifaktori fosforüleerimise taset astrotsüütides. RT-qPCR analüüsi abil selgus, et astrotsüütilise hM3Dq-DREADD-i stimuleerimine põhjustab mõõdukat induktsiooni neuronite aktiivsus-sõltuvate geenide avaldumises.

Abbreviations

A1R	A1 receptor
AAV	adeno-associated virus
Akt	v-Akt Murine Thymoma Viral Oncogene
Aldh1l1	aldehyde dehydrogenase 1 family member L1
ANLS	astrocyte-neuron lactate shuttle
Aqp4	aquaporine 4
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
BSA	bovine serum albumin
CaMK	Ca^{2+}/calmodulin-dependent protein kinase
cAMP	cyclic adenosine monophosphate
CBP/300	CREB binding protein/ homologue p300
c-Fos	cellular Fos proto-oncogene
c-Jun	cellular Jun proto-oncogene
CNO	clozapine N-oxide
CNPase	nucleoside-2′,3′-cyclic-phosphate 2′-nucleotidohydrolase
CNS	central nervous system
CREB	cAMP response element-binding protein
DAG	diacylglycerol
DCX	doublecortin
DMSO	dimethyl sulfoxide
DREADD	Designer Receptors Exclusively Activated by Designer Drugs
DTT	dithiothreitol
EGR1	early growth response protein 1
EPC	excitatory postsynaptic currents
ERK	extracellular signal-related protein kinase
FACS	fluorescent-activated cell sorting
FBS	fetal bovine serum
GABA	γ-aminobutyric acid

GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GDP	guanosine diphosphate
GFAP	glial fibrillary acidic protein
GFP	green-fluorescence protein
GLAST	glutamate/aspartate transporter
GPCR	G-protein coupled receptor
GS	glutamine synthetase
GsD	Gs coupled DREADD
GTP	guanosine triphosphate
Gαi	G protein with α i/o subtype
Gαq	G protein with αq subtype
Gαs	G protein with αs subtype
HA-tagged	hemagglutinin-tagged
HB	homogenization buffer
HBS	supplemented homogenization buffer
HBSS	Hanks Balanced Salt Solution
HEK293FT	human embryonic kidney 293 Fast growing variant if HEK293 cells
hM3Dq	human muscarinic acetylcholine receptor 3 with Gq coupled DREADD
hM4Di	human muscarinic acetylcholine receptror 3 Gq coupled DREADD
Iba1	ionized calcium-binding adapter molecule 1
ICC	immunocytochemistry
IP_3	inositol 1,4,5-triphosphate
KOR	kappa opioid receptor
KORD	kappa opioid receptor DREADD
MAP2	microtubule-associated protein 2
MAPK	mitogen-activated protein kinase
Neu-N	neuronal nuclear protein
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
NP40	nonylphenoxy polyethoxy ethanol

PBS	phosphate-buffered saline
pCREB	phosphorylated cAMP response element-binding protein
PEI	polyethyleneimine solution
PFA	paraformaldehyde
PI3K	phosphatidylinositol-3-kinases
PIP_2	phosphatidylinositol-4,5-bisphosphate
PIP_3	phosphatidolinositol-(3,4,5)-triphosphate
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PNS	peripheral nervous system
RAS	rat sarcoma
RPL22	ribosomal protein 22
Rq	endogenous muscarinic receptor
RT	room temperature
qRT – PCR	quantitative reverse-transcription polymerase chain reaction
RTK	receptor tyrosine kinase
RVC	ribonucleoside vanadyl complex
SALB	Salvinorin B
scRNA-seq	single-cell RNA sequencing
SIC	slow inward currents
SSIV	Superscript IV
Syt1	synaptotagmin 1
TF	transcription factor
TRAP	Translating Ribosome Affinity Purification
TrkB	tropomyosine related kinase B
VGCC	voltage gated Ca^{2+} channels
WPRE	Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element

Introduction

Brain tissue consists of multiple cell types, including neurons and neuroglial cells. Communication between these cells using neurotransmitters and other mediators has recently become an important area of study in neuroscience.

In this thesis, I have studied if activation of Ca^{2+} release in astrocytes can trans-activate neurons in a primary neuron-glia co-culture system.

In this work's theoretical part I am giving an overview of nervous system cell types and their functions, current knowledge in neuron-astrocyte communication, methods for cell-specific activation of signaling pathways and cell-specific gene expression analysis.

In the experimental part, I have used hM3Dq-DREADD and RiboTag methods to study changes in neuronal activity-dependent genes in neurons as response to activation of hM3Dq in astrocytes.