LUDC-IRC Postdoctoral Program 2020 – Project 6

The role of hormone sensitive lipase in brain function

PI: Cecilia Holm
Co-PI: Joao Duarte

Purpose and aims

Hypothalamic dysfunction has been suggested to precipitate the development of obesity and metabolic syndrome. On the other hand, complications deriving from unhealthy lifestyles and obesity impact the brain, and constitute a risk for cognitive impairment. **Our overall purpose is to identify targetable mechanisms and pathways that can improve neuronal function** in the hypothalamus that regulates glucose homeostasis, as well as in brain areas that are key for cognition.

Hormone sensitive lipase (HSL) activity might regulate the availability of bioactive lipids that modulate neuronal function, such as endocannabinoids. **This project aims at identifying HSL-dependent mechanisms of synaptic control that are mediated by endocannabinoids, and which are likely altered in obesity and type 2 diabetes (T2D).**

State-of-the-art

Obesity is a multifactorial complex disease that has reached pandemic proportions and is a strong risk factor for T2D, cardiovascular and cerebrovascular disease. In turn, these disorders have been proposed to contribute to the development of cognitive impairment, Alzheimer's disease (AD) and vascular dementia. Among other factors, brain insulin resistance appears to play a role in the process of cognitive deterioration, and brain insulin sensitization is a proposed therapeutic (1).

Glutamatergic dysfunction

Glutamate is the most abundant excitatory neurotransmitter in the brain, it is a key element in learning and memory, and glutamatergic dysfunction and glutamate excitotoxicity plays a role in neuropsychiatric disorders (2). Glutamatergic neurons also account for a substantial fraction of hypothalamic cells (3), and damage to glutamatergic synapses in obesity or T2D might contribute to hypothalamic nutrient and hormone sensing. Before neuronal loss, an attack on synapses is important for brain dysfunction. Our recent work sets synaptic dysfunction at the center of memory impairment and brain dysfunction in T2D (4). Astrocytes are equipped to modulate synapses, and couple neuronal activity to nutrient supply from the circulation. The metabolic support from astrocytes to glutamatergic neurotransmission was found disrupted in the Goto-Kakizaki model of T2D that displays brain insulin resistance (5,6).

Endocannabinoid system

Endocannabinoids are endogenous lipid-based modulators binding to CB1 and CB2 receptors that control neurotransmission, plasticity, learning and memory (7), and have been proposed to interact with insulin and leptin signalling. Namely:

- endocannabinoids regulate synaptic processes and cell metabolism (7);
- the CB1 receptor (CB1R) can form functional heterodimers with insulin and IGF1 receptors (8,9);
- the CB1R regulates insulin signalling in brain slices (10);
- endocannabinoids modulate leptin sensitivity in hypothalamus (11,12).

2-arachidonoylglycerol (2AG) is an endocannabinoid synthetized by lipases at postsynaptic terminals following stimulation by glutamate (figure 1), and is then released to presynaptic terminals where it activates CB1Rs, thus dampening glutamatergic activity at synapses (13). Synaptic activity also stimulates insulin-dependent GLUT4 mobilization from intracellular sources to support synaptic energy demands (14), a process that might indirectly depend on CB1Rs. In addition, activated CB1Rs directly inhibit metabolism in the

mitochondria of neurons and astrocytes (15), which may contribute to 2AG-dependent synaptic control. Altogether, this evidence suggests that the crosstalk between endocannabinoids and hormonal signalling in the hypothalamus might constitute a valuable target to improve astrocytic nutrient sensing in obesity. While global targeting of central CB1Rs might produce undesired behavioural alterations, local modulation of hypothalamic endocannabinoid synthesis might produce efficient appetite regulation. Likewise, the endocannabinoid-insulin crosstalk might regulate mechanisms related to cognition.

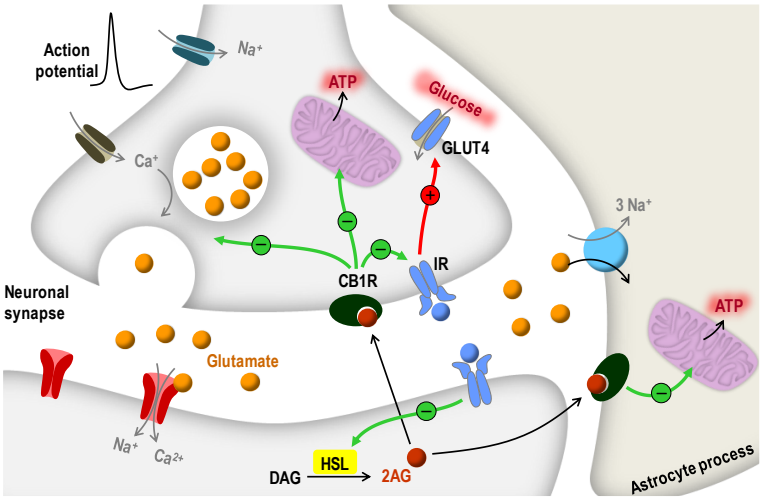

Figure 1: Putative role of HSL regulating 2AG for synaptic control. While only direct metabolic regulation is depicted, CB1Rs control many other pathways.

Hormone sensitive lipase (HSL) activity

HSL is mainly present in white adipose tissue where it is mainly responsible for degrading diacylglycerols (DAG) to monoacylglycerols (16). It is inhibited by insulin and stimulated by catecholamines. The brain is capable of metabolizing lipids, and HSL has been suggested to be expressed in the brain (17). However, since glucose is the obligatory fuel for brain metabolism and its availability is not insulin-dependent (18), any role for brain HSL is likely unrelated to direct control of brain energy metabolism. **We propose that HSL regulates the availability of bioactive lipids, namely 2AG**, and is thus key in the crosstalk between endocannabinoid and insulin signalling in the synapse. Accordingly, brain insulin resistance could result in increased HSL activity and concomitant tonic increase of 2AG levels, which in turn dampens glutamate release and synaptic fuelling via CB1Rs (figure 1). This hypothesis is in accordance to our findings in insulin resistant rats (4-6). In contrast, low HSL activity could result in low 2AG levels and thus glutamate excitotoxicity, which results in synaptic damage and neuronal death (2). Therefore, we consider that glutamatergic function in the hypothalamus or in brain areas controlling cognition may be rescued through HSL targeting.

Significance and scientific novelty

Drug development activities targeting the endocannabinoids system (ECS) require detailed knowledge of its components. Attempts to target the ECS in the treatment of obesity and associated disorders initially focused on global CB1R antagonists, e.g. rimonabant, which had beneficial effects on cardiometabolic function. However, these drugs were withdrawn in 2008 due to a number of psychiatric side effects. In this project, we will test the hypothesis that HSL, mostly known for its role in mobilization of fatty acids in adipocytes and other cell types, is a novel player in the ECS, with implications for both normal brain function and brain insulin resistance.

Our hypothesis is supported by preliminary data, obtained from detailed phenotyping of HSL knockout (KO) mice (see below). In the short run, results from the project may identify yet another role of this multifunctional enzyme as well as shed light on the puzzling phenotype of HSL KO mice, which concerning metabolic traits is similar to that observed in humans carrying a deletion in the HSL (LIPE) gene (19). In the long run, detailed knowledge about the ECS and HSL are crucial for ongoing drug development activities, where both the ECS and HSL are recognized targets (20,21).

The acknowledgement of multiple subgroups of adult diabetes highlight the need for novel and more personalized approaches to treat diabetes. For instance, **moderate obesity-associated diabetes may benefit from an approach targeting obesity development and insulin resistance at the same time**. HSL is a promising multipurpose target that can simultaneously control fatty acid mobilization, hypothalamic dysfunction, and key aspects of insulin resistance. Moreover, findings from this project might reveal that HSL modulation in the central nervous system can be repurposed for neuropsychiatric disorders.

Research plan

Animal model: HSL null mice and wild-type littermates from a colony at BMC (Lund) will be fed either a high-fat diet (HFD) or regular chow.

Metabolic characterisation: We will conduct glucose tolerance and insulin sensitivity tests, as well as glycemic clamps. Energy expenditure, food intake, locomotor activity and voluntary physical activity will be monitored using metabolic cages (27). Hormonal profiles will be characterised in detail.

Cognitive function: Learning/memory will be investigated in all studied animals with a battery of behavioral tests: Barnes Maze; Y-maze spontaneous alternation; Novel object/location recognition.

Hypothalamic function: We have recently developed an experimental setup to assess hypothalamic function in mice in a non-invasive and time-resolved manner by means of functional magnetic resonance imaging (fMRI; 28). This method will be employed to assess hypothalamic response to a glucose load.
Lipidomics: Metabolomics will be performed to investigate levels of bioactive lipids, including endoccanabinoids and oxylipins (29), and standard lipidomics by GS-MS to assess other lipids.

Identification of novel pathways: Since endocannabinoids control a wide number of cellular processes, we will conduct untargeted transcriptomics in the cortex, hippocampus and hypothalamus. We will further compare differentially expressed pathways with those observed in aging and other neurological disorders (e.g. datasets from the Allen Brain Map). Specific pathways selected from the unbiased analysis above will be further investigated by immunoblotting and qPCR from collected brain samples, and by immunofluorescence confocal microscopy on fixed brain slices for localizing proteins from altered pathways to specific brain cells.

Time plan

<table>
<tr><td colspan="4">Year 1</td><td colspan="4">Year 2</td></tr>
<tr><td>Q1</td><td>Q2</td><td>Q3</td><td>Q4</td><td>Q1</td><td>Q2</td><td>Q3</td><td>Q4</td></tr>
<tr><td colspan="3">Metabolic characterization</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="8">Cognitive function</td></tr>
<tr><td></td><td colspan="3">Hypothalamic function</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td colspan="2">Lipidomics</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td colspan="4">Identification of novel pathways (transcriptomics)</td></tr>
</table>

Implementation/organisation: Holm's lab has conducted extensive research on HSL, and has expertise on metabolic phenotyping. Duarte's lab has expertise on behavior testing (Duarte is manager of the Mouse Behavior Platform) and has implemented hypothalamic fMRI methods, which will be conducted at the 9.4 T MRI scanner of the Lund Bioimaging Center. Sequencing will be performed at the Centre for Translational Genomics, Lund or at the LUDC, Malmö, and data analysis will be in collaboration with Dr. Rashmi Prasad. A research engineer from Duarte's lab will aid in the metabolic characterization, cognitive function assessments, and sample collection for lipidomics, which will be carried out at the Swedish Metabolomics Centre using dedicated protocols (29) for endocannabinoids and oxylipins (SciLifeLab, Umeå). A Ph.D. student co-supervised by the applicants will participate in pathway confirmation experiments (immunoblotting, qPCR, confocal microscopy).

References

1. Lee JH, Jahrling JB, Denner L, Dineley KT (2018) J Alzheimers Dis 64(s1): S427-S453.
2. Nicholls DG, Brand MD, Gerencser AA (2015) J Bioenerg Biomemb 47:63-74.
3. Chen R, Wu X, Jiang L, Zhang Y (2017) Cell Rep 18(13): 3227-3241.
4. Duarte JMN, Skoug C, et al. (2019) Front Neurosci 12:1015.
5. Girault F-M, Sonnay S, Gruetter R, Duarte JMN (2019) Neurotox Res 36(2): 268-278
6. Soares AF, Nissen JD, Garcia-Serrano AM, et al. (2019) J Neurosci Res 97(8):1004-1017.
7. Metna-Laurent M, Marsicano G (2015) Glia 63(3):353-64.
8. Kim W, Lao Q, et al. (2012) Sci Signal 5(216):ra23
9. Dalton GD, Howlett AC (2012) Br J Pharmacol. 165(8):2497-511
10. Pinheiro BS, Lemos C, et al. (2016) Brain Res Bull 124:222-30.
11. Tam J, Szanda G, et al. (2017) Mol Metab 6(10): 1113-1125.
12. Thanos PK, Ramalhete RC, et al. (2008) Synapse 62(9): 637-42.

13. Iannotti FA, Di Marzo V, Petrosino S (2016) Prog Lipid Res 62:107-28.
14. Ashrafi G, Wu Z, Farrell RJ, Ryan T (2017) Neuron 93(3):606-615.
15. Duarte JMN, Ferreira SG, Carvalho RA, Cunha RA, Köfalvi A (2012) Neurochem Int 60(1):1-8.
16. Haemmerle G, Zimmermann R, et al. (2002) J Biol Chem 277(7):4806-15.
17. Etschmaier K, Becker T, et al. (2011) J Neurochem 119(5):1016-28.
18. Dienel GA (2019) Physiol Rev 99(1):949-1045.
19. Albert JS, Yerges-Armstrong LM, et al. (2014) N Engl J Med 370(24):2307-2315
20. Richey JM, Woolcott O (2017) Curr Diab Rep 17(10):99.
21. Morigny P, Houssier M, Mouisel E, Langin D (2016) Biochimie 125:259-66.
22. Osuga J, Ishibashi S, et al. (2000) PNAS 97(2):787-92.
23. Mulder H, Sorhede-Winzell M, et al. (2003) J Biol Chem 278(38):36380-8.
24. Mazier W, Saucisse N, Gatta-Cherifi B, Cota D (2015) Trends Endocrinol Metab 26(10):524-37
25. Cota D, Marsicano G, et al. (2003) J Clin Invest 112(3):423-31.
26. Powell DR, Gay JP, et al. (2015) Front Endocrinol 6:86.
27. Cavalera M, Axling U, Berger K, Holm C (2016) Nutr Metab (Lond) 13:91.
28. Mohr AA, Garcia-Serrano AM, et al. (2020) bioRxiv doi: https://doi.org/10.1101/2020.03.21.001149
29. Claeson AS, Gouveia-Figueira S, et al. (2017) Prostaglan Leukot Essent Fatty Acids 121:60-67.