The cross-talk molecular pathways of glutamate and leptin receptors

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Ionotropic glutamate receptors (NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors) are the key components of excitatory synapses in brain. They form various protein macrocomplexes that mediate inter-molecular rearrangements and form the functional regulatory protein-protein networks [1].

Leptin, the adipocytokine/neurotrophic factor, is an important regulator of body weight and metabolism. In addition to the well-described and characterized activity of leptin in the hypothalamus, it is becoming increasingly apparent that the central activity of leptin extends to including others brain regions. It's believed that this hormone has pro-cognitive and anti-depressive effects in the central nervous system. This concept is supported by studies demonstrating that leptin promotes hippocampal-dependent learning and memory, as well as studies indicating that leptin resistance is associated with deficits in hippocampal-dependent behaviors and in the induction of depressive-like behaviors [2-4]. Direct injection of leptin into the brain enhances of long-term potentiation (LTP) and facilitates the formation of spatial memory in mice [5]. Leptin reportedly may acts through some of the components of the insulin signalling cascade in particular PI3K/PKB signalling cascade [6-8] however this assumption does not seem well founded at least for hippocampal exciting synapses and requires additional research.

The class I PI3K-signaling pathway is involved in the implementation of leptin effects in the hippocampus [6-8]. Leptin has a mediated effect through PIP3 on glutamate AMPA receptors (AMPARs). This type of receptors is presented at the hippocampal synapses and is actively involved in the regulation of synaptic plasticity in the brain. AMPA receptors, which include GluR2/GluR3 subunits (GluR2-AMPAR), are involved in the processes of maintaining the basic activity of synapses and provide membrane depolarization during the LTP induction (Long Term Potentiation). AMPA receptors, which include the GluR1 subunit (GluR1-AMPAR), are introduced into the synaptic contact zone after the LTP induction and are then replaced by GluR2-AMPAR. Such recycling of different subunit composition of AMPARs depending on the synaptic activity is believed to be the basis for the synaptic transmission effectiveness control [9,10].

Leptin receptors (LepR) belong to the class I cytokine receptor family, which is known to act through JAK2 (Janus kinases). Jak2 is activated after leptin interacted with LepR. Onward Jak2 phosphorylated of tyrosine residues in LepR cytoplasmic terminus - Tyr985, Tyr1077 and Tyr1138, which is docking sites for some proteins, namely, SHP2 (SH2 (Src-like homology 2) domain-containing protein tyrosine phosphatase), STAT3 (signal transducer and activator of transcription3), STAT5 (signal transducer and activator of transcription5) respectively. Grb2 (growth factor receptor binding-2) is adapter protein that direct protein complexes formation and binds tyrosine phosphorylated sequences particularly pY (Tyr=Y) of SHP2 protein to forming leptin receptor signal transduction: LepR -> SHP2 -> Grb2 -> Ras/Raf/MAPK signaling cascade [8].

SH-2 and SH-3 (Src homology) are two types of domains that are often used by proteins to bind to other proteins in the signal transduction pathways. SH2 domains are docking to the certain short motives - YXXM motif. After this SH2-pY interaction this proteins can be activated. SH3 domains are associated with prolin rich motifs – PXXP core (Prolin rich domain, PRD) and provide spatial approach of proteins and forming temporary macrocomplexes. They are also called anchor proteins [11].

The domain organization of GeneNet database proteins that may be involved in the crosstalk molecular pathway of glutamate and leptin receptors through PI3K signaling was analyzed. A number proteins are contained both type domains, as SH2 as SH3. These proteins via its SH2 can dock to phosphotyrosine at the receptor and to a subsequent member of signal transduction via SH3 domain. In this group included protein leptin receptor signal transduction (example, Grb2, p85) and non-receptor tyrosine-protein kinases (Fyn, Lyn, Abl, Src, Yes), which included in synaptic plasticity processes.

According to the Swiss-Prot database (https://www.uniprot.org/) p85 has two SH2 domains and one SH3 domain. The regulatory p85 subunit of PI3K I class has two SH2 domains which can docking to the classical YXXM motive that is presented in the insulin receptor molecule: 1351- YTHM-1354. The sites of interaction with SH2 domains at the leptin receptor differ from the classical motive presented in the insulin receptor: 985-YATLV, 1077-YLGVT and 1138-YMPQF (for mouse LepR (P48356)).

It is known that Fyn kinase along with the SH2 domain has a SH3 domain. The Fyn annotation presented in Swiss-Prot, P39688 (Mouse), states that this protein interacts with the PRD of p85. Fyn is member of non-receptor Src family kinase and expressed at mature synapses, there is lack of its expression during early development. At shown that adult mice with mutations in Fyn gene are exhibits blunted though synaptic transmission and impaired spatial learning although mutations in other non-receptor tyrosine kinase genes (src, yes and abl) did not interfere with either the induction or the LTP maintenance [12]. Moreover, at shown that Fyn together with Lep-R direct interaction c NMDAR macrocomplexes, in particular NR2B subunits [13].

There is conclusive evidence that leptin enhances the expression of synaptic GluR1-AMPAR after NMDAR-mediated LTP induction, which was accompanied by PI3K-driven elevations in PIP3 density on synaptic membrane. In addition, the elevation levels of phosphorylated PTEN were observed [14]. PTEN (the phosphatase and tensin homolog deleted on chromosome 10) is a phosphatidylinositol D3-phosphatase that counteracts the effects of phosphatidylinositol 3-kinase, it dephosphorylates PtdIns(3,4,5)P3: PtdIns(3,4,5)P3 -> PtdIns(4,5)P2 (PIP3 -> PIP2) [15]. This eventually interrupts the signal transduction from the membrane into the cell. In a cell, PTEN is constitutively active. To deactivate it, there must be a cooperative phosphorylation of threonine and serine residues in its C terminus. Casein kinase 2 (CK2) - Ser-370, Ser-385, and Glycogen synthase kinase 3beta (GSK3beta) - Ser-362 and Thr-366 [16].

We hypothesized that Fyn may be crosstalk point of Leptin and NMDA receptors signal transduction. We implemented paired alignment to quantify the similarity of sequences SH2 domains of Fyn and proteins that integrated into leptin signal transduction - SHP2, Grb2, STAT3, STAT5 by using Smith-Waterman algorithm (http://www.ebi.ac.uk/Tools/psa/emboss_water/) (Table 1). Primary sequences were retrieved from the Swiss-prot database.

Table 1. RESULTS OF PAIRED ALIGNMENT SH2 DOMAINS

	Fyn Identity/ similarity, %	**score**
Stat3	36.1/ 55.7	64.0
Stat5	24.2/ 47.0	48.5
Jak2	27.4/ 46.4	65.5
SHP2 1 SH2	34.9 / 54.7	106.5
SHP2 1 SH2	37.9 / 53.7	135.0
Grb2	38.6 / 60.2	141.0

The highest score, as well as the degree of identity and similarity, is observed for the Fyn and Grb2 sequences. SHP2 domains also showed a high level of similarity (Table 1). Thus, we can conclude that Fyn, presumably, can be compete at pY-985 interactions either directly or by docking to pY-SHP2. It was shown that Leptin is involved in the regulation of aberrant synaptic plasticity, which was stopped by PI3K blockers, but not by MAPK blockers [6,7].

Moult with coauthors show that leptin preferentially increases the cell surface expression of GluR1-AMPAR in adult hippocampal slices. This effect was associated with an elevates PIP3 levels and the provided of CK2 and GSK3b phosphorylation that blocking activity PTEN [14].

In fact, that Fyn together with Lep-R direct interaction c NMDAR macrocomplexes and docking to proteins postsynaptic density and participate regulation AMPAR synaptic expression [17-19] allows us to propose the probably mechanism of crosstalk Leptin and NMDA receptors:

Leptin/LepR -> Jak2 -> pY-SHP2 -> Fyn -> Fyn/p85A -> p85/p100-PI3K -> [PIP2 -> PIP3]

Leptin/LepR -> Jak2 -> pY-SHP2 -> Fyn -> pY(216)-GSK3b -> pThr366-PTEN

Thus, after NMDAR-depended LTP induction starting different molecular processes that mediated structure and functional plasticity of synapse. Leptin through PI3K-signalling induced PIP3 generation that promotes LTP expression due to shutdown PTEN. This effect manifests itself quickly, which can explain the high speed of processes observed in experiments. However, subsequently leptin through PI3k/PKB shut down of GSK3b that well be advantage of the synaptic molecular network. Thus, the NMDAR and LepR association is exibitioned new facilities to regulate of synaptic plasticity (Fig. 1).

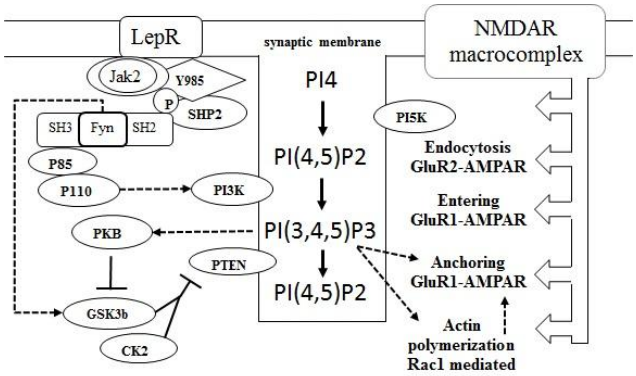

Fig. 1. The association of NMDAR and LepR during LTP.
⊣ - inhibitory effect; dotted arrow – activated effect; (P) hosphorylated residues. The description is in the text.

Although it is tempting to speculate that the Fyn kinase pathway results in the Y216 phosphorylation of GSK3β in neurons, further work is necessary to determine the validity of this hypothesis as well as verification of the direct interactions SH2 domain Fyn with pY of proteins LepR signaling cascade.

CONCLUSION

NMDA receptors mediated hippocampal excitatory neurotransmission that may be modulated of different factors in particularly hormone Leptin. After LTP induction NMDAR and Leptin receptor contributes to formed complexity system that enjoyed new emergent properties. This allows fine tuning of the regulation of synaptic plasticity in the hippocampus depending on outside signals.

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