VI. Conclusion and Prospects

Results summary

In the present study, I have characterized the distribution of VILIP-1, a member of the neuronal calcium sensor-family (VILIP family), in rat peripheral tissue and hippocampus. The VILIP sub-family is formed by highly conserved orthologues found in chick/mouse/rat/human (*VILIP-1*) showing more than 99% of amino acid identity between them (see Introduction).

Expression of VILIP-1 in rat

VILIP-1 is mainly expressed in brain, and strongly in heart, lung, liver and testis and a weaker expression was observed in ovary, kidney, spleen and pancreas. A similar expression profile was seen for NCS-1, which is in contrast to the expression of hippocalcin, which was exclusively found in rat brain.

In rat hippocampus, VILIP-1 occurs in principal cells and GABAergic interneurons but not in astrocytes, and distributes in all layers of the hippocampus.

VILIP-1-containing interneurons are morphologically and neurochemically heterogeneous.

- On the basis of colocalizing markers, they include mid-proximal dendritic inhibitory cells characterized by Calbindin-D28K immunoreactivity, and interneurons specialized to innervate other interneurons, which contain CR.
- VILIP-1 is rarely present in perisomatic inhibitory PV containing cells, and in feed-back distal dendritic inhibitory SOM/NPY-containing cells.
- Partial co-localization of mGluR1α, or α4 nAChR with VILIP-1 respectively was often found in interneurons located in the stratum oriens of CA1, but partial co-localization of α4 nAChR with VILIP-1 was also seen in the interneurons located in the border of the hilus in the dentate gyrus.

Functional activities

Interaction between VILIP-1 and α4β2 nAChR

I could show that an increase in intracellular calcium concentration, by different means, such as glutamate receptor activation or nAChR activation, induces a redistribution of endogenous VILIP-1 to specialized sites of the plasma membrane. I further clarified that both α4, and α7 nAChR activated by nicotine contributed to this redistribution, but α7 nAChR is predominant

due to its expression profile (expression in both principal neurons and interneurons) and the its high Ca^{2+} permeability.

Enhanced functional α4β2 nAChR surface expression which was induced by VILIP-1 was demonstrated in HEK cells stably transfected with human α4β2 nAChR, and cultured hippocampal neurons transiently cotransfected with α4, β2 nAChR subunits. Overexpression of VILIP-1 in hippocampal neuron culture enhances the frequency and amplitude of IPSCs via non-α7 nAChR activation, most likely α4 nAChR, suggesting that a presynaptic mechanism plays a role, but a postsynaptic mechanism could not be excluded.

Modulation of VILIP-1 expression in synaptic plasticity and possible functional impact

Group I mGluRs agonist DHPG upregulated VILIP-1 expression in rat hippocampus *in vivo* after 8 and 24 hours in a model of hippocampal synaptic plasticity.

Overexpression of VILIP-1 prolongs the half duration time of action potentials in cultured rat hippocampal neurons, indicating that it might play a role in synaptic plasticity and network activity.

Prospects

To clarify whether VILIP-1-positive cells exhibit particular functional properties related to their VILIP-1 content:

Ongoing project: studying intrinsic properties and synaptic plasticity after modulating VILIP-1 expression:

- By overexpression of VILIP-1-GFP/ GFP alone in cultured hippocampal neurons;
- Knock down (SiRNA) of VILIP-1 in cultured hippocampal neurons.

Electrophysiological characterization (synaptic plasticity and network activity) and correlative morphological and immunocytochemical studies in:

- Overexpression rat model (transgenic rats);
- Knock out mouse.

Interaction between VILIP-1 and α4 nAChR:

- Study whether VILIP-1 has an effect on high affinity proportion of α4 nAChR expression;
- Generate VILIP-1 and α4 nAChR mutants to identify the interaction motif.