Identification of signaling pathways contributing to the analgesic effect of TAT-pQYP peptide

Heloisa Espreafico Guelerman Ramos[1], Ana Maria Rodrigues Henrique[1], Allan Pradelli Roldão [1], Alexandre Martins do Nascimento[1], Beatriz Caroline de Moraes[1], Deborah Schechtman[1]

[1.] IQUSP, Departamento de Bioquímica, Instituto de Química da Universidade de São Paulo, Av. Prof. Lineu Prestes, 748 - Cidade Universitária, São Paulo - SP, Brasil;

In an inflammatory context, many chemical mediators are secreted, such as the nerve growth factor (NGF), which through its high affinity receptor, tropomyosin kinase A (TrkA), activates several signaling pathways including pain perception. A key component of pain signaling is phosphorylation/activation of PLC gamma, a substrate of TrkA. An interaction between the SH2 domain of the lipase with phosphorylated Y785, anchors PLC gamma to the receptor and is a prerequisite for the lipase phosphorylation/activation by TrkA. Once active, PLC gamma acts in a cascade that culminates in neuronal depolarization through the opening of the TRPV1 cation channel. Our laboratory, aiming at interrupting this pathway, designed a peptide, TAT-pQYP, derived from TrkA, containing PLC gamma's anchoring site. This peptide competes with TrkA binding for SH2 domain(s) of PLC gamma, preventing lipase anchoring to the receptor and decreasing mechanical hypersensitivity in an inflammatory pain model. However, in this context TAT-pQYP may modulate other protein-protein interactions, interfering in the observed analgesic effect. To this end, we used an exploratory approach to search for other proteins that bind to TAT-pQYP. Biotinylated peptides were immobilized on avidin beads and incubated with Neuro2A cell lysate. After elution of the bound proteins, samples were processed and analyzed by nanoLC-MS/MS. Mass spectrometry analysis identified approximately 2000 proteins, which were examined and filtered, excluding the most abundant ones and those that also bound to the control biotinylated peptide (TAT). To search for potential targets, we used the g:Profiler software with a 95% confidence interval for a gene ontology analysis, based on: biological processes, molecular function, cellular component, and signaling pathways (KEGG and Reactome). Additionally, we analyzed the physical interactions among the filtered proteins using StringApp (Cytoscape) since many proteins might be indirect interactors of TAT-pQYP, and looked at expression of the binding proteins in nociceptors. Finally, we found 4 proteins that also contain SH2 domains, including the ubiquitin E3 ligase CBL and SHC1, and computationally simulated the interaction of TAT-pQYP with their SH2 domains. The protein that stood out the most was the ubiquitin E3 ligase CBL, which is known to bind to TrkA, through its SH2 domain, negatively regulating the receptor by ubiquitination. Thus, these findings suggest that the interaction between the peptide and CBL could interfere with the protein\'s ubiquitination of TrkA. Data from the literature suggest that inhibiting the ubiquitination of TrkA by CBL would increase pain in an arthritis model, however, since TAT-pQYP concomitantly inhibits the PLC gamma pathway, key for pain signaling, we observe a reduction in mechanical hypersensitivity in an inflammatory pain model. The next steps will include validating TAT-pQYP interactions with proteins identified in cells, ensuring they co-localize and interact with TrkA and functional consequences of these interactions in pain signaling. Taken together, we show that inhibiting key protein interactions instead of functionally inhibiting TrkA or CBL can be a better approach for the treatment of inflammatory pain, since using this strategy we do not disrupt other pathways mediated by TrkA, such as neuronal outgrowth key for bone remodeling in arthritis.

Agradecimentos: À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)