NENS Stipend Report

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I have been in the laboratory of the Department of Pharmacology and Pharmacotherapy from the Academic Medical Center of Amsterdam from January, 5th to February, 28th of 2011. During this short period, I was enrolled in a project that aims to explore which protease-activating receptors (PAR) subtypes and signal transduction mechanisms are involved in the release of neurotrophins, such as Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF), from immortalized urothelial cells (Urotsa cells). PARs are G coupled-protein receptors (GCPR) that convert specific extracellular proteolytic activity into intracellular signals. Previous experiments from this group demonstrated that proteases, such as thrombin and trypsin, both nonselective PAR agonists, are increased during inflammation and are among the strongest stimuli inducing NGF release from Urotsa cells. Hence, our goal was to determine if urothelium could be considered as a possible source of NGF production, since the mechanisms regulating NGF expression and production are still not clear.

Thus, the first part of my lab work consisted on identifying which PAR subtypes are involved in NGF release as well as the best time point for stimulation of Urotsa cells. Cell stimulation was performed at 10 min, 30 min, 1h, 2h, 4h and 6h either with PAR1 and PAR2 activating peptides, thrombin, trypsin or bovine serum albumin (BSA, control group). At each time point, cell medium was collected for NGF quantification by ELISA. We observed that thrombin, trypsin and PAR2 activating peptide seem to contribute for increased levels of NGF, when compared to control values. PAR1 activating peptide was not able to reproduce an enhancement of NGF levels.

Several signaling pathways are thought to be activated by PAR including calcium, Gi signaling, Rho kinase, protein kinase C, PI3 kinase, MAP kinase, EGF receptor, thapsigargin. Hence, our goal was to find out which signaling pathway was being mainly activated. For that, cells were incubated with an inhibitor of a specific signaling pathway for 30 minutes. Then, thrombin or trypsin was added to the cell medium to induce stimulation. The medium was collected and NGF levels evaluated by ELISA. The MAPK, PKC and Rho Kinase signaling pathways gave us promising results, supporting the hypothesis that these signaling pathways
are crucial on the propagation of PAR effects. Nevertheless, we did not observed changes in NGF content when using a calcium quelator (BAPTA). Calcium is known to be important a mediator for activation of signaling pathways. Hence, further studies should be carried on in order to clarify this matter.

Indeed, it has been shown that during inflammation the levels of neurotrophins such NGF or BDNF are increased. Thus, in a parallel study, we decided to stimulate Urotsa cells either with BSA, BDNF, TrkA-Ig₂ and BDNF plus TrkB-Ig₂ (TrkA-Ig₂ and TrkB-Ig₂ are recombinant proteins that are able to bind to its specific neurotrophin neutralizing its effect). Cell medium was collected at 24h and 48h of stimulation. From ELISA results, we concluded that BDNF increases NGF release and, in contrast, TrkA-Ig₂ leads to a significant reduction of NGF levels on the medium. In this study, we also evaluate NGF content by Western Blot using samples from cell lysates. The results showed a clear band of 27kDa, irrespective of NGF, which was more intense on cells previously stimulated with BDNF. The intensity of the band was decreased on cells stimulated with BSA, TrkA-Ig₂ and BDNF plus TrkB-Ig₂.

Overall, this was not only a motivating and enriching scientific experience, but also socially rewarding. I would like to thank all people of the lab who helped me and gave me a lot of advices during the experiments, but specially Professor Martin Michel, who allowed me to play a part on his group, and to Peter Ochodnicky, for teaching me the know-how of cell culture and also western blot technique. On future experiments, I intent on using these two techniques to further determine possible sources of neurotrophins in the lower urinary tract in normal and pathological conditions using rat urothelial and bladder smooth muscle cells.

Finally, I am grateful to NENS and the people of Portuguese Society for Neuroscience for supporting my stay. The knowledge acquired will greatly improve my research quality and my personal career.

Regards,

Bárbara Frias
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