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Targeted profiling of neurotransmitters in the central nervous system of Marine Medaka (*Oryzias melastigma*) for environmental neurotoxicological assessments

Man-Shan Yau[1], Elva Ngai-Yu Lei[1,2], Chi-Chung Yeung[1], Margaret B Murphy[1], Ka-Leung Wong[2] and Michael Hon-Wah Lam[1]
[1]City University of Hong Kong, Hong Kong
[2]Hong Kong Baptist University, Hong Kong

Statement of the Problem: Neurotoxicology studies generally involve the neurobehavioral assessment of the integrated functioning of the nervous system, supplemented by *in vitro* neurocytotoxic assessment for the characterization of the neurotoxicity mechanism. However, associating in vitro neurocytotoxic end-points with observable *in vivo* neurodevelopmental and neurobehavioral effects is usually complicated by the lack of systemic biological processes and inter-cellular and tissue networking in *in vitro* models. It is believed that the manifestation of neurotoxicological symptoms should be accompanied by the perturbation of the normal metabolism of neurotransmitters in the CNS. While individual neurotransmitters have been investigated as biomarkers for selected environmental toxicants, there is scarcely any systematic study on how the neurotransmitter metabolome in the CNS of an *in vivo* model is perturbed by neurotoxicants. The purpose of this study is to evaluate the feasibility of using the profile of neurotransmitters in the brain tissue of a fish model for neurotoxicological assessments.

Methodology & Theoretical Orientation: Two congeners of brevetoxins, PbTx-1 and PbTx-2 were selected for triggering neurotoxic effects in the fish model marine medaka (*Oryzias melastigma*). A high-throughput LC-ESI-MS/MS analytical method has been developed for the quantitative determination of 43 major classical neurotransmitters and some of their metabolites in whole brain tissues of the fish. Chemometrics techniques, including PCA, PLS and OPLS, were used to identify discriminating factors between control fishes and dosed fishes from the metabolic profile of the targeted neurotransmitters.

Findings: OPLS analysis of the neurotransmitter profiles revealed significant gender difference of the neurotoxicological effects of PbTx-1 and -2. Most of the potential biomarkers identified are closely related to the activation of sodium channels, which is consistent with the current knowledge about brevetoxins.

Conclusion & Significance: Our results demonstrated the usefulness of the developed neurotransmitters profiling protocol for investigating *in vivo* sublethal effect of environmental contaminants to the model organism. The results of our study may also be extended to biotoxins with similar binding activity to VGSC, such as ciguatoxins.

bhmhwlam@cityu.edu.hk

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