Dynamic analysis of AKAP dependent cAMP signaling in melanotrope cells of *Xenopus laevis*

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For PKA to regulate divers processes within a cell it is believed to have specific subcellular localizations. A Kinase anchoring proteins (AKAP) are good candidates for localizing PKA near its targets. AKAPs comprise a family of proteins that are able to bind the regulatory (R) subunit of PKA. Based on the binding motif of AKAP and the PKA regulatory subunit type II (RII), an inhibitory peptide (HT31) was developed that binds PKA-RII and dislodges the enzym away from the AKAP, thereby preventing target phosphorylation. Using this inhibitor it has become clear that inhibition of RII binding to AKAP has similar effects as inhibiting PKA catalytic (C) subunits in many cellular processes.
We used St-HT31 (membrane permeable) to investigate the role of AKAP bound PKA in cAMP signaling in melanotrope cells of the pituitary gland of *Xenopus laevis*. Cyclic-AMP acting on PKA has been shown to stimulate the secretory cycle in this cell type. With radiolabeling methods we are able to measure peptide release with a high temporal resolution following the effect of AKAP/PKA disruption in a dynamic way. St-HT31 first led to an increase in peptide release followed by a sustained inibition. The stimulation was blocked by H89 and nickel, thus showing it to be both PKA and Ca^{2+} dependent.
We propose AKAP dependent PKA compartimentalization is important to cAMP signaling in *Xenopus* melanotropes and suggest that the transitory increase of secretion reflects an increase loss of RII *vs* C subunits during St-HT31 treatment. Possibly, the intact cAMP signaling system possesses excess regulatory subunits that, through a dynamic equilibrium with the catalytic subunits, are involved in setting the level of cAMP signaling. St-Ht31-induced loss of regulatory subunits would disrupt this equilibrium, leading to an increased amount of active catalytic subunits and, consequently, elevated secretion.

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