P021 Oscillations of cAMP beneath the plasma membrane of insulin-secreting cells

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To investigate the kinetics of hormone-evoked cAMP signals in insulin-secreting β-cells we developed a fluorescent biosensor that reports cAMP concentration beneath the plasma membrane ([cAMP]). A truncated form of the protein kinase A (PKA) regulatory subunit was fused with CFP and targeted to the plasma membrane. Co-expressed YFP-tagged PKA catalytic subunit also localizes to the membrane under basal conditions, but dissociates upon elevation of [cAMP]. This translocation was recorded as changes in the CFP/YFP fluorescence ratio using evanescent wave microscopy. Inhibition of phosphodiesterases with IBMX or stimulation of adenylyl cyclases with forskolin resulted in rapid and pronounced changes of the fluorescence ratio in INS-1 β-cells. Stimulation of the cells with 0.3-10 nM glucagon or glucagon-like peptide-1 resulted in pronounced oscillations of [cAMP]. Simultaneous recording of [cAMP] and the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) revealed an interplay between the two messengers. Thus, while elevation of [cAMP] evoked synchronous oscillations of $[Ca^{2+}]_i$, the [cAMP] oscillations depended on the presence of extracellular Ca^{2+}. These data provide the first demonstration of receptor-triggered oscillations of cAMP. Signalling with oscillations might help to improve low-level signal detection and to achieve specificity in downstream effects.