

**P006** cAMP oscillations restrict spatial redistribution of protein kinase A in insulin-secreting cells

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Activation of hormone receptors has been demonstrated to cause oscillations of the cAMP concentration beneath the plasma membrane ([cAMP]) of insulin-secreting cells. In this study we investigated the effect of different time-courses of [cAMP] signals on the generation of cytoplasmic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) signals and on the nuclear translocation of the protein kinase A catalytic subunit in individual INS-1  $\beta$ -cells. [cAMP] was measured with a new fluorescent translocation biosensor and ratiometric evanescent wave microscopy. Oscillations and stable elevation of [cAMP] was artificially generated by constant or pulsatile inhibition of phosphodiesterases with IBMX. Both stimulation protocols induced  $[\text{Ca}^{2+}]_i$  oscillations and in the case of oscillatory [cAMP] elevations, the  $[\text{Ca}^{2+}]_i$  responses were grouped and correlated with the periods of elevated [cAMP]. Analysis of the nuclear translocation of PKA was performed with epifluorescence microscopy and FIAsh-labelling of tetracysteine-tagged PKA-C $\alpha$  subunit. Whereas 25 min of stable [cAMP] elevation induced a pronounced rise of the nuclear/cytoplasmic FIAsh fluorescence ratio, the distribution of PKA-C $\alpha$  in the cells exposed to [cAMP] oscillations did not differ from that in non-stimulated cells. These results indicate that temporal encoding of cAMP signals may constitute a basis for differential regulation of downstream cellular targets.