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Relevance of ERK levels in Gonadotrophin-Releasing Hormone (GnRH)-Stimulated ERK Activation

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GnRH acts via G-protein coupled receptors to stimulate synthesis and secretion of luteinizing hormone and follicle-stimulating hormone. GnRH activates phospholipase C, causing calcium mobilisation and influx, activation of protein kinase C (PKC) and stimulation of mitogen-activated protein kinase cascades. GnRH is secreted in brief pulses with each pulse causing rapid and transient activation of extracellular signal regulated kinases (ERKs). This effect is largely PKC-mediated but little is known about what shapes ERK responses to GnRH receptor activation.

Here we have investigated ERK signalling using automated microscopy, a high-throughput approach which allows us to examine ERK activation (phosphorylation using an antibody to phosphorylated ERK (ppERK)), localisation (specifically nuclear to cytoplasmic distribution) and transcriptional activity using an Early growth response-1 (Egr-1) transcriptional reporter. PDBu, a PKC activator, caused a rapid and sustained ERK activation and nuclear localisation, whereas GnRH caused a similar but more transient response. The amount of ppERK was maximal for both stimuli at 5 minutes, and gradually declined thereafter to the final time point of 6 hours. In representative experiments EC_{50} values were 6 nM and 0.02 nM with 5 minutes PDBu and GnRH respectively. The resulting Egr-1 transcription became apparent from 2 hours increasing to a maximum at 6 hours. It was also more pronounced for PDBu, reaching 654±30 arbitrary fluorescence units (AFU) for PDBu and 213±5 AFU for GnRH at 4 hours, with EC_{50} values of approximately 50 nM and 0.8 nM respectively.

The cellular ERK concentration varies markedly between different cell types and individual cells (even of clonal cell lines). We used a knock-down add-back protocol to look at the effect of ERK concentration on ERK activation and the corresponding transcriptional response. Endogenous ERKs are firstly removed with siRNAs, and then varying amounts of ERK2-GFP introduced by means of an adenovirus. We ‘binned’ the individually imaged cells according to ERK2-GFP expression (mathematical sorting) and this revealed a near linear relationship between the amount of ERK2 or ppERK and the transcriptional response at 4 hours. Focussing on ERK phosphorylation at early time points we found that both stimuli caused a rapid increase in ppERK (maximal at 4-6 minutes) at approximately physiological ERK2-GFP levels. However, at higher ERK concentrations, the response was slower in onset (maximal at 12-14 minutes). As a consequence of this difference, maximal ERK activation actually occurred at submaximal ERK concentrations but only at the early time points. A similar difference in response kinetics was observed when cells with high or low ERK2-GFP expression were physically sorted, by flow cytometry, before imaging. These data are indicative of a distributive mechanism of phosphorylation, whereby in cells containing a high ERK concentration, unphosphorylated ERK competes with monophosphorylated ERK, inhibiting the production of dual phosphorylated ERK. We conclude that ERK concentration can have a pronounced effect on activation kinetics (as well as amplitude) of ERK responses, that may be particularly relevant for acute responses such as those elicited by pulsatile hormonal stimulation.