Mechanisms of Calcitonin Gene-Related Peptide-Induced Extracellular-Regulated Protein kinase Activation: Pathways to Upregulation of Inducible Nitric Oxide Synthase

Benoît Roux, Graeme Cottrell

Department of Pharmacy and Pharmacology, University of Bath, Bath, Somerset, BA2 7AY, UK

Calcitonin gene-related peptide (CGRP) is a potent vasodilatory peptide belonging to the calcitonin family of peptides, which also includes adrenomedullin, intermedin, amylin and calcitonin. CGRP has been shown to cause upregulation of inducible nitric oxide synthase (iNOS) in macrophages and trigeminal ganglion glial cells. iNOS catalyzes the oxidation of L-arginine to nitric oxide, an important signaling molecule involved in many physiological and pathological processes, such as neuronal excitotoxicity and inflammation. Like CGRP, nitric oxide is a power vasodilatory molecule and implicated in a number of vascular conditions, including migraine and sepsis. Previous studies have shown that CGRP enhances cytokine-induced upregulation of iNOS in vascular smooth muscle cells, a process that involves cAMP generation.

We hypothesized that CGRP alone would increase expression of iNOS and that this mechanism would involve activation of mitogenic signaling pathways. As a first step to understanding how CGRP activates extracellular regulated protein kinases (ERKs), we used HEK cells overexpressing CLR•RAMP1. We examined levels of phosphorylated ERK1/2 by Western blotting in the presence of inhibitors of protein kinases (PK) A (H-89, 50 µM) and C (Gö6983, 10 µM), epidermal growth factor signaling (AG1478, 1 µM) and src (PP2, 10 µM). We also determined the contribution of extracellular calcium (using Ca^{2+}-free buffer) and β-arrestins (using dominant negative mutants; β-arrestin$^{319-418}$, β-arrestin1$^{P91G\ P121E}$). We found that CGRP-dependent activation of ERKs requires PKA activity and transactivation of epidermal growth factor receptors. We next examined if CGRP-induced ERK activation causes upregulation of iNOS in primary rat thoracic aortic smooth muscle cells (TA-SMCs). Contrary to previous reports, we found that CGRP (1 µM, 0-6 h) induced iNOS expression in the absence of interleukin-1β. Further, iNOS upregulation was prevented by the MEK inhibitor U0126 (10 µM), implicating a critical role for ERK activation. Thus, modulating specific branches of the CGRP-induced ERK activation pathway may be beneficial in regulating the induction of iNOS, which has been implicated as a contributory factor in septic shock.