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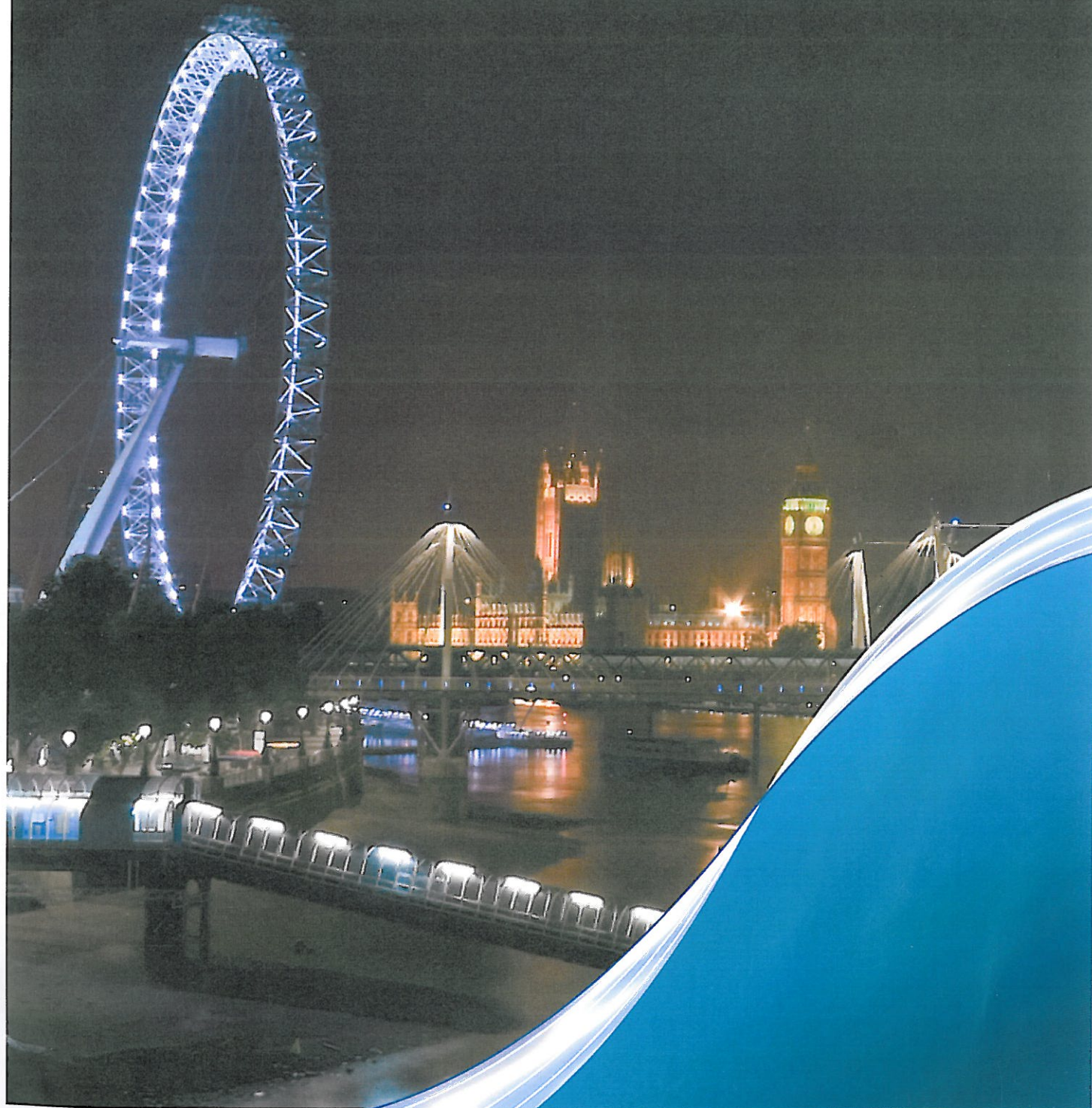
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Mechanisms of Calcitonin Gene-Related Peptide-Induced Extracellular-Regulated Protein Kinase Activation: Pathways to Upregulation of Inducible Nitric Oxide Synthase

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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 (PKA (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 Ca²⁺-free buffer) and $\square$ -arrestins (using dominant negative mutants: $\square$ -arrestin³¹⁵⁻⁴¹⁶, $\square$ -arrestin¹⁻¹³²). 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-1 $\square$. Further, iNOS upregulation was prevented by the MEK inhibitor U0125 (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.

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Morphological and structural comparison of tooth and jaw development in wild type and PACAP-deficient mice

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a multifunctional neuropeptide and it plays important role in the development of the nervous system. Tooth development shows similarities with the development of the nervous system, because teeth arise from the oral ectoderm and mesenchyme derived from the neural crest. PACAP-immunoreactive fibers are found in the tooth pulp odontoblastic and subodontoblastic layers, but there is no data about the exact effect of endogenous PACAP on tooth development.

In the first part of the study our aim was the morphological and immunohistochemical examination of tooth development in 7-day-old wild type and PACAP-deficient mice. On native histological sections, we measured the thickness of the forming dentin and enamel layers on first, second upper and lower molar teeth. We examined the structural differences between the teeth with Thermo Scientific DXR Raman microscope. We also labeled the localization of PAC1 receptors with immunohistochemistry. In the second part of the experiment we investigated the structure of the teeth and facial bones from adult wild type and PACAP-deficient mice with morphological measurement (FT-IR IMPACT 400 spectrometer, mass spectrometry and Skyscan Micro-CT).

Our results demonstrated that in PACAP-deficient mice the developing dentin layer is significantly thinner compared to wild-type mice and wild-type animals showed significantly higher PAC1 receptor expression in the odontoblastic and subodontoblastic layer compared to PACAP-deficient mice. We found significantly smaller teeth in adult PACAP-deficient mice amid morphological examinations and FT-IR spectra of facial bones showed significantly higher amid band in PACAP KO animals compared to wild-type mice. These observations suggest that PACAP plays a role in tooth development in the dentin formation as well as in the mineralization of alveolar bones, but further examinations are necessary to describe the exact molecular background of these effects. Supported by OTKA (K72592, 73044, CNK78480), TAMOP (4.2.1.B-10/2/KONV-2010-002, 4.2.2.B-10/1-2010-0029), Bolyai Scholarship, Richter Foundation, PTE-MTA "Lendület" Program.