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PKA and Epac activation counteract hypoxia-induced NO/ROS imbalance in human coronary artery endothelial cells

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In endothelial cells (ECs), severe hypoxia induces oxidative stress due to a change in the reactive oxygen species (ROS)-nitric oxide (NO) balance, leading to an endothelial dysfunction, a commonality in cardiovascular disease (e.g. hypertension, atherosclerosis, heart failure). We have previously shown that elevation of intracellular cAMP induces an increase in NO biosynthesis. This effect is mediated, in part, by the activation of PKA (cAMP dependent protein kinase) and Epac (exchange protein activated by cAMP) and contributes to the endothelium-dependent cAMP-induced vasorelaxation (16th Annual Meeting of the ECCR, *Hypertension*, in press).

Here, we have examined whether cAMP-induced NO biosynthesis can prevent hypoxia-induced NO/ROS imbalance and the involvement of cAMP-activated proteins in this process. We have also studied the hypoxia-induced changes in protein expression that can affect NO biogenesis. For these purposes, we have measured ROS generation using the fluorescent probe 2′,7′-dichlorofluorescein diacetate and flow cytometry in human coronary artery endothelial cells (HCAEC) exposed to hypoxia (2% O_2, 24 h) and in normoxic conditions. We have also quantified the amount of NO released by hypoxic and normoxic HCAEC by measuring the production of nitrites using a commercial high-sensitivity nitrite assay kit. Results are expressed as fold change compared with control values normalized to 1.

Under hypoxia, basal ROS generation was significantly increased (1.55±0.14, n=7, P<0.01) and NO synthesis was significantly reduced (0.69±0.11, n=7, P<0.01). This was accompanied with a decrease in the protein expression of endothelial NO synthase (eNOS; 0.63±0.08, n=5, P<0.05), PKA (0.70±0.13, n=5, P=0.07) and Epac1 (0.43±0.07, n=5, P<0.01). In contrast, phosphoserine-1177 eNOS was unchanged under hypoxic conditions (0.98±0.11, n=5, P>0.05), indicating that the activation of eNOS in unaltered. Incubation of HCAEC with forskolin (FSK, an adenylyl cyclase activator, 1µM), 6-Bnz-cAMP (a selective activator of PKA, 300µM) or 8-pCPT-2'OMe-cAMP (a selective Epac activator, 100µM) did not significantly modify ROS/NO levels (n≥5) under normoxic conditions (24h). However, under hypoxic conditions, incubation of HCAEC (24h) with FSK (1µM) induced a strong decrease in ROS production (1.03±0.13, n=4, P<0.05 *vs.* control with 0.1% DMSO: 1.54±0.14, n=5) and an increase in NO generation (1.36±0.42, n=4*;* control with 0.1% DMSO: 0.70±0.11, n=5). Moreover, the incubation with 6-Bnz-cAMP (300µM) and with 8-pCPT-2'OMe-cAMP (100µM) significantly diminished ROS levels (1.06±0.09 and 0.85±0.16, respectively; P<0.05 *vs.* basal values in hypoxia) and induced a significant increase in NO levels (1.62±0.48 and 1.45±0.44, respectively; P<0.05 *vs.* basal values in hypoxia).

In conclusion, PKA and Epac play an important role in hypoxia-induced NO/ROS imbalance in ECs: expression of these cAMP effectors is reduced under hypoxic conditions and their pharmacological activation diminished hypoxia-induced ROS generation and restored NO biosynthesis to normoxic levels, which suggest that these proteins are potential therapeutic targets in pathologies involving endothelial dysfunction.