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Carbon monoxide regulates intracellular calcium in human bronchial epithelial cells

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Introduction: Carbon monoxide (CO) is an important autocrine/paracrine messenger that is involved in various physiological and pathological processes. CO can be endogenously produced by haem oxygenase (HO) enzymes (HO-1, HO-2 and HO-3). Evidence has recently been obtained that CO may affect calcium homeostasis in pancreatic acinar cells[1]. This project aimed to investigate the regulatory role of CO in a P2Y receptor–mediated calcium signalling pathway in a human bronchial epithelial cell line, 16HBE14o-.

Method: To measure intracellular calcium concentration ($[Ca^{2+}]_i$), 16HBE14o- cells grown on glass coverslips were loaded with Fura-2-AM (3 μM, 45 min, 37℃) in standard Krebs–Henseleit solution. $[Ca^{2+}]_i$ was monitored via the Fura-2 fluorescence ratio (excitation 340/380 nm; emission > 510 nm). Agonist-induced D-myo-inositol-1-phosphate (IP_1) (a surrogate of 1,4,5-trisphosphate, IP_3) in LiCl-treated cells was quantified using the Cisbio IP-One kit (Cisbio Bioassays, Codolet, France). cGMP-dependent protein kinase (PKG) activity was measured with a Cyclex® assay kit (MBL International, MA, USA). The data are given as the mean ± SEM.

Results: CO-releasing molecule 2 (CORM-2) induced both calcium increase and IP_1 production in a concentration-dependent manner. This effect was suppressed by a PLC inhibitor, U73122 (10 μM). In contrast, CORM-2 exerted an inhibitory effect on UTP-induced calcium release and influx. CORM-2 did not affect the store-operated Ca^{2+} entry induced by thapsigargin. In the presence of 30 μM CORM-2, the UTP-induced calcium increase was reduced to 34.5% ± 0.7% (n = 5) of the control, but the percentage increased back to 70.6% ± 6.4% (n = 4) and 77.5% ± 7.7% (n = 3) of the control in the presence of a soluble guanylyl cyclase (sGC) inhibitor, ODQ (10 μM), or a PKG inhibitor, KT5823 (5 μM), respectively. Treating the cells with CORM-2 (30 μM) led to an increase in PKG activity (1.47 ± 0.08 - fold vs. control, n = 3), which could be blocked by KT5823 (1.13 ± 0.04 - fold vs. control, n = 3).

Conclusion: CORM-2 had dual effects on $[Ca^{2+}]_i$ modulation in 16HBE14o- cells. At higher concentrations, CORM-2 stimulated PLC/IP_3/calcium signalling pathways, whilst at lower concentrations, CORM-2 inhibited the calcium signalling evoked by P2Y receptor agonist in a sGC/PKG-dependent manner. Thus, CO may act as a regulator of calcium homeostasis in human airway epithelia.

References:

1. Moustafa A *et al*. (2014). *Am J Physiol Cell Physiol* **307**(11): C1039-C1049.

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