Selectivity of Biguanide Accumulation by Mitochondria

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The biguanides metformin, phenformin and buformin are anti-hyperglycemic drugs that function by indirect activation of AMP kinase. Mitochondrial complex I (NADH:ubiquinone oxidoreductase) has been proposed as the primary target because the drugs have consistently been observed to inhibit NADH-linked mitochondrial respiration at high (mM) concentrations. Both phenformin and buformin have been discontinued due to high incidence of lactic acidosis, consistent with mitochondrial complex I inhibition. Biguanides are protonated at neutral pH and respond to the plasma and mitochondrial membrane potentials to accumulate many fold inside the mitochondrial matrix.

Biguanide inhibition of mitochondrial complex I means that their uptake and accumulation in the mitochondrial matrix can be indirectly observed by monitoring their effect on cellular and mitochondrial oxygen consumption. In order to understand more about how biguanides exert their effects on the mitochondrion, we compared in-cellulo and in-organello data with kinetic analyses on purified mitochondrial complex I, and mitochondrial membranes. Using this strategy, we previously found that the structurally-related antimalarial biguanides proguanil and cycloguanil had no effect on cellular or intact mitochondrial respiration despite their inhibitory efficacy on the purified enzyme. Having discovered that a chemical difference between phenformin and proguanil must dictate whether or not the compound is able to reach the mitochondrial matrix, we designed a set of substituted biguanides to test which molecular features governs biguanide targeting. We are now able to pinpoint which substituents of the proguanil structure make it such a safe drug from the mitochondrial perspective.

Publications

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The regulatory role of Carbon Monoxide in Endothelial Cell Metabolism

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Carbon monoxide (CO), a product of heme degradation by heme oxygenases, plays an important role in vascular homeostasis. Recent evidence indicates that mitochondria are among a number of molecular targets that mediate the cellular actions of CO. Development of carbon monoxide releasing molecules (CO-RMs) has facilitated studies on the role of CO in diverse cellular processes. Using the Seahorse XF technology we characterized the effects of CO released from CORM-401 (10-100 μM) on mitochondrial respiration and glycolysis in intact human endothelial cells (EA.hy926). We found that CORM-401 (10-100 μM) induced a persistent increase in the oxygen consumption rate (OCR) that was accompanied by inhibition of glycolysis (extracellular acidification rate, ECAR) and a decrease in ATP-turnover. Furthermore, CORM-401 increased proton leak, diminished mitochondrial reserve capacity and enhanced non-mitochondrial respiration. Interestingly, blockade of mitochondrial large-conductance calcium-regulated potassium ion channels (mitoBKCa) with paxilline abolished the increase in OCR promoted by CORM-401 without affecting ECAR; patch-clamp experiments confirmed that CO derived from CORM-401 activated mitoBKCa channels present in mitochondria. Thus we demonstrated that CO induces a two-component metabolic response: uncoupling of mitochondrial respiration dependent on the activation of mitoBKCa channels and inhibition of glycolysis independent of mitoBKCa channels.

To investigate the acute effects of CO on endothelial cells we used life cells imaging techniques. CORM-401 (30 μM) induced a mild mitochondrial depolarization and activation of complexes I- and II-dependent mitochondrial respiration leading to ATP production through increased oxidative phosphorylation and reducing ATP production from glycolysis. Importantly, CORM-401 increased mitochondrial calcium concentration. Neither of these effects was reproduced by inactive CORM-401 (iCORM-401) supporting a direct role of CO in the endothelial metabolic response induced by CORM-401. Our results show that non-activated endothelial cells rely primarily on glycolysis and that mitochondrial membrane potential is maintained by glycolysis-derived ATP. In the presence of CO, mitochondrial Ca^{2+} increases and activates respiration that shift metabolism of endothelial cells from glycolysis- to oxidative phosphorylation-derived ATP production. Altogether, our work points out that CO regulates endothelial cells metabolism that may have physiological and pathophysiological significance.