Unspecific effect of etomoxir on mitochondrial respiration

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The liver mitochondrial dysfunction plays an important role in the progression of non-alcoholic steatohepatitis, therefore improved protocols are required to evaluate mitochondrial fitness in the course of disease. Etomoxir, an inhibitor of carnitine palmitoyltransferase-I, is often used (40-400 μM) in commercial kits to block mitochondrial fatty acid (FA) transport and to modulate fatty acid oxidation (FAO) [1, 2]. However, among others we have shed light on the unspecific effect of etomoxir on the mitochondrial fatty acid oxidation (FAO; F-pathway linked respiration) in permeabilized Huh7 liver cells and mitochondria isolated from mouse liver and brain. In our previous study (data not published) we have observed an unspecific effect of etomoxir using substrate-uncoupler-inhibitor titration (SUIT) protocols to analyze oxidative phosphorylation (OXPHOS) and electron transfer (ET) capacities of the fatty acid (F) pathway together with the anaplerotic pathways, as well as of the NADH-linked and succinate (S)-pathways separately (F, N, S) and in combination (FN, FS, FNS, NS). In the presence of palmitoylcarnitine, 200 μM etomoxir inhibited not only F-capacity, but also the NADH-linked respiration supported by glutamate or pyruvate plus malate in liver. This is the case even in brain mitochondria where F-capacity is extremely low compared to liver. While 40 μM etomoxir showed a non-significant inhibitory trend towards N-pathway. We have seen that after 30 min of incubation high concentration of etomoxir (~200 μM) might block not only the NADH-linked pathway but also the Succinate-linked respiration. These results suggest an unspecific effect of etomoxir on mitochondrial respiration involving N-and S-pathways.

The aim of this secondment is to investigate under which condition etomoxir inhibits FAO specifically, using different tissues and different source of fatty acids. For this, experiments using High-Resolution Respirometer were performed on isolated mitochondria from mouse liver and heart as well as permeabilized Huh7 liver and HEK kidney cell lines. To test the effect of etomoxir on F-pathway-linked mitochondrial respiration and clarify its specific effect on CPT-1, we used palmitoylcarnitine, palmitoyl-CoA + carnitine + low amount of malate or palmitate + CoA + carnitine.

In conclusion, our results suggest an unspecify effect of etomoxir that seems to be independent of the tissue without specific inhibition towards FAO.