Cell-permeable succinate and malonate as research tools

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BACKGROUND

- Controlling substrate supply and inhibiting or modulating selected parts of the oxidative phosphorylation pathway are key tools for mitochondrial research.
- Few mitochondrial substrates are cell-permeable which is limiting when working with intact cells.
- Certain inhibitors of mitochondrial function, such as the CII-inhibitor malonate do not pass freely through the plasma membrane.
- Cell-permeable analogues of mitochondrial substrates and modulators of the oxidative phosphorylation pathway could be valuable tools in mitochondrial research.

OBJECTIVE

- The objective of this drug discovery program was to identify cell-permeable analogues of the CII substrate succinate and the CII-inhibitor malonate for in vitro use.

METHODS

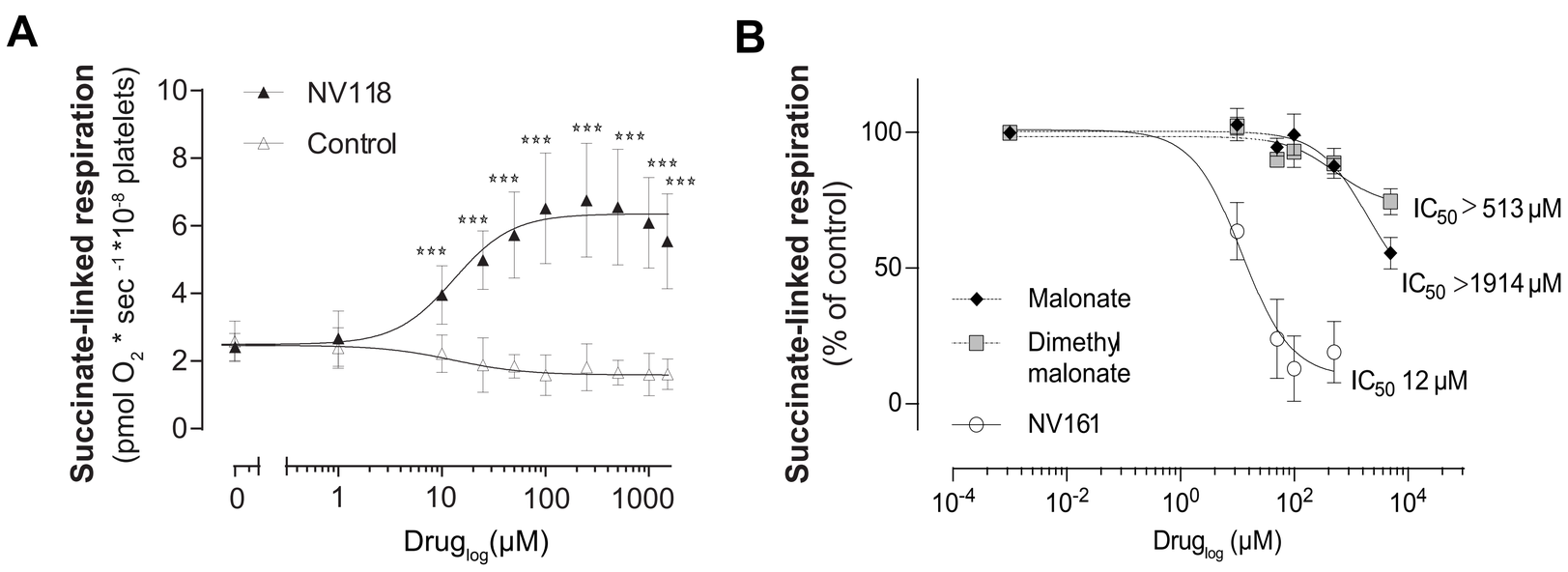
Analogues of succinate, a substrate for the mitochondrial oxidative phosphorylation pathway, were designed, synthesized and evaluated for ability to supply succinate to mitochondria in intact cells. Human platelets were utilized as primary model. Results were confirmed and further evaluated using human peripheral blood immune cells, cultured human skin fibroblasts and human heart muscle fibers. Further, analogues of the complex II-inhibitor malonate were designed and evaluated for ability to inhibit complex II function in intact cells. Data presented are from experiments with platelets from healthy donors ($200 \bullet 10^6$ cells/ml) using an Oroboros O2k high-resolution respirometer.

CONCLUSIONS

- **NV118 is a cell-permeable prodrug of succinate that once inside the cytoplasm releases succinate and thereby supports mitochondrial succinate-linked respiration.**
- **NV161 is a cell-permeable prodrug of the complex II-inhibitor malonate and decreases succinate-linked respiration at concentrations a hundredfold lower than malonate.**
- **NV118 and NV161 may prove valuable as scientific tools in mitochondrial research, enabling evaluation of complex II in intact cells and tissues**

RESULTS

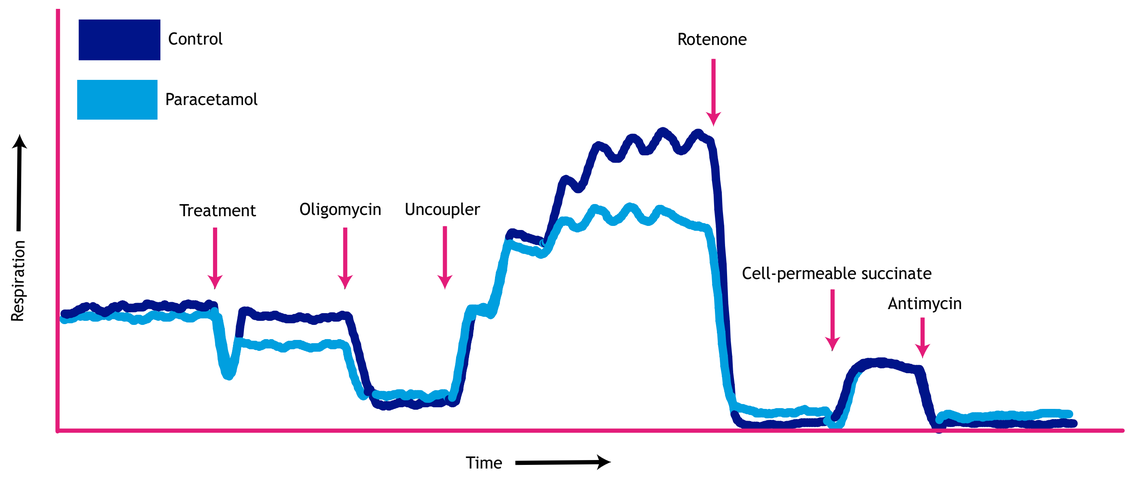
NV118 and NV161 dose-dependently support and inhibit succinate-linked mitochondrial respiration in intact human platelets



Dose-response of NV118 and NV161. Rotenone (2 µM) was added to respiring intact human platelets to inhibit complex I. **A.** To evaluate the succinate-linked respiration NV118 was titrated (from 10 µM to 1.5 mM final concentration) and rate of oxygen consumption was assessed. **B.** 100 µM of a cell-permeable succinate prodrug was added to allow for succinate-linked respiration. Malonate, dimethyl malonate or NV161 were titrated (from 10 µM to 5 mM final concentration). NV161 inhibited succinate-linked respiration at lower concentrations than malonate or dimethyl malonate.

An example of complex II-evaluation using cell-permeable succinate

In an experimental set-up designed to analyse the influence of paracetamol (acetominophen) on mitochondrial respiration, intact human platelets were treated with either 10 mM of acetominophen or vehicle. Thereafter oligomycin (1 µg/ml) was added to inhibit the ATP synthase. Maximal non-coupled respiration was reached by titrating the uncoupler FCCP. Rotenone (2 µM) was added to inhibit complex I. To evaluate the complex II function, 500 µM of a cell-permeable succinate prodrug was added. Antimycin A (1 µg/ml) was then added to account for the non-mitochondrial respiration. The cells treated with acetaminophen showed a lower maximal non-coupled respiration as compared to control, but this relative difference was not seen for succinate-linked respiration. Therefore, the cause of the impaired respiration did not seem to be related to metabolism through complex II but rather through complex I.



NeuroVive Pharmaceutical AB is a public company developing pharmaceuticals and assays in the field of mitochondrial medicine. Isomerase Therapeutics Ltd. is a drug discovery and development company with particular expertise in the discovery and development of optimised microbial natural products. A collaboration exists between the academic group Mitochondrial Medicine at Lund University and NeuroVive Pharmaceutical AB. The affiliated authors have full or partial salary from, and/or have equity interests in the respective companies.

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