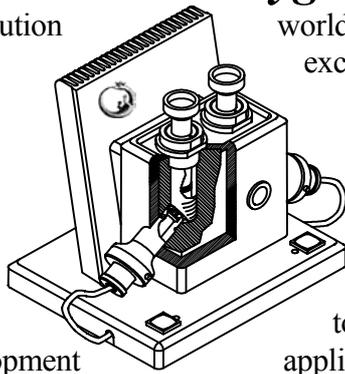


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COURSE ON HIGH-RESOLUTION RESPIROMETRY

FROM STEP TITRATION TO RAMP INJECTION: UNCOUPLING BY FCCCP WITH TIP

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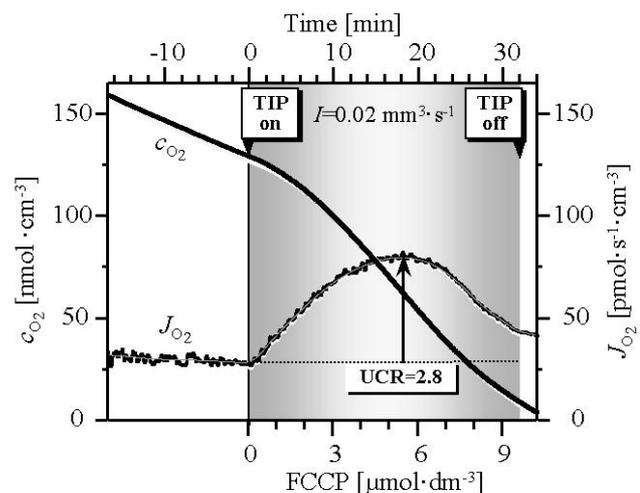
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Measurement of the uncoupling control ratio, UCR (uncoupled/coupled oxygen flux), is important for the analysis of mitochondrial function and mitochondrial defects [1,2]. Uncouplers of oxidative phosphorylation, such as DNP and FCCP, stimulate oxygen flux. Above an optimum concentration, however, respiration is inhibited [3]. Maximum oxygen flux, therefore, is underestimated if the uncoupler is added at too high or too low concentrations. As an alternative to traditional step titration, the **TITRATION-INJECTION MICROPUMP (TIP)** offers the new option of ramp injection, providing maximum resolution of the concentration dependence of oxygen flux as illustrated in the figure [4].

The FCCP concentration was increased linearly over time by ramp injection during uninterrupted monitoring of O_2 concentration, c_{O_2} , and cellular respiration, J_{O_2} , before and after switching on the TIP at time 0. The volume of the OROBOROS

Oxygraph chamber was 2 cm^3 . An FCCP injection rate of $0.005 \mu\text{M}\cdot\text{s}^{-1}$ was obtained by setting the injection flow, I , at $0.02 \text{ mm}^3\cdot\text{s}^{-1}$ with an FCCP concentration of 0.5 mM in the TIP injection syringe. This



Respiration of human umbilical vein endothelial cells (HUVEC) and stimulation by continuously increased FCCCP concentration induced by ramp injection with TIP.

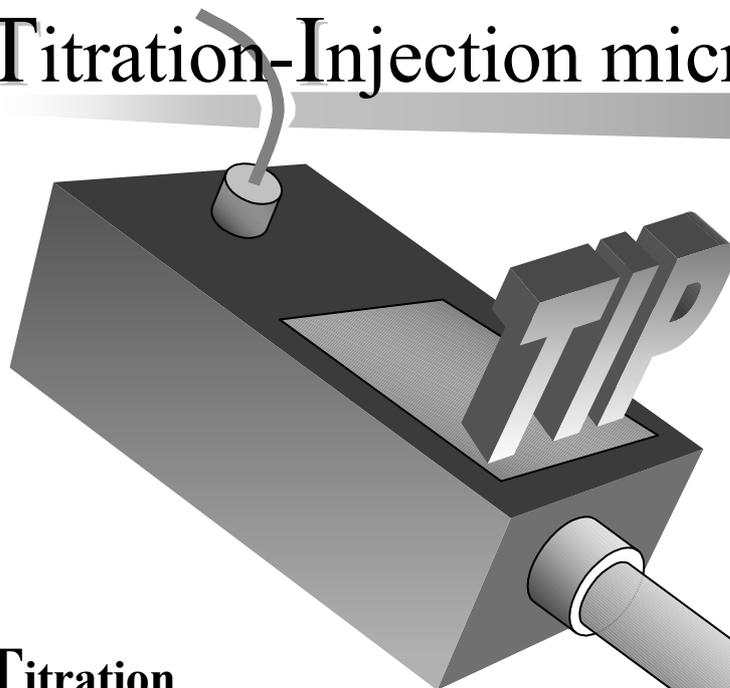
conversion factor transforms the time scale (top) directly to FCCP concentration (bottom), using DATLAB ANALYSIS.

The uncoupling control ratio, UCR, was 2.8, directly obtained from the maximum in the continuous trace of oxygen flux. Since the optimum FCCP concentration varies [3], automatic ramp injection provides a practical alternative to step titration.

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2. Gnaiger E., Rieger G., Stadlmann S., Amberger A., Eberl T., R. Margreiter (1999) Mitochondrial defect in endothelial cold ischemia/reperfusion injury. *Transplant. Proc.* **31**: 994-995.
3. Steinlechner-Maran R., Eberl T., Kunc M., Margreiter R., Gnaiger E. (1996) Oxygen dependence of respiration in coupled and uncoupled endothelial cells. *Am. J. Physiol.* **271**: C2053-C2061.
4. Gnaiger E., Reck M., Steinlechner-Maran R., Rieger G., Stadlmann S., Margreiter R. (1996) High resolution - the decisive difference in mitochondrial and cellular respirometry. In: *BioThermoKinetics of the Living Cell* (Westerhoff H.V., Snoep J.L., Wijker J.E., Sluse F.E., Kholodenko B.N., eds.), BioThermoKinetics Press, Amsterdam: 441-442.

Titration-Injection microPump - TIP



- New options for inhibitor titrations and kinetic studies.
- For steady-state injection respirometry and automatic titration regimes.
- Stand-alone instrument with keypad and display for setup programs.

Titration

Programmable, automatic titration regimes, with titration volumes of 0.05 to 250 μl , variable titration intervals and duration of titration pulse.

Injection

Ramp increase of effector concentrations by "continuous titration", or operation at quasi steady-states by continuous injection of substrates at limiting rates of consumption, providing new flexibility in experimental design by combining the technical advantages of closed and open systems.

Programmable injection flows: 0.01 to 25 $\mu\text{l}\cdot\text{s}^{-1}$.

