BACKGROUND
Pharmacological modulation of mitochondrial ATP-sensitive potassium channels (mitoKATP) in the heart has been systematically associated with cardioprotection against ischemia-reperfusion injury. Modulation of mitochondrial respiration during ischemia and/or early reperfusion has also been reported to decrease the extent of myocardial injury.

Classic mitoKATP openers (e.g., diazoxide and pinacidil) were also demonstrated to have direct effects on the mitochondrial phosphorylation, such as mild uncoupling and/or respiratory chain inhibition.

Temporary disruption of oxidative metabolism as a measure to protect the heart in pathological conditions, albeit counterintuitive, has been unequivocally associated with protection of mitochondrial function, improvement in contractile function recovery after reperfusion, and infarct size reduction.

Also, preventing the opening of the mitochondrial permeability transition pore (mPTP) by using classic pore desensitizers (e.g., Cyclosporin A, CsA), the decreasing calcium overload represents another widely recognized cardioprotective strategy in both experimental and clinical settings.

AIM
The aim of the present study was to characterize the effect of two novel mitoKATP openers (KL-1489 and KL-1495) on the respiratory rates and calcium retention capacity (CRC) in isolated rat heart mitochondria.

MATERIAL AND METHODS

1. RESPIROMETRY STUDIES

Rat heart mitochondria (RHM) were isolated from adult (8-10 months) female rats (n=6) by differential centrifugations at 4°C. Oxygen consumption was measured at 37°C using the Oroboros Oxygraph-2k system.

The Substrate-Uncoupler-Inhibitor Titration (SUIT) protocol used was as follows:

   Chamber A: GM + D_2O + ADP + O2 + FETS + Amiloride

   Chamber B: S(Rot)+ D_2O + DOPAC + O2 + FETS + Amiloride

   Respiratory control ratio (RCR) was calculated as the ratio DOPAC/O2/State 4.

2. CALCIUM RETENTION CAPACITY (CRC)

The amount of mitochondrial Ca²⁺ retained before the opening of mPTP was measured spectrophotometrically at 37°C and compared to the effect elicited by the classical pore desensitizer, cyclosporine A (CsA).

The change in extramitochondrial Ca²⁺ concentration was monitored using the fluorescent probe CaGreen (1 μM, excitation/emission, 490–530 nm).

CaCl₂ pulses (20 nmol/pulse) were added at 1 min intervals, until mitochondrial Ca²⁺ release caused by opening the PTP was observed.

CRC was calculated as the cumulative amount of Ca²⁺ taken by mitochondria before Ca²⁺ release.

RESULTS

KL-1495 elicits mitochondrial uncoupling and respiratory chain inhibition that may play a role in cardioprotection during postischemic reperfusion.

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Figure 1: Schematic representation of mitochondrial electron transport chain (ETC) and coupling/uncoupling of oxidative phosphorylation. (Adapted from Fanise et al. 2005)

Figure 2: Representative diagram of the SUIT protocol for CI-supported respiration. A. Ctrl: B. KL-1495. C. KL-1498. Oxygen concentration (O₂) and O₂ flux [mean + SEM of 3 min each, O₂ (INV)] are represented as a function of time. Additions are as follows: D – glutamate (10 mM), M – malate (2.2 mM), D – ADP (5 mM), O₂ – oxygen (10 μM). Omy - oligomycin (2.5 μg/ml), Am - amiloride A (2.5 μg/ml). Oxygen concentration (maximum 200 nmol/l) was maintained by intermittent KCl additions into the chamber (150 μM at each titration step).

Figure 3: Representative diagram of the SUIT protocol for CI-supported respiration. A. Ctrl: B. KL-1495. C. KL-1498. Additions are as follows: D – nitrone (0.5 μM), S – succinate (10 mM), D – ADP (5 mM), O₂ – oxygen (10 μM), Omy - oligomycin (2.5 μg/ml), Am - amiloride A (2.5 μg/ml).

KL-1495 ELICITS SUBSTRATE-INDEPENDENT EFFECTS OF MITOCHONDRIAL UNCOUPLING & RESPIRATORY INHIBITION

In mitochondria respiring on glutamate/malate and a significant increase of state 2 and state 4 in RHM treated with KL-1495 vs. Ctrl, was found: State 2, 123.8 ± 16.07 vs. 38.53 ± 4.146 (p<0.001), State 4, 137.2 ± 10.65 vs. 69.31 ± 0.169 (p<0.001). The compound also significantly decreased OXPHOS, 192.1 ± 46.07 vs. 409.9 ± 40.30 (p<0.05) and ETS 232.2 ± 49.78 vs. 453.8 ± 38.93 (p<0.05) and CRC 1.383 ± 0.2522 vs. 1.155 ± 0.8502 (p<0.01).

Similar results were recorded in RHM treated with KL-1495 vs. Ctrl for complex II-supported respiration, i.e., a significant increase of state 2 and state 4 respiratory rates: State 2, 332.1 ± 17.21 vs. 195.1 ± 11.58 (p<0.001), State 4, 433.9 ± 15.85 vs. 313.5 ± 21.67 (p<0.05) and an important decrease of OXPHOS, 279.6 ± 44.23 vs. 636.2 ± 54.74 (p<0.05) and ETS, 475.7 ± 27.42 vs. 707.5 ± 27.48 (p<0.001), and CRC, 0.6488 ± 0.1114 vs. 1.932 ± 0.2977 (p<0.05).

Figure 4: Representative traces of CaCl₂ pulses (20 nmol/pulse) with and without CsA (100 μM, blue tracing) and CRC data in RHM controls.

Figure 5: Representative traces of CaCl₂ pulses (20 nmol/pulse) with and without KL-1496 (150 μM, blue tracing) and CRC data.

Figure 6: Representative traces of CaCl₂ pulses (20 nmol/pulse) with and without KL-1498 (150 μM, blue tracing) and CRC data.