Characterization of Two Novel Pharmacological ATP-Sensitive Potassium Channels Modulators in Isolated Rat Heart Mitochondria

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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 (eg, diazoxide and pinacidil) were also demonstrated to have direct effects on the oxidative 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 at reperfusion, and infarct size reduction.

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

Fig. 1. Schematic representation of mitochondrial electron transport chain (ETC) and coupling/uncoupling of oxidative phosphorylation. (Adapted from Fariss et al, 2005).

AIM

The aim of the present study was to characterize the effects of two novel mitoK_{ATP} **openers** (KL-1488 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_{State2} + D_{OXP} + c + Omy_{State4} + F_{ETS} + Ama_{ROX}

Chamber B: S(Rot)_{State2} + D_{OXP} + c + Omy_{State4} + F_{ETS} + Ama_{ROX}

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

2. CALCIUM RETENTION CAPACITY (CRC) MEASUREMENT

The amount of mitochondrial Ca²⁺ retained before the opening of mPTP was measured spectrofluorimetrically 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 Ca-green (1 μM; excitation-emission, 500–530 nm).

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

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

CONCLUSION

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

RESULTS

Respiratory Function Assessment

Fig. 2. Representative diagram of the SUIT protocol for CI-supported respiration. A. Ctrl; B. KL-1495; C. KL-1488. Oxygen concentration (cO₂) and O₂ flux (pmol O₂·s⁻¹·ml⁻¹) are represented as a function of time. Additions are as follows: G – glutamate (10 mM), M – malate (0.2 mM), D – ADP (5 mM), Cyt C – cytochrome c (10 μM), Omy- oligomycin (2 μg/ml), F – FCCP (0.05 μM), Ama – antimycin A (2.5 μM). Oxygen concentration (maximum 200 nmols/ml) was maintained by intermittent H₂O₂ additions into the chamber (100 μM at each titration step).

Fig. 3. Representative diagram of the SUIT protocol for CII-supported respiration. A. Ctrl; B. KL-1495; C. KL-1488. Additions are as follows: Rot – rotenone (0.5 μM), S – succinate (10 mM), D – ADP (5 mM), Cyt C – cytochrome c (10 μM), Omy- oligomycin (2 μg/ml), F – FCCP (0.05 μM), Ama - antimycin A (2.5 μM)

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

In mitochondria respiring on glutamate/malate a significant increase of state 2 and state 4 in RHM treated with KL-1495 vs. Ctrl, was found : State 2, 123.8 ± 18.07 vs. 38.53 ± 4.146 (*p*<0.001), State 4, 137.2 ± 10.65 vs. 69.31 ± 6.189 (*p*<0.001). The compound also significantly decreased OXP/OS, 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 RCR 1.383 ± 0.2522 vs. 6.155 ± 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 OXP/OS, 279.6 ± 44.23 vs. 636.2 ± 54.74 (*p*<0.05) and ETS, 475.7 ± 27.42 vs. 757.5 ± 27.48 (*p*<0.001), and RCR, 0.6488 ± 0.1114 vs. 1.932 ± 0.2877 (*p*<0.05).

KL-1488 INDUCES RESPIRATORY INHIBITION FOR CI (BUT NOT FOR CII)-SUPPORTED RESPIRATION

Calcium Retention Capacity Assessment

KL-1495 PROMOTES WHEREAS KL-1488 HAS NO EFFECT ON THE mPTP OPENING

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

Fig. 5. Representative traces of CaCl₂ pulses (20 nmol/pulse) without and with KL-1495 (150 μM, blue tracing) and CRC data.

Fig. 6. Representative traces of CaCl₂ pulses (20 nmol/pulse) without and with KL-1488 (150 μM, blue tracing) and CRC data.