AIM

The aim of the present study was to characterize the effect of two novel mitochondrial openers (KL-1495 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: G M_max+ ΔADP + ADP + Omy + Cc + Oxy + ETS + Amo +
Chamber B: S(Res) + ΔOXPHOS + Cc + Omy + ETS + Amo +

Respiratory control ratio (RCR) was calculated as the ratio OXPHOS/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, cyclopentyl (AcsA).

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.

RESULTS

Respiratory Function Assessment

Similar results were recorded in RHM treated with KL-1495 vs. Ctrl for complex II-suppressed respiration, i.e., a significant increase of state 2 and state 4 respiratory rates: State 2, 332 ± 17.21 vs. 195 ± 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 RCR, 0.6488 ± 0.1114 vs. 1.932 ± 0.2877 (p<0.05).

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 ± 16.07 vs. 28.53 ± 4.146 (p<0.001), State 4, 137.2 ± 10.65 vs. 69.31 ± 6.189 (p<0.001). The compound also significantly decreased OXPHOS, 192.1 ± 46.07 vs. 409.2 ± 40.30 (p<0.05) and ETS 223.2 ± 49.78 vs. 453.8 ± 38.93 (p<0.05) and RCR 1.383 ± 0.2522 vs. 6.155 ± 0.8502 (p<0.01).

KL-1495 PROMOTES WHEREAS KL-1498 HAS NO EFFECT ON THE mPTP OPENING

KR-1495 elicits mitochondrial uncoupling and respiratory chain inhibition that may play a role in cardioprotection during posts ischemic reperfusion.

CONCLUSION

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.

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