Working solutions: Physiological cytosolic concentrations of K^+ and Na^+ are ~150 mM and ~10 mM, respectively (Bernardi 1999; Murphy and Eisner 2009). K/H exchange, Na/H exchange and Na/Ca exchange actively influence respiration. The most common solutions used for respiratory analysis in isolated mitochondria are based on mannitol, sucrose or KCl. Δpmt in respiring mitochondria ranges between -0.2/-0.25 V. It is estimated that the Donnan potential in resting isolated mitochondria is around -30 mV to -100 mV in Tris-sucrose medium (Mitchell and Moyle 1969; Pietrobon *et al*. 1982; Zoratti *et al.* 1982; Jung *et al.* 1988). When sucrose is replaced by increasing concentrations of KCl, this potential tends to 0 mV (Jung *et al.* 1988; Nicholls 1974). In the same line, the increase in KCl concentration lowers the contribution of ΔΨmt in favor of ΔμH+ which shifts from near 0 mV to -60 mV in the KCl solution (Mitchell and Moyle 1969; Zoratti *et al.* 1982; Jung *et al.* 1988).

In addition, Ca^{2+} is directly involved in respiratory control through the activation of TCA cycle dehydrogenases (Denton 2009). Mitochondrial Ca^{2+} is exchanged by Na^+ and Na^+ equilibrates to μH+ in respiring mitochondria (Murphy and Eisner 2009; Jung *et al.* 1992). Hence, the alteration of ΔμH+ (e.g. by means of the solution selected) can alter oxygen consumption through imbalance of cation cycling across the inner mitochondrial membrane and influence of TCA cycle turnover.

Therefore, the election of an appropriate working solution is a key determinant for the analysis of respiration and its components.

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