Effects of Monovalent Cations on Ca\(^{2+}\) Transport in Mitochondria; A Comparison between Brown Fat and Liver Mitochondria from Rat *

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Brown fat mitochondria belong to the class of mitochondria\(^1\) that shows Na\(^+\)-induced Ca\(^{2+}\) release.\(^2,3\) We have here investigated the effect of this Na\(^+\) response on the ability of the mitochondria to maintain a defined concentration of Ca\(^{2+}\) in the medium (i.e. in the cell: the cytosol) in an attempt to clarify the regulatory role of the mitochondrial Na\(^+\)/Ca\(^{2+}\) exchange.

Results. In Fig. 1a (upper trace) we have followed Ca\(^{2+}\) uptake and release in brown fat mitochondria. Addition of Ca\(^{2+}\) (20 nmol/mg protein) led to a transient increase of free Ca\(^{2+}\) in the medium: the added Ca\(^{2+}\) was rapidly taken up; the uptake followed approximately first-order kinetics and it was possible to read off the time constant \(k\) from the curve (cf. Harris and Zaba\(^4\)). After the net uptake had ceased, the Ca\(^{2+}\)-uptake inhibitor red (RR) was added; this revealed a slow efflux of Ca\(^{2+}\) (rate 1). The further addition of 20 mM NaCl led to a stimulation of Ca\(^{2+}\) efflux (rate 2). (Rate 2—rate 1) is thus the Na\(^+\)-induced Ca\(^{2+}\) release rate. A similar trace is depicted for liver mitochondria in the lower trace of Fig. 1a. It should be noted that the efflux seen here under our conditions after ruthenium red addition is much lower than that seen under other conditions.\(^1\)

In Fig. 2a we have compared the Na\(^+\)-induced Ca\(^{2+}\) release in brown fat and liver mitochondria as a function of Na\(^+\) concentration. Na\(^+\)-induced Ca\(^{2+}\) release has been reported not to occur in liver mitochondria\(^1\) but, under our conditions, a slight effect of Na\(^+\) could be observed also in liver—this observation was facilitated by the much lower Ca\(^{2+}\) efflux after ruthenium red addition (Fig. 1a lower trace, rate 1).

The Na\(^+\) stimulation of efflux also manifests itself when Ca\(^{2+}\) uptake is not inhibited. This is demonstrated in Fig. 1b. After uptake of the first Ca\(^{2+}\) addition, addition of Na\(^+\) caused the mitochondria, as a consequence of the Na\(^+\)-induced release, to

\[ \frac{\Delta [\text{Ca}^{2+}]}{\text{t}} = \lambda \mu M \]

\[ \Delta [\text{Ca}^{2+}]_0 \]

Fig. 1. Ca\(^{2+}\) uptake and release in brown fat and liver mitochondria. Ca\(^{2+}\) and Na\(^+\) were added as the chloride salts. \(k\) indicates the measurement of the time constant for Ca\(^{2+}\) uptake.\(^*\) RR: ruthenium red. \(s\) is the baseline shift upon addition of Na\(^+\). \(\Delta [\text{Ca}^{2+}]_0\) indicates the change in the steady-state extramitochondrial Ca\(^{2+}\) concentration caused by Na\(^+\) addition. Conditions as described in Experimental.

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regulate subsequently the steady-state to a higher level (Δ[Ca$^{2+}$]$_0$). This was observed both in brown fat (upper trace) and liver (lower trace). This is a true new steady-state, as a small amount of Ca$^{2+}$, added later, was fully taken up by the mitochondria (Fig. 1b). These reactions are principally similar to what has been observed in heart mitochondria$^5$ and may reflect the physiological role of the Na$^+$-induced Ca$^{2+}$ release: a series of reactions are controlled by the cytosolic Ca$^{2+}$ concentration.

In Fig. 2b, we have compared the Na$^+$ responsiveness of brown fat and liver mitochondria measured as a change in free Ca$^{2+}$ (Δ[Ca$^{2+}$]$_0$) as a function of Na$^+$ concentration. It appears that Na$^+$ is nearly as good a regulator of free Ca$^{2+}$ in liver as in brown fat and this may imply that also in liver Na$^+$-induced Ca$^{2+}$ release is of physiological significance and that the observed α-adrenergic agonist stimulation of Ca$^{2+}$ release in liver cells$^6$ may be related to this mechanism.

It is evident from the initial Ca$^{2+}$ uptake rates seen in Fig. 3 that the higher rate of Na$^+$-induced Ca$^{2+}$ release in brown fat may, to a certain extent, be compensated by a more rapid (re-)uptake of Ca$^{2+}$, thus at least in part explaining the apparent similarity between the two tissues depicted in Fig. 2b.

We have also observed, both in brown fat and liver mitochondria, that addition of K$^+$ led to a decrease in the rate of Ca$^{2+}$ uptake (i.e. the time constant $k_i$ became larger). As seen in Fig. 3, this effect was maximal at K$^+$ concentrations which are probably much below those in the cytosol, and changes in the cytosolic K$^+$ cannot be expected to
influence the steady-state level of Ca$^{2+}$. It is, however, important to consider this effect in in vitro studies of Ca$^{2+}$ metabolism.

Thus both in brown fat and liver mitochondria, changes in cytosolic monovalent cation concentrations will be reflected in changes in cytosolic Ca$^{2+}$ levels, probably with regulatory functions.

**Experimental**. Brown fat mitochondria were prepared from adult rats which had lived at 5°C for at least 3 weeks. The general method as described by Cannon and Lindberg was used; the medium was 250 mM sucrose, 20 mM Tris HCl, 0.1 mM ethylene glycol-bis[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid (EGTA), 0.1% bovine serum albumin (fatty acid free), pH 7.2. Albumin was not included in the final washing medium, but was added to the suspension medium to a final concentration of 2% after samples had been removed for protein determination by the biuret method. Liver mitochondria were prepared by the same method, with the omission of the initial high speed centrifugation step. Calcium uptake and release were followed as previously described in a dual-wavelength spectrophotometer (Aminco DW-2) by the use of arsenazo III \( \{2,2'\text{-dihydroxy-3,6-bisulfo-2,7-naphthalene-bis}[azo]\text{-dibenzene arsonic acid}\} \) (grade 1, Sigma) as indicator at 675–685 nm with a 3 nm slit. The medium for brown fat mitochondria was 125 mM sucrose (batch-purified with Dowex 50 WX8, H$^+$ form), 20 mM Tris–HCl, pH 7.2 and contained 4 μM rotenone, 0.5 mM GDP (Tris-salt), 0.2% bovine serum albumin (fatty acid free), 5 mM l-glycerol-3-phosphate (dicyclohexylammonium salt) and 0.1 mM arsenazo III. Mitochondria were added to a final concentration of 0.5 mg/ml and the volume was 1 ml. When present, the ruthenium red concentration was 1 μM. The temperature was 20°C. With liver mitochondria the sucrose concentration was 250 mM, and 5 mM succinate (Tris-salt) was substituted for l-glycerol-3-phosphate. In order to determine changes in steady-state Ca$^{2+}$ levels, it was found necessary to use NaCl of “Suprapur” grade (Merck). Other details as in the figure legends.

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