Na^+ RELEASES Ca^{2+} FROM LIVER, KIDNEY AND LUNG MITOCHONDRIA

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1. Introduction

Intracellular Ca^{2+} has assumed increasing importance as a regulator of a variety of cellular processes, including muscle contraction, secretion and cell division [1]. A prime candidate for a controller of intracellular Ca^{2+} levels, by virtue of its high affinity Ca^{2+} uptake mechanism, is the mitochondrion. Such a role for the mitochondrion has gained support from the demonstration that mitochondrial Ca^{2+} release is under separate control from uptake [2]. Two systems capable of releasing mitochondria Ca^{2+} are the Na^+-induced release system [3] and the Ca^{2+}-induced transition in permeability of the inner membrane [4]. In elucidating the role of these release systems in Ca^{2+} homeostasis it is important to know the extent of their occurence in mitochondria from different tissues. Such a study for the Na^+ system has concluded that this system was most active in brain, heart and adrenal cortex, present in striated muscle, but absent in kidney, liver, lung, smooth muscle and hepatoma [5]. We report that the Na^+ system is indeed present in mitochondria from liver, kidney and lung. We have not studied smooth muscle or hepatoma.

2. Materials and methods

Sprague-Dawley female retired breeder rats were used. All types of mitochondria were prepared as in [6], except that the homogenization medium (4 ml/g wet wt tissue) contained 250 mM sucrose, 10 mM Tris–HCl, 0.5 mM K^+EGTA (pH 7.4). For lung and brain mitochondria this procedure does not give a completely pure preparation. However, results can be obtained with crude homogenates which are essentially identical to those obtained with pure mitochondria (except for kidney, where residual Na^+ must be washed out). This indicates that mitochondrial purity is not a critical factor in the demonstration of Na^+-induced Ca^{2+} release.

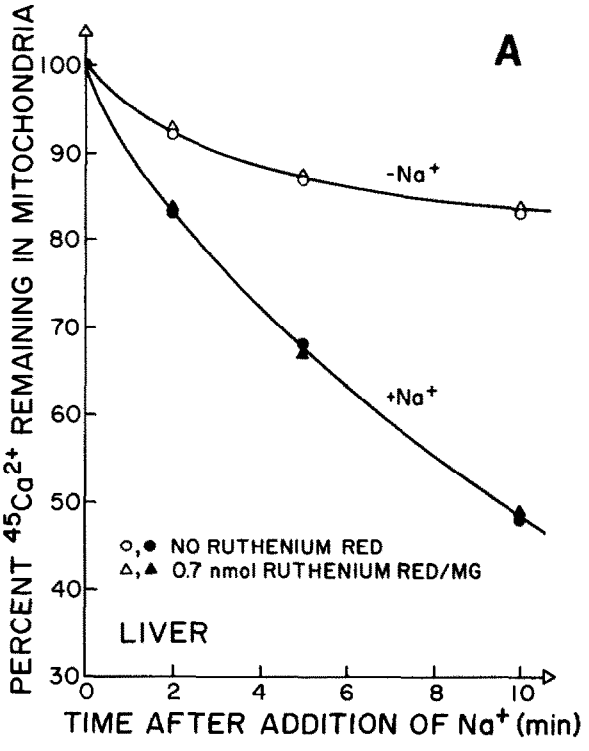
The methodology for measuring Na^+-induced efflux of ^{45}Ca was as in [4]. The standard buffer for Ca^{2+} release studies contained 250 mM sucrose, 20 mM Tris–MOPS (pH 7.1), 40 mM KCl, 2 mM K^+P_i, 2 mM $MgCl_2$, 0.8 mg/ml BSA, 2 mM K^+ATP and 5 mM K^+ succinate. Typically, to 12 ml standard buffer was added 12 mg mitochondria, followed immediately by 20 nmol rotenone. $CaCl_2$ (80 nmol) containing ^{45}Ca (2×10^6 dpm/μmol) was added, and the suspension was incubated at 30°C for 3 min in a shaking water bath. K^+EGTA (10 μmol) was then added, and a 0.5 ml aliquot was immediately centrifuged for 1.5 min in a Brinkmann 3200 bench centrifuge. Immediately following EGTA addition the remaining suspension was divided, typically into 4 and added (at $t = 0$) to separate flasks containing the agents whose effect on Ca^{2+} efflux was to be studied. Aliquots (0.5 ml) from each flask were centrifuged at time intervals. After 10 min the contents of all flasks were recombined, and 10 μg A23187 added. This ionophore releases the remaining mitochondrial Ca^{2+} and hence allows the 'zero mitochondrial Ca^{2+}' point to be determined. After 10 min incubation with A23187 the final 0.5 ml aliquot was centrifuged. Radioactivity in the supernatants was estimated by counting in Aquasol (New England Nuclear). These counts were used to estimate the amount of Ca^{2+} accumulated by the mitochondria, and the time course of Ca^{2+} release.

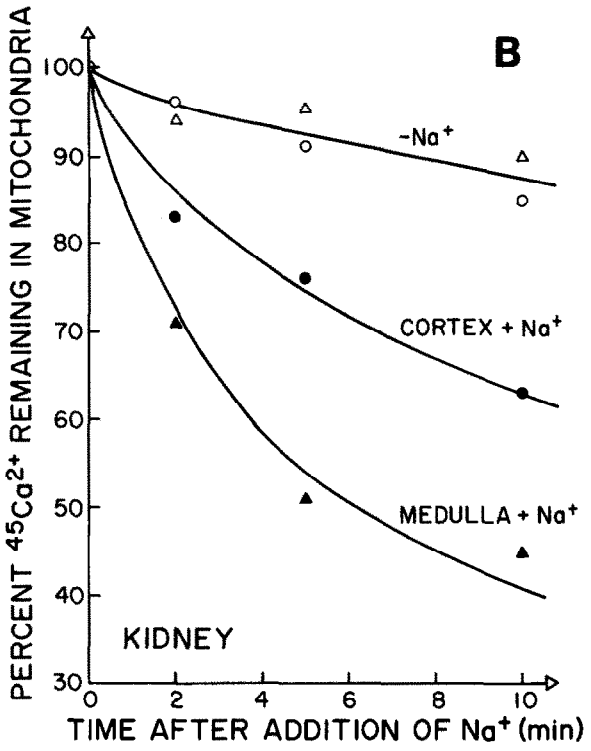
Abbreviations: BSA, bovine serum albumin; EGTA ethylenebisoxoethylenenitrolotetraacetic acid; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; MOPS, 4-morpholinepropanesulfonic acid

3. Results and discussion

Figure 1 shows the time course of Ca^{2+} release from mitochondria of liver, kidney and lung induced by the addition of 10 mM NaCl. KCl at 10 mM caused no greater degree of release than no addition (fig.1C). Both the spontaneous release and the Na^+-induced release were unaffected by the presence of ruthenium red (fig.1A). Mitochondria from kidney medulla released Ca^{2+} much faster than mitochondria from kidney cortex (fig.1B).

We are not certain why no Na^+-induced Ca^{2+} release from mitochondria from these organs was detected [3]. One factor could be that the Ca^{2+} release caused by the Ca^{2+}-induced transition was not sufficiently inhibited [4], which gave a high rate of ruthenium red-insensitive efflux even in the absence of Na^+, at least





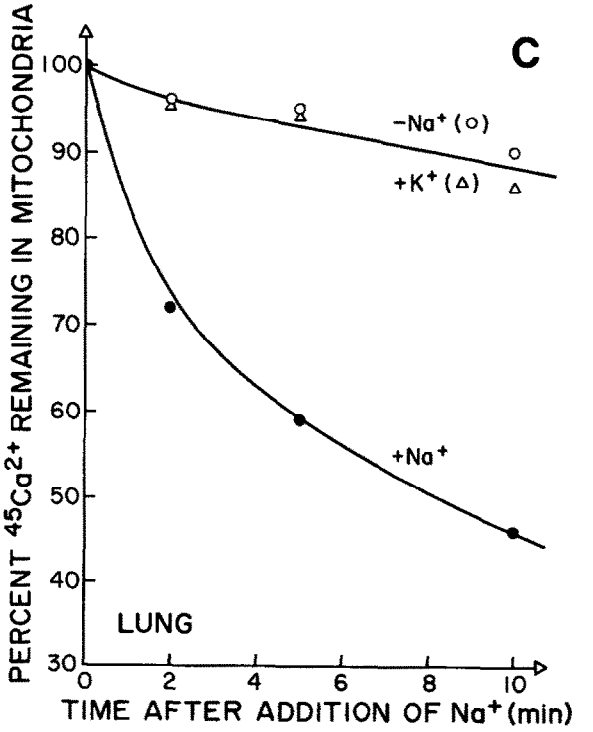


Fig.1. The Na^+-induced release of low levels of Ca^{2+} from mitochondria from: (A) liver; (B) kidney; (C) lung. Ca^{2+} uptake and release was measured as in section 2. Mitochondria were given 6.7 nmol $CaCl_2$/mg protein. After the 3 min incubation period the % uptake was: (a) liver, 79%; (b) kidney cortex, 85%; medulla, 82%; (c) lung, 87%. These % of 6.7 nmol/mg correspond to the 100% mitochondrial Ca^{2+} at $t = 0$. Na^+-dependent Ca^{2+} release was induced by the presence of 10 mM NaCl.

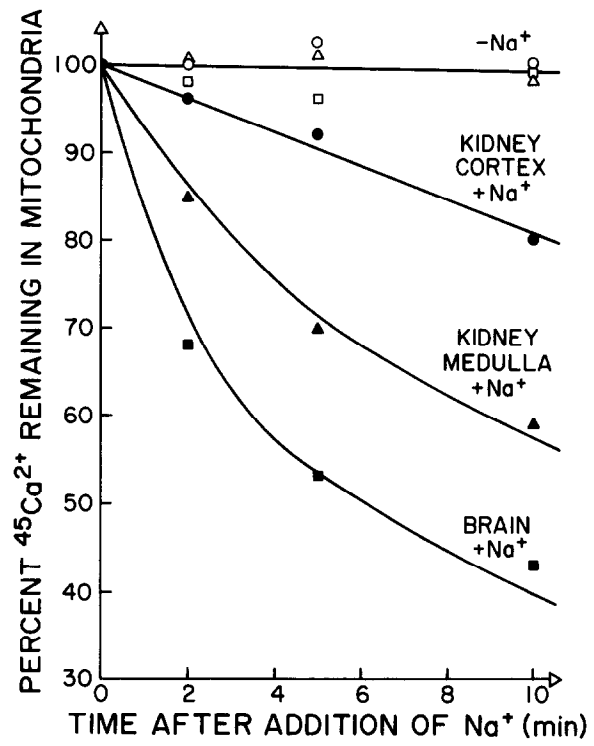


Fig.2. The Na^+-induced release of high levels of Ca^{2+} from mitochondria of kidney and brain. The procedure was as before, except that mitochondria were given 150 nmol $CaCl_2$/mg protein. After the 3 min incubation period the % uptake was: kidney cortex, 90%; kidney medulla, 93%; brain, 54%. Na^+-dependent Ca^{2+} release was initiated by the presence of 20 mM NaCl.

in the case of liver and kidney [3]. In our system this efflux was maximally inhibited by the presence of ATP and Mg^{2+}, in addition to rotenone. To obtain an estimate of V_{max} for Na^+-induced Ca^{2+} release, mitochondria were loaded with high levels of Ca^{2+} (150 nmol/mg) and the rate of release induced by 20 mM NaCl was measured (fig.2). This level of Na^+ was found to maximally stimulate Ca^{2+} release. High levels of Ca^{2+} were used in order to promote the saturation of the Ca^{2+} efflux site and to overwhelm the endogenous (unlabelled) Ca^{2+}. Still higher levels of Ca^{2+} were found to affect the values of V_{max} found by this method. The initial rate of Na^+-dependent Ca^{2+} efflux gave the estimates of V_{max} shown in table 1. The values found for brain and heart are comparable to those in [3,5].

Table 1
Estimated V_{max} for Na^+-induced Ca^{2+} release

	V_{max} (nmol Ca^{2+} . min^{-1} . mg $protein^{-1}$)
Liver	1.6
Lung	2.4
Kidney cortex	2.7
Kidney medulla	11
Brain	16
Heart	10

It is particularly important to know of the presence of Na^+-induced Ca^{2+} release in mitochondria from these tissues, since this opens the possibility of Na^+-induced Ca^{2+} release being involved in the Ca^{2+}-mobilizing action of some hormones. It has already been shown that the glycogenolytic action of vasopressin, angiotensin II and phenylephrine on liver is accompanied by a significant depletion of mitochondrial Ca^{2+} [6]. The rate of this depletion was found to be of the order of 0.3 nmol Ca^{2+} . min^{-1} . mg $protein^{-1}$ and therefore well within the Ca^{2+} release capability of the Na^+-induced release system.

Acknowledgement

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