THE ACTIVATION BY POTASSIUM OF THE SODIUM–CALCIUM CARRIER OF CARDIAC MITOCHONDRIA

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1. Introduction

In 1976 it was reported that cardiac mitochondria contain a Na^+–Ca^{2+} antiporter [1], and subsequent work has established that this carrier is present also in mitochondria of several other tissues [2–4]. The properties of the antiporter indicate that it is quite distinct from the carrier (uniporter) that catalyzes Ca^{2+} influx [5,6] and that its natural role is to catalyze the efflux of Ca^{2+} from mitochondria [1–3,5]. The simultaneous catalysis of Ca^{2+} flux by the uniporter and antiporter generates Ca^{2+} recycling across the inner membrane, and a steady state is attained when the activities of the two Ca^{2+} carriers are equal. [1,2,7,8].

It has been proposed that the function of the cycle in cardiac mitochondria is the control of the intramitochondrial free $[Ca^{2+}]$ in accordance with the regulatory requirements of certain dehydrogenases of the citric acid cycle [9,10]. However, to evaluate the capacity of the cycle to control the transmembrane distribution of Ca^{2+}, and in particular the intramitochondrial free $[Ca^{2+}]$, it is clearly necessary to establish the factors that influence the activities of the uniporter and antiporter. This report presents evidence that the antiporter, but not the uniporter, is strongly activated by K^+. Both the Na^+–Ca^{2+} and Ca^{2+}–Ca^{2+} exchange activities of the antiporter are affected.

2. Methods

2.1. *Preparation of mitochondria*

Mitochondria were prepared from female Wistar rat hearts, and their protein content was determined as stated in [1].

2.2. *The measurement of Ca^{2+} fluxes*

Mitochondria (containing 3 mg protein) were loaded with Ca^{2+} at 25°C in 3 ml medium containing 120 mM choline chloride plus KCl (as stated in the legends), 10 mM *N*-2-hydroxyethyl piperazine *N'*-2-ethane sulphonate (Tris salt, pH 7.2), 3 μg rotenone, 60 nmol $CaCl_2$ and 5 mM succinate (Tris salt). After 2–3 min, when almost all the Ca^{2+} had been accumulated, further uptake was stopped by the addition of 3 nmol ruthenium red, a specific inhibitor of influx [1,11].

One minute after ruthenium red had been added, Na^+–Ca^{2+} exchange was begun by the addition of 10 mM NaCl, and the net efflux of Ca^{2+} was monitored with a Ca^{2+}-selective electrode as in [1].

Ca^{2+}–Ca^{2+} exchange was started by the addition of 20 μM $CaCl_2$ 1 min after ruthenium red. When Ca^{2+} influx was measured, the $CaCl_2$ contained 0.1 μCi $^{45}Ca^{2+}$. When Ca^{2+} efflux was measured, the mitochondria were loaded with 0.1 μCi $^{45}Ca^{2+}$ of known specific activity. The time courses and initial rates of Ca^{2+}–Ca^{2+} exchanges were determined with the La^{3+}-stop technique, detailed in [5].

3. Results and discussion

In the experiment reported in fig.1, cardiac mitochondria were preloaded with Ca^{2+} and then inhibited with ruthenium red to prevent Ca^{2+} flux via the uniporter [11]. At zero time in the figure net efflux of Ca^{2+} was induced by the addition of Na^+. Considerable evidence indicates that the Ca^{2+} efflux under these conditions is catalysed by a Na^+–Ca^{2+} antiporter [1–3,5,6,8,9]. Fig.1 (upper two curves) shows that the Na^+-induced efflux of Ca^{2+} is stimulated ~2.4-

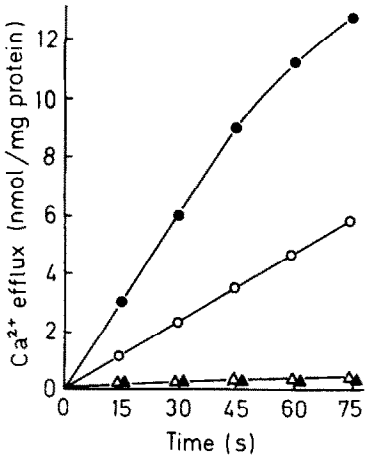

Fig.1. The stimulation of the Na^+-induced efflux of Ca^{2+} from cardiac mitochondria by K^+. The efflux of Ca^{2+} was measured (as in section 2) in the presence of ruthenium red and the following concentrations of NaCl and KCl: (•) 10 mM NaCl, 15 mM KCl; (○) 10 mM NaCl, KCl absent; (▲) 15 mM KCl, NaCl absent; (△) KCl and NaCl absent.

fold by 15 mM K^+.

Fig.1 also shows the very slow loss of accumulated Ca^{2+} that occurs in the absence of added Na^+. In [6] this Na^+-independent efflux of Ca^{2+} was inhibited very poorly by lanthanides, in contrast to the Na^+-dependent efflux, from which it was concluded that the Na^+-independent efflux is not due to residual

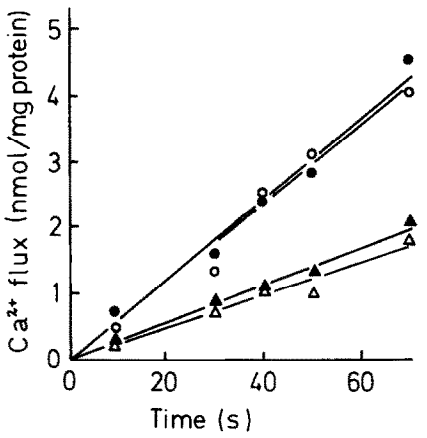

Fig.2. The stimulation by K^+ of the Ca^{2+}–Ca^{2+} exchange across the inner membrane of cardiac mitochondria. The influx and efflux of Ca^{2+} (measured as in section 2) refer to the unidirectional influx and unidirectional efflux, respectively (where unidirectional influx minus unidirectional efflux equals net flux). (○) Ca^{2+} influx with 30 mM KCl; (•) Ca^{2+} efflux with 30 mM KCl; (△) Ca^{2+} influx with 6 mM KCl; (▲) Ca efflux with 6 mM KCl.

activity of the Na^+–Ca^{2+} antiporter in the absence of Na^+. It is evident from fig.1 that K^+ does not change significantly the Na^+-independent efflux.

The inability of K^+ to promote Ca^{2+} efflux in the absence of Na^+ indicates that K^+ cannot substitute for Na^+ as a substrate for the Na^+–Ca^{2+} antiporter. The data suggest, therefore, that K^+ activates, directly or indirectly, the Na^+–Ca^{2+} exchange.

It seemed possible that K^+ might affect the fluxes of Na^+ that follow exchange of Na^+ with Ca^{2+}, and

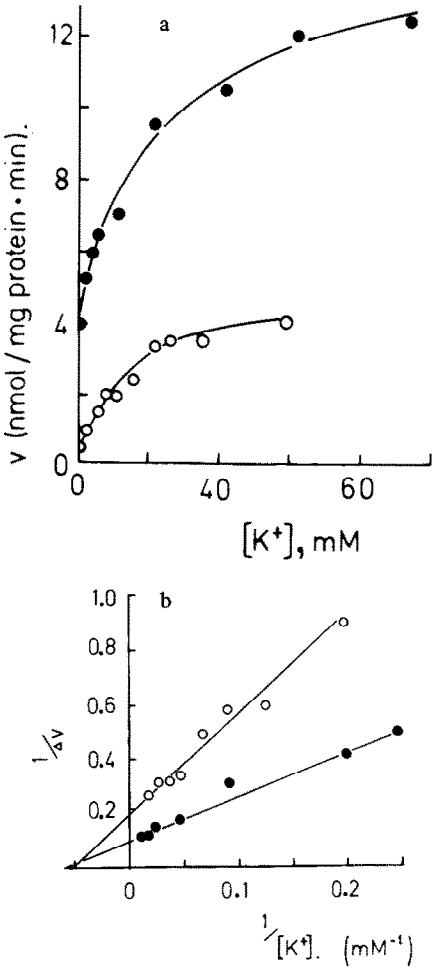

Fig.3. The dependence of the initial rates of Na^+–Ca^{2+} and Ca^{2+}–Ca^{2+} exchanges on the extramitochondrial $[K^+]$. (•) Na^+–Ca^{2+} exchange; (○) Ca^{2+}–Ca^{2+} exchange. (a) The initial rates (v) of Na^+-induced and Ca^{2+}-induced effluxes of Ca^{2+} were determined as in section 2. (b) A double reciprocal plot of the increase in initial rates of the exchanges versus $[K^+]$. Each value (Δv) is calculated from the data of fig.3a, and is the difference between the velocities observed in the presence and absence of K^+.

thereby activate the Na^+–Ca^{2+} exchange indirectly. This possibility was checked by investigating the ability of K^+ to activate Ca^{2+}–Ca^{2+} exchange by the antiporter. The catalysis of 1:1 Ca^{2+}–Ca^{2+} exchange by the antiporter in the presence of ruthenium red has been demonstrated [5]. The exchange is measured by following in parallel experiments the influx of $^{45}Ca^{2+}$ and the efflux of $^{45}Ca^{2+}$, both of known specific radioactivity [5].

Fig.2 shows the influxes and effluxes of Ca^{2+} on the addition of external Ca^{2+} (at $t = 0$) to mitochondria preloaded with Ca^{2+} and inhibited with ruthenium red. The fluxes were measured with 30 mM K^+ (upper two curves) and 6 mM K^+ (lower two curves) present. At both $[K^+]$, the ratio of the rates of Ca^{2+} influx to Ca^{2+} efflux was close to 1, which indicates that a true Ca^{2+}–Ca^{2+} exchange is measured in both cases. The rate of Ca^{2+}–Ca^{2+} exchange is approximately doubled when the $[K^+]$ is increased from 6 mM to 30 mM.

The most straightforward explanation of the data of fig.1,2 is that K^+ combines with the antiporter and modifies its Ca^{2+}–Ca^{2+} and Na^+–Ca^{2+} exchange activities. If this is correct, activation by K^+ should be a saturable phenomenon with a defined dissociation constant of activation. This prediction was investigated as reported in fig.3, which shows the activation of the antiporter as a function of $[K^+]$. Under these experimental conditions, K^+ maximally activates the Na^+–Ca^{2+} exchange ~3-fold and the Ca^{2+}–Ca^{2+} exchange ~13-fold (fig.3a). A double reciprocal plot of the increase in activity of the two exchanges against the $[K^+]$ is given in fig.3b. Both plots are linear and yield essentially the same K_m value (17–19 mM).

Other experiments (not shown) have revealed that an increase in $[K^+]$ over 0–70 mM (maintaining constant ionic strength with choline chloride) does not detectably increase the rate of Ca^{2+} influx via the Ca^{2+} uniporter. The selective activation of the antiporter by K^+, therefore, provides a further distinction between the uniporter and antiporter to those already noted [1,5,6] and underlines the existence of two distinct systems for Ca^{2+} transport in cardiac mitochondria.

At physiological $[K^+]$, the antiporter will be fully activated, and K^+ cannot be regarded as a regulator of the carrier. Nevertheless, the marked activation by K^+ must clearly be taken into account when conducting in vitro experiments designed to assess the in vivo capacity of the carrier to extrude Ca^{2+}.

Acknowledgements

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