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THE Ca^{2+}-Na^{+} ANTIPORTER OF HEART MITOCHONDRIA OPERATES ELECTRONEUTRALLY

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ABSTRACT The transmembrane potential of heart mitochondria during Na^{+}-induced Ca^{2+} release has been monitored continuously with a Tetraphenylphosphonium-sensitive electrode. The data obtained indicate that the exchange process does not generate an electrical current, and provides direct support for the concept of a 2 Na^{+} per 1 Ca^{2+} exchange.

INTRODUCTION

In mitochondria isolated from various tissues a ruthenium red insensitive Ca^{2+}-Na^{+} antiporter has been described (1,2,3). This antiporter mediates the efflux of Ca^{2+}, and thus participates in the regulation of the Ca^{2+} content of the organelles and of the cytosol. Experimental difficulties have so far prevented the direct measurement of the stoichiometry of the Ca^{2+}-Na^{+} antiporter. The kinetic data of Crompton et al. (4,5) have provided indirect indications for a ratio of $\geq$ 2 Na^{+} for 1 Ca^{2+}. In this article, direct evidence will be presented that the stoichiometry of the antiporter is 2 Na^{+} for 1 Ca^{2+}. The conclusion has been reached from experiments in which the transmembrane potential of mitochondria during the operation of the Ca^{2+}-Na^{+} antiporter has been continuously recordered using a TPP^{+}-selective electrode.

ABBREVIATIONS: TPP^{+} : Tetraphenylphosphonium - ion
$\Delta\psi$: mitochondrial membrane potential

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EXPERIMENTAL PROCEDURES

Mitochondrial Preparation. Rat heart mitochondria were prepared by the methods of Crompton et al. (4). The protein concentration of the suspension was estimated by a modified biuret procedure (6) with bovine serum albumin (Sigma Chemical Co.) as a standard.

Measurement of Ca^{2+}-Fluxes. Ca^{2+}-activities were measured with a Ca^{2+}-selective liquid membrane electrode of the neutral ionophore type (7) in a system analogous to that described by Affolter and Sigel (8).

Measurement of the Transmembrane Potential ($\Delta\psi$). The $\Delta\psi$ was monitored continuously with a TTP^{+}-selective electrode as described by Kamo et al. (9) in a system analogous to that used for the Ca^{2+} electrode measurements. The non-specific binding of TTP^{+} to the membrane was checked by using appropriate de-energizing inhibitors.

RESULTS AND DISCUSSION

Figure 1 shows a typical experiment in which uptake and release of Ca^{2+} have been recorded with the Ca^{2+}-selective electrode. After the accumulation of 25 nmol Ca^{2+}/mg mitochondrial protein the uptake is abolished by the addition of 300 nmol ruthenium red, and the efflux of Ca^{2+} is induced shortly thereafter by the addition of 15 mM Na^{+}. The efflux velocity in the particular experiment shown is 9 nmol Ca^{2+}/min/mg mitochondrial protein. Addition of 0.8 µg Antimycin A/mg mitochondrial protein prior to the addition of Na^{+} lowers the efflux velocity to a third, in agreement with previous findings by Crompton et al. (4).

Figure 2 shows the measurement of the $\Delta\psi$ during the uptake and the subsequent efflux of Ca^{2+} induced by Na^{+}. After mitochondria have rapidly developed a transmembrane potential of 165 mV, negative inside, the addition of Ca^{2+} induces a sudden decrease of the $\Delta\psi$, which tends to approach a plateau at about 156 mV, when the uptake is essentially complete. The lower steady state of the post Ca^{2+} $\Delta\psi$ level is evidently due

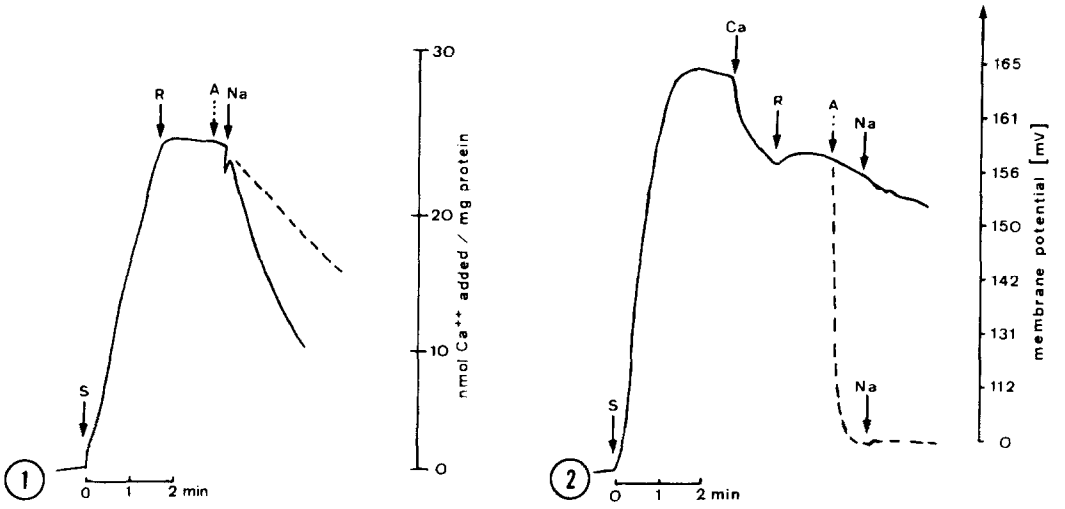

Figure 1 Na^+ induced Ca^{2+} efflux:
The incubation medium contains: 130 mM KCl, 10 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate), 1 µg Rotenone / ml, 0.5 mg mitochondrial protein / ml, 15 µM $CaCl_2$. Final pH = 7.0, temperature 25°C. Further additions are as follows. At the point "R", 150 pmol ruthenium red / ml; at point "Na", 15 mM NaCl; at point "A", 0.4 µg Antimycin A / ml.

Figure 2 The membrane potential during Ca^{2+} uptake and Na^+-induced efflux: same conditions as in figure 1, but instead of 15 µM $CaCl_2$, 5 µM TPP-Cl was present. At point "Ca", 15 µM $CaCl_2$ was added.

to the energy dissipating "cycling" of Ca^{2+} (10), since ruthenium red blocks the Ca^{2+} induced decrease of the $\Delta\psi$ trace, and in general, although not very evidently in the experiment shown in Fig. 2, reverses it substantially. It is clear from Fig. 2, however, that the steady-state $\Delta\psi$ trace after addition of ruthenium red does not change upon addition of the Na^+-induced Ca^{2+} efflux. Indeed, the slope of the TPP^+-selective electrode after addition of Na^+ is perfectly superimposable to that observed in the absence of Na^+ (not shown). It is clear, then, that no net charge movement occurs during the exchange of Ca^{2+} against Na^+, in agreement with the concept of an electroneutral exchange process.

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