ENERGY-DEPENDENT CONTRACTION OF SWOLLEN MITOCHONDRIA: ACTIVATION BY NIGERICIN

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Summary - The rate, extent, and efficiency of the energy-dependent contraction of heart mitochondria swollen in Na+ or K+ nitrate are all strongly activated by nigericin, an antibiotic which is known to support cation/H+ exchange in natural and model membranes. In the absence of nigericin, the cation selectivity sequence of energy-dependent contraction (Na+>Li+>K+>choline+) is identical to that of passive swelling in acetate salts, a reaction which is presumed to be dependent on an endogenous cation/H+ exchanger. These results strongly favor an osmotic mechanism for energy-dependent contraction which depends on electrogenic H+ ejection, H+/cation exchange, and electrophoretic anion efflux.

The presence of a Na+/H+ exchanger in the mitochondrial inner membrane has been deduced from studies of H+ equilibration following O_2 pulses in intact mitochondria (1) and submitochondrial particles (SMP) (2), and this exchange has been invoked to explain the rapid rates of passive osmotic swelling seen in Na+ acetate and phosphate (3-5). Studies from this and other laboratories also suggest that energy-dependent contraction of swollen mitochondria is an osmotic reaction which depends on the activity of this endogenous cation/H+ exchanger (3, 6-8). Azzone et al (9, 10) have rejected this mechanism, however, based on the following arguments: (a) The energy-dependent extrusion is inhibited by nigericin, an exogenous cation/H+ exchanger, (b) The contraction and cation extrusion do not show cation selectivity, and (c) The rate of cation extrusion exceeds that of cation/H+ exchange, as usually estimated from passive swelling experiments. The present communication summarizes preliminary experiments which indicate that: (a) Nigericin strongly activates energy-dependent contraction of swollen heart mitochondria, (b) The identical cation selectivity sequence (Na+>Li+>K+>choline+) is seen for energy-dependent contraction and for passive swelling in acetate salts,

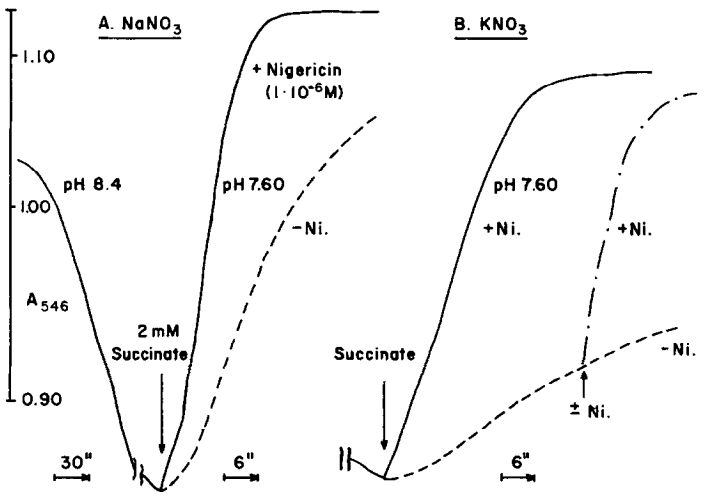

Fig 1 - Stimulation of respiration-dependent contraction by nigericin. Nagarse beef heart mitochondria were suspended at 0.5 mg/ml in a medium of either $NaNO_3$ (100 mM, Part A) or KNO_3 (100 mM, B) containing 2 mM Tris (pH 8.4), rotenone (2 ug/ml), and sucrose (added with the mitochondria, 5 mM). Swelling was recorded using an Eppendorf photometer equipped with a circular cuvette maintained at 35°. After a decrease in A_{546} of about 0.2, the recorder was switched to the indicated fast chart speed and Tris succinate (2 mM final) was added to bring the pH to 7.60. Where indicated, nigericin was added to a final concentration of 1 X 10^{-6}M just prior to the succinate or during the contraction as shown.

and (c) The rate of passive swelling in Na+ acetate is strongly dependent on the concentration of matrix K+ and on the presence of divalent cations, factors which suggest that the exchanger may be subject to considerable metabolic regulation and that direct comparison of rates of passive swelling and energy-dependent contraction may be misleading.

Activation of Energy-Dependent Contraction by Nigericin - Isolated beef heart mitochondria suspended in 100 mM Na+ or K+ nitrate above pH8 swell spontaneously at 35°C (7). Mitochondria swollen under these conditions contract in an energy-dependent reaction when respiration is initiated or ATP supplied (7). The rate and extent of contraction are both greater in $NaNO_3$ than KNO_3 (Fig 1) and the reaction shows a strong pH dependence with an optimum at pH 6.8. Nigericin markedly stimulates both the rate and extent of respiration-dependent contraction in Na+, and especially in the K+ medium (Fig 1) and shifts the pH optimum to 7.5.

Table I - Efficiency of Succinate-Dependent Contraction

	$NaNO_3$ (pH 7.64)		KNO_3 (pH 7.70)	
	No Addn.	Nigericin (1.5×10^{-7}M)	No Addn.	Nigericin
Rate of Contraction with succinate addition ($\Delta A_{546} \cdot min^{-1} \cdot mg^{-1}$)	0.35	1.02	0.17	0.65
Cation Extrusion rate (uatoms C+. min^{-1}. mg^{-1})	0.30	0.87	0.14	0.55
Respiration rate during contraction phase (uatoms O_2. min^{-1}. mg^{-1})	0.20	0.14	0.22	0.20
Efficiency (ΔNa+ or K+/O)	1.5	6.2	0.6	2.8

Experiments were carried out as described for Fig 1 with a YSI oxygen electrode in a lucite plunger to record respiration at the same time as succinate-dependent contraction. Cation extrusion was estimated from a calibration curve relating A_{546} to the cation content of the matrix (14).

The reaction is inhibited by antimycin and dependent on nigericin concentration, with activation detectable at 10^{-9}M and optimal just above 10^{-7}M.

This result is in direct conflict with reports from Azzone's laboratory (9, 10) that nigericin inhibits respiration-dependent contraction. The basis for the discrepancy appears to reside with the high levels of nigericin employed (9) and their use of valinomycin to induce the initial mitochondrial swelling. Valinomycin inhibits nigericin-activated contraction in KNO_3 under the conditions of Fig 1B (50% inhibition at 10^{-8}M). Energy-dependent contraction is readily visible following valinomycin-induced swelling (11, 12), but the rate and extent of contraction are considerably less than for mitochondria swollen and contracted under the conditions of the present study.

Activation of energy-dependent contraction by nigericin would be predicted by the osmotic contraction model (Ref 7, Fig 1), since the exogenous cation/H+ exchange would be operating in parallel with the endogenous exchanger. These results also complement studies which have shown that nigericin activates

Table II - Cation Selectivity Sequence for Passive Swelling of Heart Mitochondria in Acetate and for Succinate-Dependent Contraction in Chloride Salts.

	Rate of Passive Swelling - Acetate Salts	Rate of Succinate Dependent Contraction - Chloride Salts	
	(ΔA_{546}. min^{-1})		
Na+	0.25	0.31	(0.14)
Li+	0.07	0.14	(0.17)
K+	0.01	0.10	(0.20)
TMA+ or Choline+	<0.01	0	(0.12)

In the swelling experiments, the initial rate of swelling of heart mitochondria (0.5 mg/ml) was recorded at 25° in a medium of 100 mM of the indicated acetate salt, containing Tris acetate (2 mM, pH 7.2), sucrose (5 mM), rotenone (2μM), antimycin (0.4 ug/mg), and oligomycin (2 ug/mg). For the contraction series, mitochondria were suspended at 0.5 mg/ml in 100 mM of the indicated chloride salt containing Tris Cl^- (2 mM, pH 7.2), sucrose (5 mM), and rotenone (2 uM). Swelling was initiated by addition of p-chloromercuribenzoate (50 nmoles/mg). After a decrease in A_{546} of 0.2 (2-6 minutes, depending on the cation), dithioerythritol (100 nmoles/mg) was added followed by Tris succinate (2 mM) and the rate of contraction was recorded. Respiration rates (uatoms . min^{-1}. mg^{-1}) are given in parentheses.

cation uptake in SMP (2, 5, 13). Since the membrane of SMP has the opposite orientation to that of the intact mitochondrion, ion extrusion in the intact mitochondrion should correspond to ion uptake in SMP.

In studies to be reported more completely elsewhere, we have also established that there is a burst of respiration equivalent to the State 3 rate during succinate-supported contraction in Na NO_3 (conditions of Fig 1A) which returns to the controlled rate when the contraction phase ceases, and that there is less succinate-dependent H+ ejection during the contraction phase than in the absence of a volume change. The efficiency of contraction in Na+ exceeds that in K+ in the absence of nigericin and efficiency in both Na+ and K+ is markedly increased by the addition of nigericin (Table I). The sum of the Na+ extruded plus H+ ejected approaches a limit of about 8 per 0 consumed. This value is compatible

Table III - Initial Rates of Passive Swelling of Heart Mitochondria in Na+ Acetate.

Preparation and Additions	K+ content nmoles/mg)	Swelling Rate ($\Delta A_{546} \cdot min^{-1}$) Non-energized	Succinate Respiration
Fresh, untreated BHM	88	0.26	.63
" + CCP (1×10^{-6})		0.30	.25
" + valinomycin (1×10^{-6})		0.10	.66
" + Ca^{+2} (50 μM)		0.30	.66
" + Mn^{+2} (2 mM)		0.07	.51
Aged BHM (24 hours, 0-4°)	20	0.13	--
Aged 24 hours in 100 mM KCl	120	0.26	--

The initial rates of swelling were estimated at 25° for beef heart mitochondria (BHM) (0.5 mg/ml) in a medium of Na+ acetate (100 mM) containing Tris acetate (2 mM, pH 7.2) and sucrose (5 mM) with the additions indicated. K+ content was estimated by atomic absorption spectroscopy. Non-energized preparations contained rotenone (2 μM), antimycin (0.4 ug/mg) and oligomycin (2 ug/mg). CCP is m-chlorocarbonylcyanidephenylhydrazone.

with recent re-evaluations of the limiting value for the $H^+/2e^-$ ratio (15), if one assumes that H+/Na+ exchange is responsible for cation extrusion.

Cation Selectivity Sequence for Contraction - Study of the relative effectiveness of various cations in the contraction reaction is complicated by the fact that prolonged periods of incubation at elevated pH are necessary in order to obtain swelling in nitrate salts other than Na+ or K+ under the conditions of Fig 1. Swelling in $LiNO_3$, for example, requires nearly twice the incubation time of Na+ or K+ to reach the same volume increase. With this complication in mind, a sequence of Na+>Li+>K+ is observed for contraction rates under the conditions of Fig 1. Alternatively, a satisfactory rate of swelling can be obtained in chloride salts of Li+, choline, and tetramethyl ammonium (TMA+) following addition of p-chloromercuribenzoate (CMB, 50 nmoles/mg) at 25°, pH7.2 (16). Mitochondria swollen under these conditions contract in an energy-dependent reaction upon removal of the bulk of the mercurial with dithioerythitol (DTE, 100 nmoles/mg). As shown in Table II, the cation selectivity sequence for the rate of contraction under these conditions is: Na+>Li+>K+>TMA+ or choline+. The rate and extent of

contraction in Na+, Li+, and K+ chloride are stimulated by nigericin under these conditions, as in the nitrate salts (Fig 1). This marked dependence of the contraction reaction on the nature of the accumulated cation is certainly at odds with the statement of Azzone et al (9) that "extrusion is independent of the chemical structure of the cation". Further experiments are in progress in an attempt to identify the basis of this discrepancy. In addition, the observed cation selectivity sequence is identical to that for passive osmotic swelling in the acetate salts of these cations, a reaction which is presumed to depend on the endogenous cation/H+ exchanger (1-6).

Rate of Cation Extrusion as Compared with the Rate of Exchanger-Mediated Cation Uptake - Freshly isolated beef heart mitochondria swell passively at a maximum observed rate of 0.45 A_{546} min^{-1} at 35°C in isotonic Na+ acetate under conditions otherwise identical to those of Table III. These same mitochondria contract at a rate of 0.8 in Na+ nitrate with succinate (conditions as in Fig 1A). Since little passive swelling is observed in K+ acetate (3-5) the discrepancy between the swelling and the contraction rates (which we postulate to depend on the identical exchanger) are even more obvious. However, this rate discrepancy alone does not constitute sufficient reason to reject a common mechanism for the two processes, since it is by no means certain that the turnover of the exchanger is rate limiting in either reaction. Further examination of the factors affecting the rate of passive osmotic swelling in Na+ acetate has revealed that the initial rate is very dependent on the concentration of K+ in the matrix and on the presence of divalent cations (Table III). The reaction is strongly inhibited by valinomycin and by ageing at 0-4° in a K+-free medium, conditions which lead to loss of matrix K+. Ageing under conditions in which there is no loss of matrix K+ results in retention of a high rate of swelling in Na+ acetate. Passive swelling is stimulated to a somewhat variable degree by uncouplers and by Ca^{+2} at low (50 μM) concentrations (Table III) whereas Mn^{+2} strongly inhibits the reaction.

Since mitochondrial K+ is retained under conditions of massive Na+ and acetate accumulation, we conclude that Na+/K+ exchange is not involved in the process, but that matrix K+ may regulate the Na+/H+ exchange in a previously unsuspected manner. The effects of Ca^{+2} and Mn^{+2} also suggest that divalent cations may have a regulatory role in the permeability and the exchange of monovalent cations across the inner membrane (6). It is clear that, until all the factors which contribute to monovalent cation movement can be identified, a direct comparison of rate of passive cation influx with rate of energy-dependent efflux will not be particularly useful in delineating mechanisms.

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