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NIGERICIN-INDUCED TRANSIENT CHANGES IN RAT-LIVER MITOCHONDRIA

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Addition of nigericin to mitochondria oxidizing succinate in a choline- and Tris-supplemented, low-KCl medium leads to a transient matrix acidification, followed by a return of pH_{in} to values very close to pH_{out}. The initial inhibition of stimulated respiration is gradually relieved as pH_{in} returns to higher values. Matrix realkalinization depends on the operation of the H^+ pumps and on the electrogenic influx of cations and efflux of anions. The process leads to replacement of much of the matrix K^+ by other cations. Throughout the acidification/realkalinization cycle $\Delta\tilde{\mu}_H$ variations, if any, are small, even though there are profound changes in the relative contributions of its two components, $\Delta\psi$ and ΔpH.

Introduction

Nigericin is commonly used to catalyze an electroneutral H^+/K^+ exchange across membranes and to modulate the chemical and electrical components, $\Delta\mu_H$ and $\Delta\psi$, of the proton electrochemical gradient, $\Delta\tilde{\mu}_H$ (e.g., Refs. 1–3). Its properties as an ionophore depend on the presence in its structure of a carboxyl group and of several appropriately placed ethereal oxygens. At low concentrations (say, below $1 \cdot 10^{-7}$ M (Ref. 4)), the molecule diffuses across membranes only in an uncharged form, i.e., when the carboxyl group either carries a proton or a monovalent metal cation, which is held by interaction with the oxygens as well as with the carboxylate anion. For steric reasons, K^+ is bound with the highest affinity. Rb^+, Na^+, Cs^+ and Li^+ can also be complexed, with progressively decreasing affinity [5].

Shuttling back and forth across the membrane, nigericin will thus catalyze the transport of M^+ and H^+ in opposite directions if a chemical concentration gradient exists for one or both of the two species. The flows are strictly coupled, in the sense that a given net flow of M^+ in one direction can only occur if an equal and opposite flow of protons also takes place. The driving force for such an exchange is given by the difference of the chemical potential gradients for the species undergoing the exchange, $\Delta\mu_M - \Delta\mu_H$ (cf. Ref. 6). Net flows of ions are thus predicted to stop when the driving force is zero, i.e., when $\Delta\mu_H = \Delta\mu_K$. In biological organelles such as mitochondria, nigericin will therefore catalyze an acidification of the matrix space as long as initially $\Delta\mu_K > \Delta\mu_H$. In low-potassium media, a reversed, i.e., acidic-inside, ΔpH may easily be formed. Apparently, this possibility has not been always given due consideration.

ΔpH decline or inversion is accompanied by an increase of $\Delta\psi$, as the system reacts to maintain $\Delta\tilde{\mu}_H$ at the normal level (e.g., Refs. 7–9). The kinetics of the ΔpH and $\Delta\psi$ changes have however

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Abbreviations: $TPMP^+$, Triphenylmethylphosphonium ion; DMO, 5,5-dimethyl-2,4-oxazolidinedione; Mops, 4-morpholinepropanesulfonic acid; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

received scarce attention. In bacterial chromatophores, Jackson et al. [10] observed that the nigericin-induced variations of H^+ and K^+ concentration persist indefinitely. In erythrocytes, Henderson et al. [5] found that the nigericin-induced pH changes were followed by a slow return to the initial pH. The rate of return was accelerated by replacing choline chloride with LiCl, CsCl or NaCl and the reacidification of the medium was paralleled by an influx of cations into the red cells. In this case, the primary K^+/H^+ exchange catalyzed by nigericin is followed by a secondary H^+/M^+ exchange, whereby the protons taken up by the cells in exchange for endogenous K^+ are re-exported and substituted by other metal ions, possessing a lower affinity for nigericin and therefore transported more slowly. Henderson et al. [5] also reported that in respiration-inhibited mitochondria, the nigericin-induced H^+/K^+ exchange and matrix acidification was followed by a slow realkalinization of the matrix without K^+ influx. The effect was attributed to a slow exchange of matrix anions for external OH^-.

It has been known for a long time that addition of nigericin to mitochondria suspended in a low-K^+ medium results in inhibition of stimulated respiration. The present investigation was prompted by the observation of the transient nature of this respiratory inhibition. It will be shown that the acidification of the matrix of respiring mitochondria is transient, and it is accompanied by a transient rise of $\Delta\psi$, while the subsequent decline of $\Delta\psi$ and H^+ efflux from the matrix are paralleled by proton-pump-driven cation influx and anion efflux.

Materials and Methods

Rat-liver mitochondria were prepared by a standard procedure [11] and protein concentration was determined by the biuret method. Ionophores, inhibitors of mitochondrial functions and adenine nucleotides were obtained from Sigma or Boehringer.

pH variations were measured using a Beckman glass electrode connected to a pHM 26 pH meter and to a Texas Instruments chart recorder. Calibration by addition of known amounts of HCl permitted the calculation of the amounts of protons taken up or released by mitochondria incubated in weakly buffered media (see legends to figures). Potassium concentration levels were followed using a Schott K^+-sensitive electrode. Respiration was monitored with a Clark electrode (Yellow Springs) in a close thermostatted vessel with magnetic stirring.

Determination of $\Delta\psi$ and ΔpH. The transmembrane electrical field, $\Delta\psi$, was evaluated from the distribution of the lipophilic ion triphenylmethylphosphonium ($TPMP^+$) (e.g., Ref. 6 and references therein). The concentration of $TPMP^+$ in the incubation medium was continuously followed using a $TPMP^+$-sensitive membrane electrode, described elsewhere [12]. The response time of the electrode varied depending on the individual membranes used, a typical time for half response being 10–20 s. Monitoring the medium $TPMP^+$ concentration allows one to calculate the amount of probe taken up by the mitochondria at any given time, and hence, knowing the matrix volume and neglecting binding (see below), the matrix $TPMP^+$ concentration. During transients, ΔpH at any given time was calculated from the extent of proton uptake or release, from measurements of initial (steady state) ΔpH and from an estimate of the matrix-buffering power, mentioned in Results.

Under steady-state conditions, (State 4 or after the end of the nigericin-induced fluxes), $\Delta\psi$ was determined from the accumulation of the radioactively labeled ion, [^{14}C]$TPMP^+$, and ΔpH from the distribution of a weak acid, [^{14}C]DMO. The procedures used were as described elsewhere [13], with the following modifications. [^{3}H]Glycerol (approx. 0.5 μCi/ml) and 5 mM glycerol were included in the medium in order to obtain a measure of the total pellet volume after centrifugation (see Ref. 6). In the presence of unlabeled glycerol, no metabolization or accumulation of the labeled material was detectable. Mitochondria (4–6 mg/ml) were incubated directly in 5-ml plastic liquid-scintillation vials (Packard), which were centrifuged in an adapted SE-12 rotor in a Sorvall RC-5B centrifuge at 10×10^3 rpm for 6 min. After the supernatant had been removed and the pellet dissolved in 1% sodium deoxycholate, scintillation fluid (Insta Gel, Packard) was added directly into the vials. The amounts of protein in the pellet and in the supernatant were determined for each ex-

periment in parallel samples, and calculations were based on the pellet protein. Since the binding of $TPMP^+$ to mitochondria has not yet been assessed in a satisfactory way [6], no correction for it has been introduced in the calculation of $\Delta\psi$.

A weak base is theoretically the probe of choice for measuring inverted (acid-inside) ΔpH. However, we have found that methylamine, ethylamine and triethylamine are apparently accumulated even when a large basic-inside ΔpH exists across the mitochondrial membrane. This apparent uptake is due in part to binding and in part to accumulation into contaminating lysosomes. In view of the difficulty of introducing a suitable correction for methylamine binding or accumulation, the weak acid DMO has been used for measurements of ΔpH close to zero.

Measurement of mitochondrial matrix volume. The classical method, based on the use of a permeant and an impermeant marker (e.g., Ref. 6, and references therein) was used. Mitochondria were separated by centrifugation as described above for $\Delta\psi$ and ΔpH determinations. [^{3}H]Glycerol was used as the permeant marker. $^{45}Ca^{2+}$ was used as the impermeant marker. The incubation medium was supplemented with 2 mM EGTA and EDTA, and 1 nmol/mg Ruthenium red in order to prevent Ca^{2+} uptake by the mitochondria. $^{45}Ca^{2+}$ was added immediately before centrifugation. Control experiments have shown that the accumulation of $^{45}Ca^{2+}$ in Ruthenium red- and EGTA-treated mitochondria is negligible over a time period of several minutes.

Results and Discussion

Fig. 1 (solid traces) shows that nigericin had an inhibitory effect on uncoupler-stimulated respiration by mitochondria suspended in a low-K^+ medium. Inhibition was not immediate, requiring instead a few seconds. The curvature of the oxygraph recordings indicates that the inhibition was transient: the rate of respiration continued to increase, approaching the rate induced by FCCP in the absence of nigericin after several minutes (not shown). In Fig. 1 (left), the dashed lines show the behavior of respiration observed in separate experiments, in which FCCP was added at the indicated, variable times after nigericin. The effect of

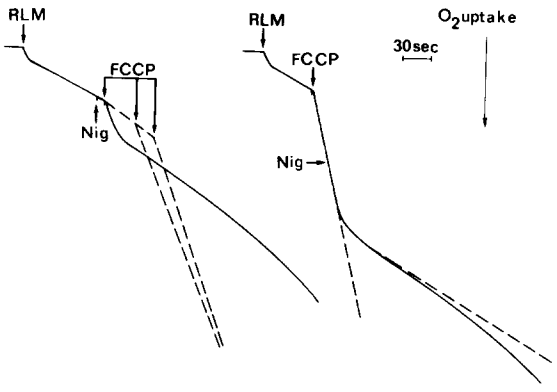

Fig. 1. Uncoupler-stimulated respiration in nigericin-treated mitochondria. Medium composition: 0.18 M sucrose/10 mM Tris-Mops/10 mM choline chloride/5 mM succinate-Tris/1 mM P_i/0.5 mM EGTA-Tris/2 μM rotenone/0.1 mM KCl. pH, 7.4, *T*, 25°C; rat-liver mitochondria (RLM), 1 mg protein/ml. Where indicated: nigericin, 13.8 pmol/mg; FCCP, 500 pmol/mg. Similar traces were obtained when respiration was stimulated by ADP. Dashed lines in the left part refer to separate experiments in which FCCP was added at variable times after nigericin. In the right part, they serve only to facilitate appreciation of rate variations. Nig, nigericin.

FCCP clearly depends on the time elapsed after the administration of nigericin to mitochondria. If FCCP is added about 1 min after nigericin, respiration is immediately stimulated to values close to those of a control without nigericin. The behavior observed when respiration was stimulated by ADP/phosphate rather than by an uncoupler was identical, except that respiration recovered from nigericin-induced inhibition more rapidly (i.e., the curvature of the solid lines was more pronounced).

The phenomenon of nigericin-induced inhibition of stimulated respiration by mitochondria suspended in a low-potassium medium, known since 1958 [14], has generally been attributed to the efflux of phosphate and respiratory substrates following matrix acidification. We have performed a number of experiments (not shown) which confirm the link between matrix acidification and respiratory inhibition. Nigericin induced no respiratory inhibition when electrons were fed into the respiratory chain directly from the cytosolid side, utilizing ascorbate and TMPD as redox mediators. When the nigericin-catalyzed H^+/K^+ exchange occurred in the presence of methylamine, which

decreased the extent of matrix acidification, the extent of inhibition was reduced and, at high (50 mM) concentrations of methylamine, almost abolished. Finally, the inhibition decreased as the medium potassium concentration increased, i.e., as matrix acidification decreased.

Given the link between respiratory inhibition and matrix acidification, the behavior seen in Fig. 1 suggests that the nigericin-induced pH variations are also transient. This is confirmed by the observations presented in Fig. 2, which shows the kinetics of the H^+ and K^+ fluxes occurring after addition of nigericin to respiring mitochondria suspended in a weakly buffered low-K^+ medium containing succinate, choline and Tris. The medium K^+ concentration increased monotonically to a certain level and then remained constant. In contrast, the H^+ concentration decreased rapidly reaching a negative peak in a few seconds. Extensive reacidification of the medium then ensued, i.e., most of the protons that had entered the mitochondrial matrix in eachange for K^+ moved back to the outer phase. During the process of matrix realkalinization, the K^+ efflux continued. Qualitatively similar results were obtained with phosphorylating or uncoupled mitochondria. In

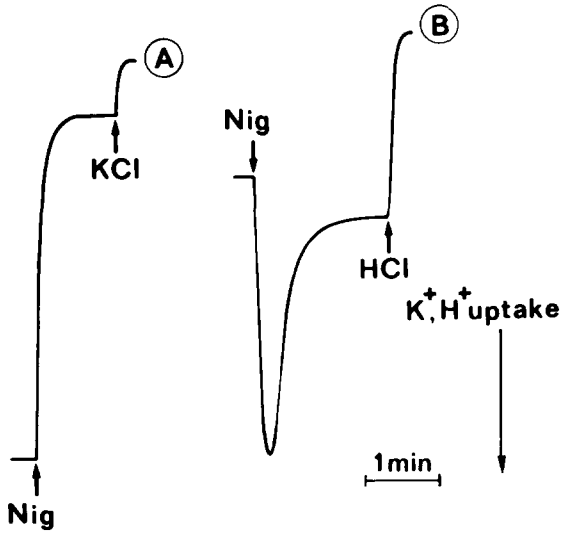

Fig. 2. Nigericin-induced K^+ and H^+ fluxes. Medium composition as in Fig. 1, except that Tris-Mops and P_i were omitted and sucrose was 0.2 M. *T*, 25°C; rat-liver mitochondria, 6 mg protein/ml. Where indicated: nigericin, 13.8 pmol/mg; HCl, 25.8 nmol/ml; KCl, 100 nmol/ml.

the presence of uncoupler, however, the rate of matrix realkalinization was much slower (not shown).

The observations of Fig. 1 thus find an explanation: the time required for the onset of inhibition must be related to the time needed for the H^+/K^+ exchange to proceed until causing a pronounced decrease of the matrix pH. Recovery of respiration is determined by the return of this parameter to higher values, a process which occurs more rapidly in the absence than in the presence of uncouplers. If respiration is stimulated at different times after the addition of nigericin, the extent of stimulation varies following the acidification/realkalinization cycle.

The quantitative aspects of the H^+/K^+ exchange were as follows: (i) the H^+/K^+ ratio, based on the amounts of H^+ and K^+ transported at the peak of the medium alkalinization phase, was always lower than 1 (approx. 0.65 on the average); (ii) depletion of endogenous phosphate (by incubation in isotonic KCl and washing) led to an increase of the H^+/K^+ ratio to about 0.85, indicating that a fast phosphate efflux is largely responsible for the low H^+/K^+ stoichiometry observed; (iii) the extent of H^+ influx into the mitochondria, measured as in (i), and the rate of matrix realkalinization decreased with the increase of the K^+ concentration in the medium in the range 0.1–3 mM. However, (iv) the total amount of K^+ lost by the mitochondria, 55–60 nmol/mg protein, was independent, within experimental error, of the K^+ concentration in the same range. This behavior will be discussed below. Notice also that the medium pH did not quite return to the initial values.

The phase of H^+ efflux from the mitochondria may be due to several different processes, namely H^+/cation or OH^-/anion exchange, involving or not the redox H^+ pumps. A simple reversal of the nigericin-mediated H^+/K^+ exchanges can be ruled out, since potassium efflux continued during matrix realkalinization. An H^+/M^+ exchange of the type first described by Henderson et al. [5] can also be excluded, since the realkalinization occurs also in media containing no metallic cations other than K^+ (Na^+ contamination below 20 μM).

Carrier-mediated electroneutral exchange processes in general should occur equally well in

the presence or absence of respiration if an appropriate driving force exists. However, Fig. 3 shows that H^+ extrusion (matrix realkalinization) was strongly inhibited when respiration was restricted by omission of substrates and by respiratory inhibitors (compare traces A and B). The behavior of nonrespiring mitochondria is strongly reminiscent of that reported by Henderson et al. [5]. Furthermore, addition of antimycin after the process of H^+ extrusion had already begun led to an immediate inhibition of medium acidification (trace C). Inhibition of the H^+ pumps affected both H^+ and K^+ fluxes: in contrast to the results with respiring mitochondria, the total amount of K^+ lost decreased with the increase of the medium K^+ concentration, going from 45 nmol/mg protein at 0.1 mM K^+ to 32 nmol/mg protein at 3 mM K^+. Thus, depletion of mitochondrial K^+ after addition of nigericin is more complete in the case of respiring rather than nonrespiring mitochondria.

This is easily explained if one consideres that the thermodynamic equilibrium condition in the presence of nigericin is $\Delta\mu_H = \Delta\mu_K$. In nonrespiring mitochondria, attainment of this condition requires an extent of H^+/K^+ exchange which decreases as $|K^+|_{out}$ increases. In respiring mitochondria, however, when matrix realkalinization occurs, the driving force for a nigericin-mediated H^+/K^+ exchange is reconstituted, and K^+ efflux continues, through a succession of states where the equality $\Delta\mu_H = \Delta\mu_K$ holds approximately, until, in the end, $\Delta\mu_H = \Delta\mu_K = 0$. Thus, at low $|K^+|_{out}$, virtually all the mitochondrial potassium is lost. This will of course happen whatever the mechanism of matrix realkalinization.

If the extruded protons are expelled by the redox H^+ pumps, the question arises as to the ionic species moving to maintain macroscopic electroneutrality. An uncompensated electrogenic H^+ extrusion would in fact be expected to cause a large increase of the transmembrane electrical field, $\Delta\psi$. However, it will be shown below (Fig. 6) that $\Delta\psi$ decreases during matrix realkalinization. The electrogenic movement of protons from the matrix to the outer phase must therefore be compensated either by an influx of cations or by an outflow of anions. Besides K^+, essentially the only cations present in our usual media were choline and Tris. Fig. 4 shows that their replacement by sucrose resulted in a marked depression of the H^+ extrusion. The extent of H^+ efflux as calculated with

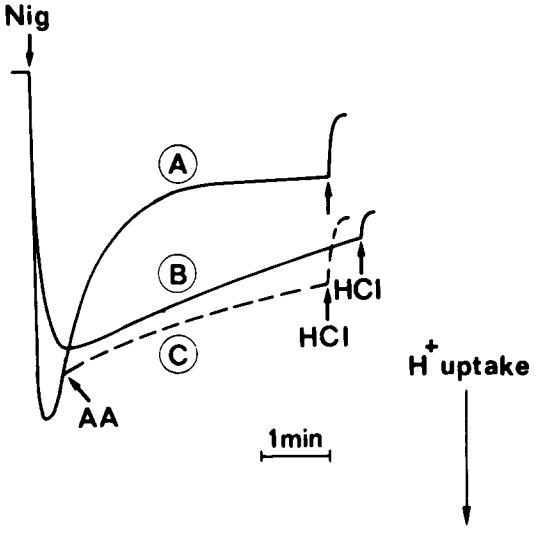

Fig. 3. The effect of respiratory inhibitors on nigericin-induced H^+ fluxes. Medium composition: traces A and C: as in Fig. 2; trace B: succinate omitted, +10 mM Tris. *T*, 25°C; rat-liver mitochondria, 6 mg/ml. Addition for trace B and where shown: antimycin A, 0.5 μg/mg. Where indicated: nigericin, 13.8 pmol/mg; HCl, 25.8 nmol/ml.

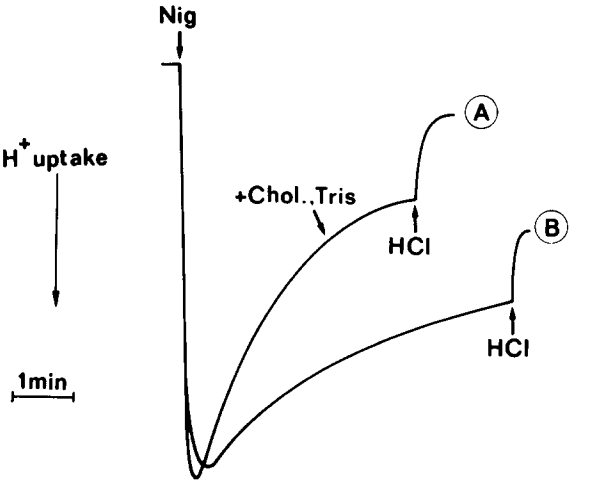

Fig. 4. The effect of choline and Tris on nigericin-induced H^+ fluxes. Medium composition: 0.22 M sucrose, 2 mM succinate/K^+, 0.5 mM EDTA/K^+, 2 μM rotenone. In trace A: +10 mM choline chloride, 5 mM Tris-HCl. *T*, 25°C; rat-liver mitochondria, 6 mg/ml. Where indicated: nigericin 13.8 pmol/mg; HCl, 25.8 nmol/ml.

respect to the H^+ influx following the addition of nigericin, 2 min after the latter, decreased from about 53 to about 21% in the experiment shown (with approx. 6 mM K^+). Qualitatively, the same effect was obtained with choline or Tris alone. The latter was however less effective on a molar basis. It thus appears that H^+ extrusion is accompanied by the influx of choline and/or Tris cations.

That an influx of osmotically active species occurs is confirmed by the measured variations of matrix volume. The matrix volume of State 4 mitochondria was, under the prevailing experimental conditions, 0.9 μl/mg protein. The nigericin-induced K^+ efflux was accompanied by a shrinkage of the matrix space, with a decrease to about 0.4 μl/mg protein. The subsequent phase of H^+ efflux was accompanied by an increase of the matrix volume from 0.4 to 0.8 μl/mg protein after 5 min. Abolition of H^+ extrusion by antimycin and depression of it by omission of Tris and choline were accompanied by a marked inhibition of the increase in matrix volume after the initial shrinkage. Thus, it appears that the addition of nigericin to respiring mitochondria in a medium containing choline and/or Tris results in a replacement of mitochondrial K^+ by these ions.

That nigericin induces an uptake of choline by energized mitochondria is directly shown by the experiment utilizing [^{14}C]choline presented in Table I. Notice that choline is found in the mitochondrial matrix even in the absence of nigericin. Indeed choline is well-known to permeate the mitochondrial membrane, entering the matrix where it can be oxidized by choline dehydrogenase (e.g., Refs. 15 and 16). This permeation has been proposed to occur in exchange for an H^+ expelled by the pumps [17]. Choline oxidation is enhanced by uncouplers [16,18], indicating that the ion can cross the membrane under low $\Delta\psi$ conditions, even though at a lower rate [17]. A decrease in $\Delta\psi$, the driving force, is expected to lower the rate of all electrogenic ion movements. This explains the behavior observed in the presence of uncouplers: matrix realkalinization still occurs, but at a slower rate than with coupled mitochondria. Since Tris has effects similar to those of choline, it may be concluded that it too must be able to permeate, at least in the presence of a high $\Delta\psi$ (see below). The fact that energized mitochondria show negligible swelling in choline- or Tris-containing media then suggests the existence of nonspecific H^+/cation exchange systems which prevent attainment of an electrochemical equilibrium distribution for these ions.

TABLE I

NIGERICIN-INDUCED [^{14}C]CHOLINE UPTAKE BY MITOCHONDRIA

Medium composition as in Fig. 2, plus 5 mM glycerol, [^{3}H]glycerol (1 μCi/ml) and [^{14}C]choline (0.3 μCi/ml). Nigericin (13.8 pmol/mg) was added to mitochondria (4 mg/ml) after 2 min of incubation. The suspensions, and controls without nigericin, were then centrifuged at the indicated times. Matrix volumes were determined in a parallel experiment. Amounts of choline in the matrix were calculated from the radioactivity found in the pellet and the known specific activity of choline, after correction for the intermitochondrial space in the pellet. (Values are averages of duplicate measurements.) Nig, nigericin.

Time of incubation (min)		nmol choline/mg protein	
initial	+ Nig	+ Nig	− Nig
2	–	–	29.8
2.5	0.5	33.4	31.3
3	1	34.9	31.6
6	4	42.2	32.0
8	6	44.7	33.2

Fig. 4 also shows that matrix realkalinization still occurred even in the absence of any permeant cations (trace B), even though it was slower and more limited in extent. Another mechanism must therefore be operating as well, presumably involving an electrogenic expulsion of anions accompanying the pumped protons. Given that phosphate is lost largely during the initial, fast, matrix acidification phase, the most likely candidate for the role of electrogenically expelled anion is succinate, the respiratory substrate used in these experiments. Alternatively, an electroneutral exchange of matrix succinate for phosphate lost from the matrix during the initial acidification phase may occur. Such an exchange would have no effect on either ΔpH or $\Delta\psi$, but it might be followed by the electrogenic expulsion of the phosphate thus reintroduced into the matrix. The electrical movement of phosphate anions across mitochondrial membranes has been observed and identified to take place via the phosphate carrier [19]. To our knowledge, there is no

report in the literature of an electrophoretic movement of succinate across the mitochondrial membrane. However, it might conceivably occur via the dicarboxylate or phosphate carriers, which would act in an unusual manner because of the high electrical field (see below).

An indication that succinate may well be involved comes from the experiments shown in Fig. 5. Here, succinate as substrate was replaced by the ascorbate/TMPD couple, which allows electrons to enter the respiratory chain at the level of cytochrome *c*. Mitochondria can therefore pump protons through Complex IV [20] and maintain a $\Delta\psi$ of about 150 mV (not shown). The respiratory chain above Site III is blocked by antimycin. The disadvantage of this replacement is that oxidation of ascorbate results in a continuous medium pH drift, which masks the underlying H^+ fluxes. In spite of this complication, it is easily seen from Fig. 5 (compare traces A and C) that the presence of succinate resulted in a considerable enhancement of the process of H^+ extrusion. The effect of 5 mM succinate was comparable to that produced by 10 mM LiCl (trace B).

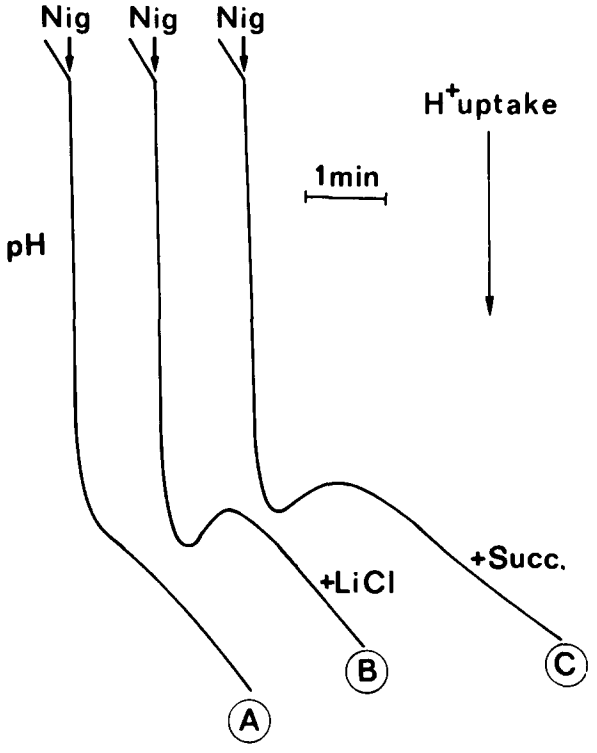

Fig. 5. The effects of succinate and LiCl on matrix realkalinization. Medium composition: 0.2 M sucrose, 10 mM choline chloride, 0.5 mM EDTA-Tris, 5 mM Tris-HCl, 2 μM rotenone, 2 mM ascorbic acid, 50 μM TMPD, 0.5 μg/mg protein antimycin A. In trace B: +10 mM LiCl; Trace C: −Tris-HCl, +5 mM succinate-Tris. *T*, 25°C; rat-liver mitochondria, 6 mg/ml. Where indicated: nigericin (Nig), 13.8 pmol/mg.

Therefore, both cation influx and succinate efflux apparently take place. It follows that the nigericin-induced inhibition of respiration must be at least partially due to a direct modulation of the activities of the enzymes of the respiratory chain by the matrix pH, and not only to substrate depletion. In fact, there is a recovery of the rate of respiration even though the matrix substrate concentration presumably declines, at least initially, due to succinate efflux.

An important question related to the acidification of the mitochondrial matrix concerns the extent of conversion of ΔpH into $\Delta\psi$ and the maximal dimension of $\Delta\psi$ in nigericin-treated mitochondria. Due to the transient nature of this phenomenon, an analysis is possible only by assessing these parameters by suitable electrodes ($TPMP^+$ and pH). The quantitative analysis of the traces is easily done in the case of $\Delta\psi$. In the case of ΔpH, the calculation depends on the assessment of the matrix-buffering power. Mitchell and Moyle [21] reported a matrix buffering power of about 15 nmol H^+/mg protein per pH unit in the pH range between 7 and 8, with an increase to about 30 nmol H^+/mg protein per pH unit between pH 7 and 6. In the higher pH interval, we obtained similar estimates by comparing H^+ movement data and ΔpH determinations by weak acid (DMO) distribution measurements [22]. We therefore used an average value of 20 nmol H^+/mg protein per pH unit to calculate transient ΔpH values.

In the experiment of Fig. 6, State 4 mitochondria maintained a $\Delta\psi$ of 191 mV and a ΔpH of 22 mV (basic-inside; by DMO distribution). Addition of nigericin caused a considerable uptake of both $TPMP^+$ ($\Delta\psi$ increase) and protons (ΔpH decrease and inversion). Apparently, the two processes were not exactly in phase. However, this is due to the slower time-response of the $TPMP^+$ electrode. The negative peak of the pH trace corresponds to a $\Delta\Delta$pH of about 57 mV (ΔpH = approx. 35 mV, acid-inside). The peak of the $TPMP^+$ trace is

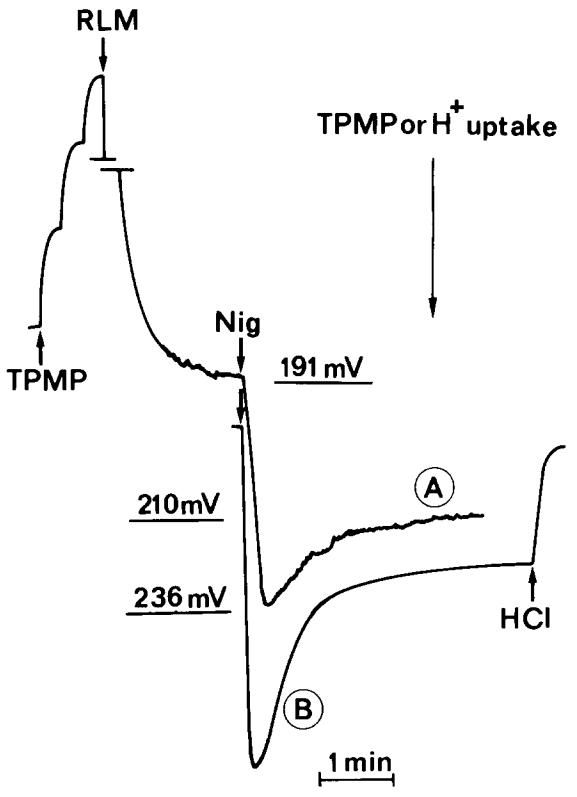

Fig. 6. The nigericin-induced ΔpH-$\Delta\psi$ interconversions. (A) $TPMP^+$ trace. (B) pH trace. Medium composition as in Fig. 2. Rat-liver mitochondria (RLM), 6 mg/ml. Final $TPMP^+$ concentration was 60 μM; only the last three additions (10 μM each) are shown for clarity. Essentially identical results were obtained using 1 mg/ml rat-liver mitochondria and 5 μM $TPMP^+$ to follow $\Delta\psi$. Nigericin, 13.8 pmol/mg; HCl, 40 nmol/ml.

reached somewhat later; at that moment a $\Delta\psi$ of 236 mV and a ΔpH of 25 mV (acid-inside) may be calculated from the traces. $\Delta\psi$ decline and matrix realkalinization then proceed together, reaching eventually a value of 210 mV for $\Delta\psi$ and about zero for ΔpH.

The discrepancy in the response times of the pH and $TPMP^+$ electrodes hampers the direct comparison of ΔpH and $\Delta\psi$ values in the initial part of the transient. It appears however that at least after the $TPMP^+$ trace peak, the variations of the two parameters mirror each other, decline of one corresponding to the increase of the other. The quantitative agreement was relatively good, considering the assumptions and approximations made, in all experiments performed. After the point of maximal recorded $\Delta\psi$, calculated $\Delta\Delta\psi$ and $\Delta\Delta$pH values never differed by more than 15 mV, even though transient $\Delta\Delta$pH values as large as 90 mV were obtained at low K^+ concentrations. While this agreement may not be significant in view of the methods used, greater significance may be attached to steady-state measurements performed before and after the transient, utilizing radioactive distribution probes (Table II). $\Delta\tilde{\mu}_H$ is approximately the same in these two steady states, the increase of $\Delta\psi$ corresponding to the size of the State 4 ΔpH, which essentially disappears. We may therefore conclude that if any large $\Delta\tilde{\mu}_H$ variations occur during the nigericin-induced transient, they are short-lived.

TABLE II

$\Delta\psi$ AND ΔpH IN STATE 4 MITOCHONDRIA BEFORE AND AFTER THE NIGERICIN-INDUCED TRANSIENT

Medium composition as in Fig. 2. $\Delta\psi$ and ΔpH were measured from the distribution of [^{14}C]$TPMP^+$ and [^{14}C]DMO in State 4 and 6 min after the addition of 13.8 pmol/mg of nigericin.

	ΔpH (mV)	$\Delta\psi$ (mV)	$\Delta\tilde{\mu}_H$ (mV)
State 4	22	190	212
+ Nig (after 6 min)	4	207	211

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