Potassium-specific Uncoupling by Nigericin*

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SUMMARY

The uncoupling activity of nigericin in rat liver mitochondria was found to be proportional to the external concentration of potassium. Rubidium (40% as effective as potassium) was the only other alkali metal cation that permitted significant uncoupling. The ability of nigericin selectively to inhibit (at low concentrations) and to uncouple (at high concentrations) only certain substrates was investigated with particular reference to glutamate and malate. The rate-limiting reaction for glutamate oxidation in the presence of nigericin was substrate level phosphorylation, not glutamate uptake. Nigericin was compared with other uncouplers with respect to induced ion movements and relative effectiveness in overcoming respiratory inhibition by guanidine compounds. Nigericin potentiated rather than reversed the guanidine inhibition. Possible mechanisms for the involvement of the potassium complex of nigericin in the uncoupling process are discussed.

Nigericin uncouples oxidative phosphorylation in rat liver mitochondria when present at concentrations 10-fold greater than those required to inhibit respiration and ATPase (1-4). Striking features of this uncoupling activity are its requirement for high external potassium and its apparent selectivity for certain substrates. In view of interpreting the relationship between the inhibition and uncoupling processes, it is notable that certain compounds sharing nigericin’s inhibitory effects at low concentrations, such as dianemycin and monensin (2, 4-6), do not induce ATPase or respiratory uncoupling at higher levels.

This paper reports further characterization of the uncoupling activity of nigericin and relates this information to current theories of the mechanism of coupling and our present knowledge of the structure of nigericin and its analogues. The K⁺ complex of nigericin is implicated in the uncoupling process, and two alternative mechanisms for its involvement in one-way charge transfer are discussed.

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METHODS

Mitochondria were prepared (7) from liver of male rats (150 to 200 g) by homogenizing in 250 mM mannitol, 70 mM sucrose, and 1 mM EDTA. Two subsequent washes and final suspension of the mitochondria were done in the same medium without EDTA.

Changes in the oxidation-reduction state of intramitochondrial pyridine nucleotides were monitored at 340 - 374 nm with an Amino-Chance dual wave length spectrophotometer. Oxygen uptake was measured simultaneously by means of a vibrating platinum electrode inserted in the same cuvette. Respiration and phosphorylation were also measured manometrically (8). ATPase was assayed by the method of Lardy and Wellman (9).

Simultaneous measurements of K⁺, H⁺, O₂, and light-scattering were carried out using an apparatus described by Pressman (10, 11).

Glutamate, malate, and α-ketoglutarate were assayed enzymatically (12), after complex were deproteinized with an equal volume of 0.6 n perchloric acid and neutralized with KOH. Conditions of incubation before assays were performed are given in the legends of the figures. Enzymes used in assays were obtained from Boehringer Mannheim. Proteins were measured by the biuret procedure.

RESULTS

Potassium Requirement—Fig. 1 illustrates the potassium dependence of uncoupling by high concentrations of nigericin. As a diagnostic test for classical uncoupling, oxidative phosphorylation supported by β-hydroxybutyrate was inhibited by aurovertin, and the degree of restoration of oxygen consumption was measured in the presence of nigericin and increasing amounts of KCl. Maximal stimulation of respiration was achieved with 80 mM KCl. This experiment was repeated with oligomycin as the inhibitor and with different monovalent cations (at 45 mM) (Fig. 2). Per cent restoration of oxygen consumption was plotted as a function of the ionic radius of the different alkali metal cations. Rubidium was found to be the only other alkali metal capable of supporting the uncoupling function of nigericin, and it was just 40% as effective as potassium. A similar cation specificity has been reported for nigericin-induced ATPase (2, 5) and for nigericin effects on artificial membranes, red blood cells (4, 5, 13, 14), and chloroplasts (15).

Substrate Specificity—A striking feature of nigericin activity at both inhibitory and uncoupling concentrations is its discriminatory behavior toward substrates (1, 4, 5, 16, 17). At high
Both of these effects of nigericin and prevents the anion loss sively (3, 19-22, 25). However, high external potassium reverses influx in controlling anion movements has been debated extensively (3, 19-22, 25). In addition, it has been shown by Azzone and Calderon (33) that the structural organization of the mitochondrion might be the basis of discrimination, since some dehydrogenases were contained within the matrix space, whereas others (succinic, beta-hydroxybutyric, proline) were a part of the inner membrane (26) and therefore accessible to their substrates without transport into the matrix. However, if this were the case, oxidation by these enzymes should be relatively insensitive to agents that block substrate penetration across the inner membrane. On the contrary, studies by Robinson (30) show that malate and succinate oxidation are equally affected by butylmalonate, a compound which has been shown to inhibit the dicarboxylic acid exchange system (31, 32).

Some impairment of permeability to anions apparently still exists under conditions favorable for nigericin uncoupling. However, a lower lowering of anion permeability does not provide sufficient basis to explain the particular sensitivity of certain substrates. It was suggested by Lardy and Ferguson (29) that the structural organization of the mitochondrion might be the basis of discrimination, since some dehydrogenases were contained within the matrix space, whereas others (succinic, beta-hydroxybutyric, proline) were a part of the inner membrane (26) and therefore accessible to their substrates without transport into the matrix. However, if this were the case, oxidation by these enzymes should be relatively insensitive to agents that block substrate penetration across the inner membrane. On the contrary, studies by Robinson (30) show that malate and succinate oxidation are equally affected by butylmalonate, a compound which has been shown to inhibit the dicarboxylic acid exchange system (31, 32).

An alternative explanation for substrate specificity was suggested by Henderson, McGivan, and Chappell (4, 5), implicating phosphate deprivation as the discriminating factor. Evidence consistent with this view was obtained by Estrada-O. and Calderon (33). In addition, it has been shown by Azzone and Earnest (34) that lack of PI in the medium of uncoupled mitochondria causes inhibition of respiration with the same substrate specificity as nigericin. This effect was attributed to the requirement for phosphate in substrate level phosphorylation (35, 36). A preferential loss of phosphate over other anions in the presence of nigericin might be expected from the results of Palmieri, Quagliariello, and Klingenberg (21), who demonstrated that the phosphate uptake system is the most sensitive of the anion exchange system (31, 32).

The abbreviations used are: TTFB, tetrachlorotrifluoro-
methylbenzimidazole; PN, diphospho- and triphosphopyridine nucleotides; TEA, triethanolamine.
TABLE I

**Substrate specificity of uncoupling of oxidative phosphorylation induced by nigericin in mitochondria**

The reaction mixture contained: 2 mM ATP; 13 mM phosphate-TEA buffer, pH 7.4; 3 mM MgCl₂; 15 mM KCl; 140 mM sucrose; and 2.2 mg of nitrogen in 0.5 ml of 0.25 M sucrose. Hexokinase and 50 μmoles of glucose were tipped from the side arm at zero time. All substrates were added to give concentrations of 10 mM. When l-malate was present with glutamate it was 5 mM. Final volume, 3 ml; temperature, 30°. ΔQₒ₂(N) represents the change in microliters of O₂ per min per mg of nitrogen.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Glutamate + malate</th>
<th>β-hydroxybutyrate</th>
<th>Glutamate</th>
<th>Malate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔQₒ₂(N)</td>
<td>P:O Ratio</td>
<td>ΔQₒ₂(N)</td>
<td>P:O Ratio</td>
</tr>
<tr>
<td>None</td>
<td>238</td>
<td>2.8</td>
<td>162</td>
<td>1.8</td>
</tr>
<tr>
<td>Nigericin (1.4 × 10⁻⁵ M)</td>
<td>207</td>
<td>0</td>
<td>156</td>
<td>0</td>
</tr>
<tr>
<td>Oligomycin (2 μg per ml)</td>
<td>31</td>
<td>0</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Atractylate (5 μg per ml)</td>
<td>41</td>
<td>0</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Oligomycin + nigericin</td>
<td>221</td>
<td>0</td>
<td>162</td>
<td>0</td>
</tr>
<tr>
<td>Atractylate + nigericin</td>
<td>228</td>
<td>0</td>
<td>154</td>
<td>0</td>
</tr>
</tbody>
</table>

*Fig. 3. The effect of nigericin on mitochondrial oxygen consumption and pyridine nucleotide reduction with succinate and glutamate as substrates. The reaction mixture contained 80 mM KCl, 20 mM Tris-Cl, 7.2 mg of mitochondrial protein, in a total volume of 3.2 ml at pH 7.3 and 20°. Nigericin was added to give 1.2 × 10⁻⁴ M. The numbers on the oxygen tracing indicate the rates of oxygen consumption in nmoles of O₂ per min. BSA, bovine serum albumin.*

*Fig. 4. Legend is the same as Fig. 3, except that the amount of mitochondrial protein was 5 mg.*

*Fig. 5. Nigericin uncoupling of glutamate and malate oxidation. Reaction mixture contained 80 mM KCl, 20 mM TEA-Cl, and 5 mg of mitochondrial protein in a total volume of 3.2 ml at pH 7.3 and 20°.*

Translocations to inhibition by lowering of the pH gradient across the mitochondrial membrane. From this evidence, it is plausible to suggest that phosphate supply may become rate-limiting for those substrates whose oxidation pathways include substrate level phosphorylation, primarily or secondarily. However, it does not explain inhibition of malate oxidation, or glutamate-malate synergism. The oxidation of these two substrates was therefore examined.

It is known that cysteine sulfinic acid transaminates with oxalacetate to give aspartate and sulfopyruvate (37), neither of which are further oxidized. We therefore decided to test the ability of cysteine sulfinate to overcome the inhibition by nigericin of malate oxidation, as an indication of the importance of the transaminating function alone. Fig. 6 shows that cysteine sulfinate is equally as effective as glutamate in stimulating malate oxidation in the presence of low concentrations of nigericin (Fig. 6a) and only slightly less effective under uncoupling conditions (Fig. 6b). In the latter case, glutamate oxidation by the glutamic dehydrogenase pathway may be contributing to the observed rate (38). From this evidence it is concluded that glutamate-malate synergism in the presence of nigericin is mainly the result of increased malate oxidation, and that glutamate oxidation remains inhibited. This would be expected if the block by nigericin is at the level of the phosphate-requiring suc-
Fig. 6. Comparison of ability of cysteine sulfinate and glutamate to stimulate malate oxidation. a, inhibited by nigericin. Reaction medium contained 15 mM KCl, 100 mM sucrose, 10 mM TEA-Cl (pH 7.4), 5 mM TEA-phosphate, 3 mM MgCl₂, and 3.3 mM TEA-malate. Protein (4 mg) was present in a total volume of 3.2 ml. Respiration was induced by 10⁻⁶ M monazomycin and inhibited by 7 × 10⁻⁸ M nigericin. Initial rates of oxygen consumption caused by subsequent addition of glutamate or cysteine sulfinate were plotted after correction for the inhibited rate with malate alone. ○, glutamate; ■, cysteine sulfinate. b, uncoupled by nigericin. Reaction medium contained 50 mM KCl, 100 mM sucrose, 10 mM TEA-Cl (pH 7.4), 5 mM TEA-phosphate, 3 mM MgCl₂, and 3.3 mM TEA-malate. Protein (5.1 mg) was present in a total volume of 3.1 ml. Respiration was induced by 10⁻⁶ M monazomycin and inhibited by 7 × 10⁻⁸ M nigericin. Initial rates of oxygen consumption caused by subsequent additions of glutamate or cysteine sulfinate were plotted. Temperature was 20°C.

**TABLE II**

<table>
<thead>
<tr>
<th>Experiment and substrate</th>
<th>Respiration inducer</th>
<th>Respiration inhibitor</th>
<th>KCl Concentration</th>
<th>Pi Concentration</th>
<th>Malate used</th>
<th>Glutamate used</th>
<th>α-Ketoglutarate produced</th>
<th>Glutamate × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Glutamate + malate</td>
<td>Monazomycin (1.3 × 10⁻⁴ M)</td>
<td>None</td>
<td>50</td>
<td>5</td>
<td>50</td>
<td>54</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Monazomycin (1.3 × 10⁻⁴ M)</td>
<td>Nigericin (7 × 10⁻⁴ M)</td>
<td>15</td>
<td>5</td>
<td>07</td>
<td>48</td>
<td>40</td>
<td>83</td>
</tr>
<tr>
<td>2 Glutamate + malate</td>
<td>Monazomycin (1.3 × 10⁻⁴ M)</td>
<td>None</td>
<td>50</td>
<td>3.3</td>
<td>38</td>
<td>34</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>TTFB (7 × 10⁻⁷ M)</td>
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<td>50</td>
<td>3.3</td>
<td>38</td>
<td>30</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Nigericin (1.1 × 10⁻⁴ M)</td>
<td>None</td>
<td>50</td>
<td>3.3</td>
<td>38</td>
<td>33</td>
<td>30</td>
<td>89</td>
</tr>
<tr>
<td>3 Glutamate</td>
<td>Salicylanilide (2.7 × 10⁻³ M)</td>
<td>Sucrose (200 mM)</td>
<td>0</td>
<td>5</td>
<td>18</td>
<td>8</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salicylanilide (2.7 × 10⁻³ M)</td>
<td>Glucose (200 mM)</td>
<td>0</td>
<td>5</td>
<td>48</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Glutamate + malate</td>
<td>Salicylanilide (2.7 × 10⁻³ M)</td>
<td>Sucrose (200 mM)</td>
<td>0</td>
<td>5</td>
<td>115</td>
<td>52</td>
<td>52</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Salicylanilide (2.7 × 10⁻³ M)</td>
<td>Glucose (200 mM)</td>
<td>0</td>
<td>5</td>
<td>122</td>
<td>92</td>
<td>52</td>
<td>57</td>
</tr>
</tbody>
</table>

carnitine-CoA synthetase step in α-ketoglutarate oxidation, and it would also be predicted that accumulation of α-ketoglutarate should occur during glutamate plus malate oxidation. In fact, Table II shows that approximately 80% of the glutamate utilized in the presence of nigericin accumulates as α-ketoglutarate. Other respiratory stimulators that do not normally show substrate specificity (monazomycin, TTFB, salicylanilide) did not cause α-ketoglutarate accumulation under the same conditions. It is further shown in Fig. 7 that conditions known to overcome nigericin inhibition, such as high potassium (1–3) or high phos-
FIG. 7. Effects of KCl and phosphate on α-ketoglutarate accumulation. a, KCl effect on nigericin inhibition. Reaction medium contained 15 or 50 mM KCl and 170 or 100 mM sucrose, respectively, 10 mM TEA-Cl (pH 7.4), 5 mM TEA-phosphate, 3 mM MgCl₂, and 3.3 mM each of TEA-glutamate and TEA-malate. Mitochondrial protein (8 mg) was present in a total volume of 3.2 ml with 10⁻⁶ M monazomycin and 7 × 10⁻⁹ M nigericin. Samples were incubated for 12 min at 30°C with constant shaking. b, comparative effects of phosphate on nigericin and TTFB uncoupling. Reaction medium contained 50 mM KCl, 100 mM sucrose, 10 mM TEA-Cl (pH 7.4), 1.7 mM MgCl₂, and 3.3 mM each TEA-glutamate and TEA-malate. TEA-phosphate was present at the concentrations indicated. Protein (9.4 mg) was present in a total volume of 3.0 ml with TTFB (7 × 10⁻⁹ M) or nigericin (10⁻⁹ M) as designated. Samples were incubated for 15 min at 30°C with constant shaking. The numbers shown are averages of duplicates from a representative experiment. Substrate levels were determined as described under "Methods."

It is apparent from these results that inhibition of glutamate oxidation by nigericin results primarily from a block of α-ketoglutarate metabolism due to lack of phosphate.

The malate dehydrogenase reaction is known to be very sensitive to the relative concentration of substrate and the product, oxalacetate (39, 40). In the presence of nigericin, the ratio of malate to oxalacetate is apparently unfavorable to the forward reaction, either because of limited uptake of malate, or increased retention of oxalacetate, or both. Since either glutamate or cysteine sulfinate allows the forward reaction to proceed, the concentration of oxalacetate must be an important controlling factor under these conditions. It should also be mentioned that malate dehydrogenase activity is inhibited by lowering the pH from 8 to 7 in the case of the isolated pig heart enzyme (40). Therefore the internal acidification caused by nigericin may directly inhibit malate dehydrogenase activity.

Inhibition by Guanidine—We investigated the activity of nigericin in the presence of various guanidines because several reports indicated that certain of these compounds inhibit the different energy-conserving sites selectively (42-47). Since it has also been suggested that potassium uptake is supported with different efficiency by the three phosphorylation sites (48), it was of interest to see whether nigericin showed any apparent discrimination among the coupling sites.

Octylguanidine inhibits oxidation of pyridine nucleotide-linked substrates at relatively low concentrations without blocking succinate oxidation (42). In Fig. 9, the ability of various uncouplers to overcome the octylguanidine block of β-hydroxybutyrate oxidation is compared. Nigericin is the least effective of the three different uncoupling agents tested. All were present at concentrations that stimulated succinate oxidation to the same degree. Dinactin and TTFB both caused marked oxidation of inhibiting phosphate penetration as in the case of nigericin, although the mechanism of this inhibition is not obvious.

Inhibition by Sucrose—Hypertonic sucrose selectively inhibits mitochondrial oxidations in a manner similar to nigericin (16, 41). It was found (Fig. 8) that even hypotonic sucrose (100 mosm) inhibited glutamate oxidation when KCl was not present. To determine whether lack of potassium was the important factor in sucrose inhibition, glucose was tested and found to be much less inhibitory than sucrose. The two sugars were compared with respect to α-ketoglutarate accumulation (Table II) and a significant difference was observed, suggesting that sucrose was also affecting substrate level phosphorylation, possibly by inhibiting phosphate penetration as in the case of nigericin.
Fig. 10. A comparison of the abilities of nigericin, dinactin, and TTFB to overcome the phenethylbiguanide (DBI)-induced block of β-hydroxybutyrate oxidation. The reaction mixture was the same as that described in the legend of Fig. 9 except that 6 mg of protein were present.

Phenethylbiguanide inhibits the oxidation of succinate more rapidly than PN-linked substrates, probably because succinate is more efficient at providing energy for phenethylbiguanide inhibition by displacing the inhibitor from the mitochondria (49). The increased reduction caused by nigericin might indicate that uptake of the guanidine was favored in this case, rather than release.

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Fig. 11. Inhibition of nigericin-induced ATPase by synthalin and octylguanidine. Reaction mixtures contained 15 mM KCl, 120 mM sucrose, 10 mM Tris-Cl, 6 mM Tris-ATP, 1.4 × 10⁻⁶ M nigericin, and 1.2 mg of mitochondrial protein in a total volume of 1.0 ml at pH 7.4 and 30°.

Pyridine nucleotides, indicating that increased flow of electrons was occurring through Site 1, even though the less sensitive oxygen trace showed only a small increase in respiration rate. In contrast, nigericin caused an initial reduction of pyridine nucleotides and an almost undetectable increase in respiration. It has been shown that one other uncoupler (dinitrophenol) overcomes biguanide inhibition by displacing the inhibitor from the mitochondria (49). The increased reduction caused by nigericin might indicate that uptake of the guanidine was favored in this case, rather than release.

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The possibility was considered that nigericin was unable to penetrate the membrane effectively in the presence of the guanidine because of an electrostatic barrier (see "Discussion"). This seemed unlikely because when succinate was added, its oxidation was effectively uncoupled by nigericin. But a permeability barrier to nigericin cannot be completely ruled out, since succinate itself could alter the penetration of nigericin by neutralizing a positive surface charge.

Another guanidine, synthalin, has a reported specificity for the third site of energy conservation (45). Nigericin, TTFB, and dinactin affected synthalin inhibition in a manner similar to their effect on phenethylbiguanide.

All three inhibitors were tested for their ability to block nigericin-stimulated ATPase. Octylguanidine and synthalin were more effective (Fig. 11), but phenethylbiguanide also inhibited the ATPase at concentrations comparable to those required to block substrate oxidation. This is in contrast to dinitrophenol-stimulated ATPase which is unaffected by all three compounds (42, 44).

These results are difficult to explain in terms of complete site specificity of either the guanidines or nigericin.

Ion Movements—Possible explanations for nigericin uncoupling include hydrolysis of a high energy intermediate, increased K⁺ conductance, increased H⁺ conductance, or nonspecific disruption of the membrane structure. One apparent means of distinguishing between some of these alternatives was to compare K⁺, H⁺, and light-scattering changes produced by nigericin with those caused by a variety of other uncoupling agents. Although this approach was severely limited because nigericin uncoupling requires high external potassium and the K⁺-sensitive electrode is accurate only at low potassium concentrations (optimally below 10 mM) (11, 50), a compromise medium containing 15 mM K⁺ allowed some uncoupling by nigericin and K⁺ movements were still measurable. Further, the solvent used for nigericin, dimethylformamide-ethanol 1:3, alone caused an apparent efflux of K⁺ from the mitochondria. It was found (Fig. 12) that no immediate net uptake or release of K⁺ was caused by nigericin under these uncoupling conditions that could not be accounted for by solvent artifact. However, the results should be compared with those obtained when dinactin was added in the presence of low concentrations of nigericin. Succinate oxidation is stimulated but no net uptake or release of K⁺ occurs. The inability to observe net movements of K⁺ or H⁺ does not eliminate
as do other members of this class of antibiotics (54).

nigericin and is not known to have a functional carboxyl group.

lower affinities for potassium (53). An exception to this last charge (4, 52, see below).

for potassium equal to nigericin3 but with no uncoupling ability. However, this compound is structurally very different from

monensin) but without uncoupling ability have considerably

the uncoupling activities of dianemycin, nigericin, TTFB, and the combination of low nigericin plus dianemycin. The reaction mixture contained 15 mm KCl, 120 mm sucrose, 5 mm TEA-Cl, 5 mm succinate, 3 X 10–7 M rotenone, and 3.6 mg of mitochondrial protein in a total volume of 5 ml at pH 7.2 and 25°. Release of ions and swelling are indicated by a downward deflection.

the possibility that increased conductance of either ion may be important in the uncoupling process (19). A nonspecific detergent action is not suggested by the data, since shrinking, not swelling, occurred.

Preliminary experiments have been performed with an artificial bilayer membrane system which show increased conductance (cf. Reference 51) when both nigericin and potassium are present at high concentrations, with specificity for K+ similar to that for uncoupling in mitochondria.2 This would support the idea that uncoupling by nigericin may result from a one-way movement of charge (4, 52, see below).

DISCUSSION

Three facts suggest that the potassium complex of nigericin is important in the uncoupling process: (a) uncoupling activity is proportional to the concentration of external potassium; (b) the uncoupling process shows strong specificity for potassium over other alkali metal cations; (c) compounds with activity similar to nigericin at low concentrations (dianemycin, X-206, monensin) but without uncoupling ability have considerably lower affinities for potassium (53). An exception to this last statement is boronycin, a compound with affinity and specificity for potassium equal to nigericin,1 but with no uncoupling ability. However, this compound is structurally very different from nigericin and is not known to have a functional carboxyl group as do other members of this class of antibiotics (54).

The complete structures of the metal complexes of nigericin and monencin have been determined by Steinrauf, Pinkerton, and Chamberlin (55) and Agatarap et al. (56) and are similar but not identical. The binding of silver by nigericin, but not monencin, involves interaction between the carboxyl oxygen and the metal, a possibly significant difference in the light of studies by Pressman (53). These studies showed that nigericin and dianemycin bind metal cations only when the carboxyl group was unprotonated, as indicated by the pH dependence of rubidium transport into an organic phase. Pressman concluded that hydrogen bonding between the unprotonated carboxyl and the hydroxyl group at the other end of the molecule was an essential force in maintaining a stable cation complex. As a further extrapolation from this theory, it is suggested that the importance of hydrogen bonding for stabilizing the cyclic complex may be less in the case of nigericin, where the carboxyl group is held in position by electrostatic interaction with the cation, than in the case of monensin (and presumably dianemycin), where the carboxyl does not associate with the cation. A mechanism for uncoupling is implied in this possibility, since nigericin, unlike others of its class, might thus be capable of carrying a proton on its carboxyl while still binding K+ thereby allowing one-way transfer of hydrogen ion by means of this positively charged species. Recycling of the neutral K+ complex would cause dissipation of a membrane potential form of conserved energy (19). A critical test for the existence of this positively charged species would be to determine whether a lipid-soluble anion could increase the organic/water partition coefficient of nigericin’s potassium complex at low pH. Experiments of this nature have been performed by Pressman (14, 53) but only with rubidium at low concentrations as a cation, whereas potassium at high concentrations may be required to demonstrate the postulated species. It might also be possible to locate the carboxyl proton of nigericin by nuclear magnetic resonance, and determine whether it was retained in the presence of potassium under certain conditions.

An alternative hypothesis deserving equal consideration has been suggested by Henderson et al. (4) and Mueller and Rudin (51). Assuming that the anion form of nigericin is somewhat permeable under uncoupling conditions, one-way charge transfer could be accomplished by movement of this negatively charged species, thus collapsing a membrane potential. A critical test of this idea would be a demonstration of greater lipid solubility of the nigericin anion than that of dianemycin, monencin, and particularly boronycin.

It is impossible to distinguish between these two theories on the basis of observable ion movements in whole mitochondria since net movements of either K+ or H+ associated with actual uncoupling are masked by Kf—Hf and K+-K+ exchanges occurring under all circumstances. Studies with an artificial membrane and bulk organic phase systems may provide the conditions necessary to make the distinction.

Uncoupling mechanisms other than those suggested by the assumption of a membrane potential (19), such as discharge of a chemical high energy intermediate or modification of a conformational state, are not eliminated by the evidence presented so far, but neither do they provide explanations that take into account the known structure and ion-binding properties of nigericin.

The observations on nigericin’s substrate specificity at both inhibitory and uncoupling concentrations appear to support the theory (4) that a phosphate requirement for conversion of succinyl-CoA to succinate becomes rate-limiting in the presence of nigericin, at least in the case of glutamate. Under certain conditions, general lowering of anion permeability as a result of internal acidification and K+ loss may also become rate-limiting for certain substrates, depending on the kinetic characteristics of their enzymes and carrier systems.

Phosphate has also been shown to be an important requirement for anion uptake via exchange-carrier systems (26, 57). But both succinate and malate exchange for phosphate, and therefore the need for Pi in this context does not appear to provide a basis of discrimination. However, proline and β-hydroxybutyrate

1 P. J. F. Henderson, personal communication.

2 A. Yoshimoto, and H. A. Lardy, manuscript in preparation.
apparently move into the mitochondrion as uncharged molecules (5, 58) and do not require phosphate for exchange. This may explain in part their relative insensitivity to nigericin inhibition.

The results obtained with octylguanidine, phenethylbiguanide, and synthalin suggest that the uncoupling form of nigericin is not as effective as other uncouplers or ion transport inducers at displacing guanidines or otherwise overcoming the guanidine inhibition. This result would be explained if the uncoupling form of nigericin was positively charged and had reduced permeability in a membrane altered by the presence of guanidine cations (cf. Reference 18).

The possibility exists that uncoupling by nigericin may involve binding of divalent cations, such as calcium. However, inhibition. This result would be explained if the uncoupling form of nigericin may in-

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REFERENCES

47. Scheafer, G., and Bojanowski, D., in Abstracts of the Sixth Meeting of the Federation of European Biochemical Societies, Madrid, 1969, no. 967.
Potassium-specific Uncoupling by Nigericin
S. M. F. Ferguson, Sergio Estrada-O and Henry A. Lardy


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