SHORT COMMUNICATIONS

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Inhibition of uptake and oxidation of succinate in rat-liver mitochondria

Recently evidence has been given that succinate accumulates within the mitochondria during Ca^{2+} or K^+ uptake, while under conditions in which the cation uptake is inhibited succinate uptake is also decreased[1,2]. Particularly in the presence of low concentrations of succinate (1–3 mM), it is possible to show that the rate of respiration is higher during K^+ uptake than in the active or in the uncoupled state[2–4]. The known inhibition of respiration by high concentrations of uncouplers has been related recently to a lack of penetration of substrate into mitochondria[1–6]. On the basis of these results, an inhibition of succinate oxidation should have been expected by conditions which induce K^+ loss from the mitochondria. However, GRAVEN, ESTRADA AND LARDY[7] reported no inhibition of respiration with succinate after addition of nigericin, even though K^+ was lost.

In this paper it is shown that nigericin does inhibit respiration, with succinate *plus* rotenone as substrate, and that this inhibition is due to a decrease of the intramitochondrial concentration of succinate.

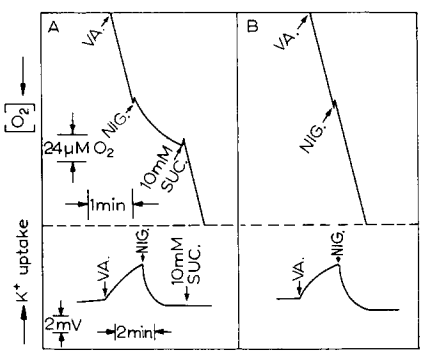

Fig. 1. Effect of nigericin on the O_2 consumption and the transport of K^+ by rat-liver mitochondria at different succinate concentrations. The incubation mixture consisted of 0.25 M sucrose, 20 mM Tris–HCl buffer, pH 7.2, 1 μg/ml rotenone, 4 mM KCl, 4 mM acetate, 1 mM succinate in A or 10 mM succinate in B. Where indicated, 0.1 μg/ml valinomycin, $0.6 \cdot 10^{-3}$ μmole/ml nigericin or 10 mM succinate was added. All the anions were neutralized with Tris. Temperature: 25°. The oxygen uptake was recorded polarographically in a final volume of 1 ml, containing 2.2 mg protein. K^+ was measured with a K^+-sensitive electrode: the final volume, containing 15 mg protein, was 5 ml and the medium was oxygenated. An upward deflection in the K^+ tracing represents a decrease in the concentration of K^+ in the medium. Abbreviations: VA., valinomycin; NIG., nigericin; SUC., succinate.

Fig. 1A shows that, in the presence of 1 mM succinate, nigericin inhibits the valinomycin-stimulated O_2 consumption. This inhibition is released by 10 mM succinate and does not occur if 10 mM succinate is present from the beginning (Fig. 1B). The respiration, in the active or uncoupled state, is also inhibited by nigericin in

Abbreviation: TMPD, tetramethyl-*p*-phenylenediamine.

the presence of 1 mM succinate, but not in the presence of an excess of substrate. As GRAVEN, ESTRADA AND LARDY[7] used a concentration still higher than 10 mM, this is probably the reason why they fail to find any inhibition. The K^+-sensitive electrode tracings (Fig. 1) indicate that nigericin induces a K^+ loss which is not reversed or prevented by 10 mM succinate.

TABLE I

EFFECT OF NIGERICIN ON SUCCINATE UPTAKE INDUCED BY K^+ *plus* VALINOMYCIN

In addition to the oxygenated medium, consisting of 0.25 M sucrose and 20 mM Tris–HCl, pH 7.2, the reaction mixture contained 1 μg rotenone, 1 mM [^{14}C]succinate (specific activity 181–200 counts/min per 10^{-9} mole), 2 μg antimycin, 0.2 mM TMPD, 1.5 mM ascorbate, 0.1 μg valinomycin, 5 mM KCl, 5 mM acetate, 3 mg protein in Expts. 1 and 2, 2.6 mg protein in Expt. 3, and (where indicated) $0.6 \cdot 10^{-3}$ μmole nigericin. All the anions were neutralized with Tris. Final volume: 1 ml. Temperature: 25°. At the end of the incubation time, the mitochondria were separated by the rapid centrifugal filtration[8,9]. The adherent supernatant was determined by [^{14}C]carboxyldextran and subtracted from the values of the total water in the pellet. The intramitochondrial water was 5.0, 5.1 and 3.4 μl/mg respectively in Expts. 1, 2 and 3 in the absence of nigericin; 2.2, 2.6 and 1.8 μl/mg, respectively, in Expts. 1, 2 and 3 in the presence of nigericin. Radioactivity was measured in a Tri-Carb scintillation counter.

Time of incubation (sec)		*Intramitochondrial succinate (mM)*		
Before nigericin addition	*After nigericin addition*	*Expt. 1*	*Expt. 2*	*Expt. 3*
45	—	6.3	6.8	6.2
90	—	8.3	8.4	6.1
45	45	2.3	1.5	1.5

Our interpretation of these results is that, in the presence of low concentrations of substrate, the rate of respiration depends upon succinate uptake. Further evidence for this hypothesis is given by the experiments reported in Table I, in which the intramitochondrial concentration of [^{14}C]succinate has been measured before and after the addition of nigericin. The addition of nigericin to mitochondria which have been allowed to accumulate succinate causes a marked decrease in the intramitochondrial concentration of succinate to approximately the level of the external concentration. This result agrees with the finding of HARRIS, VAN DAM AND PRESSMAN[1] that nigericin inhibits succinate uptake. With 10 mM succinate, nigericin also abolishes the preexisting gradient between the intra- and extramitochondrial concentration of succinate (not shown), but, in this case no inhibition of respiration supervenes (Fig. 1B) because an intramitochondrial concentration of 10 mM succinate is expected to give the maximal rate of respiration (see ref. 10).

The effect of nigericin on the intramitochondrial concentration of succinate has been studied mostly by using tetramethyl-*p*-phenylenediamine (TMPD) *plus* ascorbate as the energy source, in the presence of succinate and antimycin. This system offers the advantage that no products of succinate oxidation are formed within the mitochondria and the total succinate present in the incubation mixture remains constant during the time. However, in short time incubation experiments (5–15 sec), the same effect of nigericin, shown in Table I, was also observed with succinate *plus* rotenone as substrate.

Recently KIMMICH AND RASMUSSEN[11] have reported an inhibition of O_2 uptake with 1–5 mM succinate, under conditions which give rise to K^+ loss, *e.g.* using a combination of dinitrophenol and valinomycin. However, they concluded on the basis of an experiment with malate that high concentrations of substrate do not influence this inhibition and took this as evidence that a decrease of the intramitochondrial concentration of K^+, rather than of oxidable substrate, is responsible for the inhibition of respiration. In contrast to this conclusion, we have found that the respiratory inhibition by dinitrophenol *plus* valinomycin is also released or prevented by an excess of succinate and that a decrease of the intramitochondrial concentration of succinate, besides a K^+ loss, occurs when agents inducing ion transport are added to mitochondria pre-treated with 0.1 mM dinitrophenol.

The results reported above are not consistent with the interpretation that the inhibition of respiration by nigericin or by the combination of dinitrophenol and valinomycin are due simply to a loss of K^+[7,11], which should be required for the functioning of the dehydrogenases[7,11] or for electron transport at a point(s) prior to cytochrome *c*[11].

The release of the respiratory inhibition by high concentrations of succinate, without changing the intramitochondrial concentration of K^+, and the demonstration that succinate concentration is actually decreased within the mitochondria during the inhibition give evidence that the entry of succinate is rate-limiting in these conditions. Thus, these data are in agreement with and further support the recent concept of a metabolic regulation in mitochondria by substrate transport[1–6].

Department of Biochemistry,
University of Bari, Bari (Italy)

FERDINANDO PALMIERI
MARIO CISTERNINO
ERNESTO QUAGLIARIELLO

1 E. J. HARRIS, K. VAN DAM AND B. C. PRESSMAN, *Nature*, 213 (1967) 1126.
2 E. QUAGLIARIELLO, F. PALMIERI AND M. CISTERNINO, *Boll. Soc. Ital. Biol. Sper.*, 43 (1967) 297.
3 E. QUAGLIARIELLO AND F. PALMIERI, *Boll. Soc. Ital. Biol. Sper.*, 43 (1967) 300.
4 E. J. HARRIS, M. P. HÖFER AND B. C. PRESSMAN, *Biochemistry*, 6 (1967) 1348.
5 K. VAN DAM, *Biochim. Biophys. Acta*, 131 (1967) 407.
6 K. VAN DAM AND E. J. HARRIS, *4th Meeting Federation European Biochem. Soc., Oslo, 1967*, Abstracts, p. 12.
7 S. N. GRAVEN, S. ESTRADA AND H. A. LARDY, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 654.
8 M. KLINGENBERG, E. PFAFF AND A. KRÖGER, in B. CHANCE, *Rapid Mixing and Sampling Techniques in Biochemistry*, Academic Press, New York, 1964, p. 333.
9 M. KLINGENBERG AND E. PFAFF, in J. M. TAGER, S. PAPA, E. QUAGLIARIELLO AND E. C. SLATER, *Regulation of Metabolic Processes in Mitochondria* (BBA Library, Vol. 7), Elsevier, Amsterdam, 1966, p. 180.
10 E. C. SLATER AND W. D. BONNER, *Biochem. J.*, 52 (1952) 185.
11 J. A. KIMMICH AND H. RASMUSSEN, *Biochim. Biophys. Acta*, 131 (1967) 413.

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