Biochem. J. (1980) **186**, 637–639
Printed in Great Britain

Effect of 2-n-Heptyl-4-hydroxyquinoline *N*-Oxide on Proton Permeability of the Mitochondrial Membrane

Klaas KRAB and Mårten WIKSTRÖM
Department of Medical Chemistry, University of Helsinki, Siltavuorenpenger 10A, SF-00170 Helsinki 17, Finland

(*Received 26 November 1979*)

The respiratory-chain inhibitor 2-n-heptyl-4-hydroxyquinoline *N*-oxide catalyses trans-membrane proton transport driven by a pH gradient in isolated rat liver mitochondria. This effect explains the apparent blockade of net proton translocation by this compound in mitochondria respiring with ferrocyanide as described by Papa, Lorusso, Guerrieri, Boffoli, Izzo & Capuano [(1977) in *Bioenergetics of Membranes* (Packer, Papageorgiu & Trebst, eds.), pp. 377–388, Elsevier/North-Holland, Amsterdam] and by Lorusso, Capuano, Boffoli, Stefanelli & Papa [(1979) *Biochem. J.* **182**, 133–147].

2-n-Heptyl-4-hydroxyquinoline *N*-oxide is an inhibitor of electron transfer through the cytochrome bc_1 segment of the respiratory chain. In addition, this compound has long been known to act as an uncoupling agent when added at high concentrations (Slater, 1967). More recently, the latter property of this antibiotic has become a central issue of controversy in the discussion of proton translocation linked to oxidation of ferrocyanide by antimycin-inhibited mitochondria (Papa *et al.*, 1977; Wikström, 1977, 1978; Lorusso *et al.*, 1979).

Papa and co-workers reported that this proton translocation is sensitive to high concentrations of the inhibitor (Papa *et al.*, 1977; Lorusso *et al.*, 1979), and claim that this inhibitory effect is not correlated with an increase in the proton conductance of the mitochondrial membrane as suggested by Wikström (1978). Instead, they concluded that the observed proton translocation linked to oxidation of ferrocyanide is due to antimycin-insensitive electron transfer in the cytochrome bc_1 segment, which is blocked by high concentrations of 2-n-heptyl-4-hydroxyquinoline *N*-oxide.

Lorusso *et al.* (1979) measured the effect of this antibiotic on proton conduction through mitochondrial membranes in a system where uptake of H^+ into the mitochondria was driven by an electrical membrane potential. The latter was induced by K^+ efflux catalysed by the ionophore valinomycin. However, in the experimental conditions where H^+ translocation linked to ferrocyanide oxidation is usually demonstrated (see e.g., Wikström, 1977), it is essential that the membrane potential is collapsed by a permeant ion (K^+ with valinomycin, or Ca^{2+}). Under such conditions the backflux of protons is driven by a pH gradient (ΔpH).

In the present paper we demonstrate that 2-n-heptyl-4-hydroxyquinoline *N*-oxide mediates ΔpH-driven transfer of H^+ across the mitochondrial membrane at ratios of the antibiotic to protein where net proton translocation linked to oxidation of ferrocyanide is abolished, but where there is little effect on the membrane-potential-driven H^+ uptake. It is concluded that 2-n-heptyl-4-hydroxyquinoline *N*-oxide may transfer H^+ analogously to weak permeant acids, and that this property is responsible for the observed blockade of net proton translocation linked to oxidation of ferrocyanide observed by Papa *et al.* (1977) and Lorusso *et al.* (1979).

Experimental and Results

Preparation of mitochondria and measurements of mitochondrial protein and of pH were carried out as described previously (Krab & Wikström, 1979). pH-jump experiments were performed essentially as described by Mitchell & Moyle (1967).

Fig. 1 shows that, in the absence of 2-n-heptyl-4-hydroxyquinoline *N*-oxide (trace *a*), addition of HCl to the anaerobic mitochondrial suspension results in a fast pH change, the extent of which is determined by the external buffering power of medium and mitochondria. This is followed by a slow decay towards a more alkaline pH, which is due to equilibration of protons across the mitochondrial membrane. The final pH value is determined by the total buffering power, including that of the inside (matrix) phase (cf. Mitchell & Moyle, 1967).

In the presence of increasing amounts of 2-n-heptyl-4-hydroxyquinoline *N*-oxide the decay phase in the alkaline direction is accelerated (trace

0306-3283/80/020637-03 $1.50/1

b), until the final value is reached nearly instantaneously at a concentration of the antibiotic corresponding to 6 nmol/mg of mitochondrial protein (trace c). In the experiment of trace (d) the proton-conducting uncoupler carbonyl cyanide p-trifluoromethoxyphenylhydrazone was present instead of the antibiotic, for comparison. These experiments were performed in 150 mM-KCl in the presence of valinomycin, so that no membrane potential can develop. Analogous experiments in the absence of valinomycin (not shown), where H^+ influx is limited by an electrical membrane potential, yielded a much less pronounced acceleration of the alkaline decay by the antibiotic.

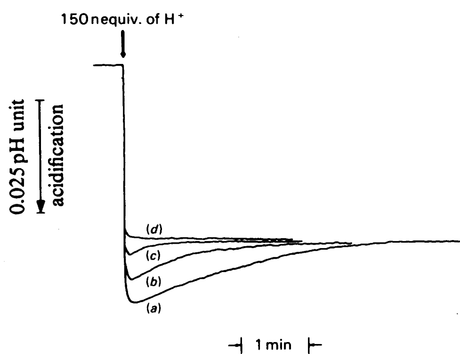

Fig. 1. *Stimulation of ΔpH-driven proton uptake by 2-n-heptyl-4-hydroxyquinoline N-oxide in rat liver mitochondria*

A medium containing 150 mM-KCl, 1 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonate] and 0.68 μg of valinomycin/ml, pH 7.2, at 25 °C was made anaerobic by bubbling with ultrapure argon (containing less than 3 p.p.m. of O_2), and then anaerobic rat liver mitochondria (6.2 mg of protein/ml) were added. The experiments were carried out anaerobically under a stream of argon in a glass vessel that was sealed except for a narrow port for additions. After 20 min of equilibration, 150 n-equiv. of HCl was added as indicated (as 5 μl of an anaerobic 30 mM-HCl solution). In trace (a) no further additions were made. In traces (b) and (c) 3 and 6 nmol of 2-n-heptyl-4-hydroxyquinoline N-oxide were present respectively. Trace (d) was obtained in the presence of 0.33 μM-carbonyl cyanide p-trifluoromethoxyphenylhydrazone. The incubation volume was 3 ml. The four traces represent different incubations with the same batch of rat liver mitochondria. Several experiments of the kind shown ensured that there was no time-dependent change of the mitochondria with respect to the measured parameters. Identical results were also obtained in experiments where the conditions corresponding to (a)–(d) were achieved by subsequent additions to the same incubation, or where the entire experiment was performed aerobically in the presence of rotenone.

In parallel experiments (not shown) we confirmed the report by Lorusso *et al.* (1979) that net H^+ translocation linked to ferrocyanide oxidation is indeed abolished by 2-n-heptyl-4-hydroxyquinoline *N*-oxide in the concentration range 3–10 nmol/mg of mitochondrial protein. This is precisely the range in which the antibiotic accelerates H^+ uptake driven by ΔpH to a point that is indistinguishable from the effect of an uncoupling agent (cf. Fig. 1). It should also be noted that the amount of antibiotic added to reach these effects is of the same order of magnitude as the observed net proton translocation in the absence of antibiotic (see Lorusso *et al.*, 1979).

Discussion

Our experiments show that, when 2-n-heptyl-4-hydroxyquinoline *N*-oxide is present in sufficiently high concentrations, it catalyses ΔpH-driven H^+ uptake into mitochondria. This suggests a mechanism in which the antibiotic acts in a similar way to weak permeant acids (e.g. P_i or acetate). Protons are picked up by the anionic form of the compound on the outside of the membrane, transferred across the membrane as the uncharged acid, and this is followed by proton release on the inside in an overall process driven exclusively by ΔpH. Catalysis of membrane-potential-driven H^+ flux by this antibiotic appears to be much less efficient as judged from experiments similar to those in Fig. 1, but without valinomycin (and see Wikström, 1978; Lorusso *et al.*, 1979). This may be due to a much smaller mobility of the charged form of the compound in the membrane as compared with that of the uncharged acid.

The presently discussed effect of the antibiotic requires much higher concentrations than those that inhibit electron transfer in the cytochrome bc_1 segment (0.17 nmol/mg of protein; Slater, 1967). However, at high concentrations 2-n-heptyl-4-hydroxyquinoline *N*-oxide will function as an effective uncoupling agent provided that the membrane potential is collapsed, e.g. by K^+ plus valinomycin. Since these are exactly the conditions in which H^+ translocation linked to ferrocyanide oxidation is studied, we conclude that the inhibition of net proton translocation by the antibiotic observed by Lorusso *et al.* (1979) is due to uncoupling. Since these authors only tested the antibiotic for membrane-potential-induced H^+ translocation, they did not detect the particular type of uncoupling shown in the present work.

We thank Ms. Hilkka Vuorenmaa for technical assistance, and the European Molecular Biology Organization (EMBO) and the Sigrid Jusèlius Foundation for financial support.

References

Krab, K. & Wikström, M. (1979) *Biochim. Biophys. Acta* **548**, 1–15

Lorusso, M., Capuano, F., Boffoli, D., Stefanelli, R. & Papa, S. (1979) *Biochem. J.* **182**, 133–147

Mitchell, P. & Moyle, J. (1967) *Biochem. J.* **104**, 588–600

Papa, S., Lorusso, M., Guerrieri, F., Boffoli, D., Izzo, G. & Capuano, F. (1977) in *Bioenergetics of Membranes* (Packer, L., Papageorgiou, G. C. & Trebst, A., eds.), pp. 377–388, Elsevier/North-Holland Biomedical Press, Amsterdam

Slater, E. C. (1967) *Methods Enzymol.* **10**, 48–57

Wikström, M. K. F. (1977) *Nature (London)* **266**, 271–273

Wikström, M. K. F. (1978) in *The Proton and Calcium Pumps* (Azzone, G. F., Avron, M., Metcalfe, J. C., Quagliariello, E. & Siliprandi, N., eds.), pp. 215–226, Elsevier/North-Holland Biomedical Press, Amsterdam