ON THE MECHANISM OF INHIBITION OF THE RESPIRATORY CHAIN BY 2-HEPTYL-4-HYDROXYQUINOLINE-*N*-OXIDE

G. IZZO, F. GUERRIERI and S. PAPA

Institute of Biological Chemistry Faculty of Medicine and *Centre for the Study of Mitochondria and Energy Metabolism, CNR, University of Bari, Italy*

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1. Introduction

Alkyl-hydroxyquinoline-*N*-oxide inhibits, like antimycin A, electron flow in the mitochondrial respiratory chain between *b* and c_1 cytochromes [1–3]. Although these two inhibitors are widely used their mechanism of action is not yet fully understood [2,3]. It has been proposed that alkyl-hydroxyquinoline-*N*-oxide and antimycin share the same reaction site [2–6]. The concentration in the inner mitochondrial membrane of specific binding sites for 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) is identical with that of antimycin binding sites, antimycin antagonizes the binding of HQNO and the inhibition of forward electron flow by HQNO is apparently additive with that by antimycin [6]. Both inhibitors induce a characteristic oxidant-dependent reduction of *b* cytochromes [3–8].

In this paper a kinetic analysis of the effect of HQNO and antimycin on the aerobic oxidation and reduction of *b* cytochromes is reported. The results provide evidence for difference in the reaction sites of the two inhibitors.

2. Methods

Heavy beef-heart mitochondria were prepared as in [9]. Oxido-reduction of cytochromes was monitored with a Johnson Foundation dual wavelength spectrophotometer, equipped with regenerative stopped-flow apparatus [10]. The mixing ratio was 1:70, the reaction time during the continuous-flow phase ranged in different experiments from 5–10 ms.

3. Results and discussion

Figures 1 and 2 show the effect of saturating concentrations of antimycin and HQNO on the response to oxygen pulses of *b* and *c* cytochromes of mitochondria whose respiratory carriers were pre-reduced by succinate in anaerobiosis. HQNO caused, like

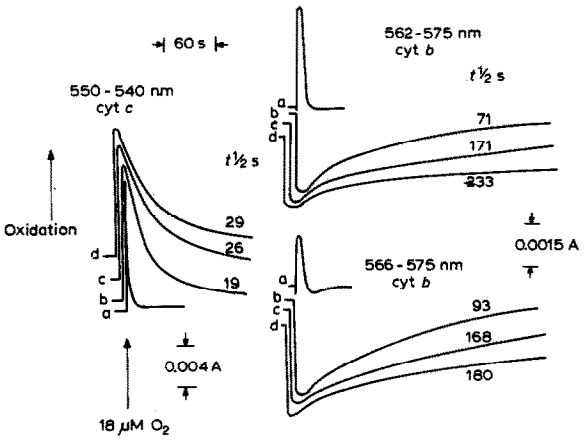

Fig.1. Effect of antimycin and HQNO on redox transitions of *b* and *c* cytochromes induced by oxygenation of anaerobic beefheart mitochondria. Mitochondria (2 mg protein·ml^{-1}) were incubated in the main-syringe of the flow-apparatus at 25°C in: 200 mM sucrose, 30 mM KCl, 3 mM K-succinate, 0.5 μg·mg protein^{-1} rotenone; 0.5 μg·mg protein^{-1} valinomycin and 2 μg·mg protein^{-1} oligomycin, pH 6.8. Oxygen was delivered by the side-syringe of the flow-apparatus as: 200 mM sucrose and 30 mM KCl. (a) control; (b) 1 nmol·mg protein^{-1} antimycin; (c) 10 nmol·mg protein^{-1} HQNO; (d) antimycin plus HQNO. The numbers on the traces refer to the $t_{1/2}$ (s) of cytochrome *c* re-reduction and cytochrome *b* reoxidation in anaerobiosis.

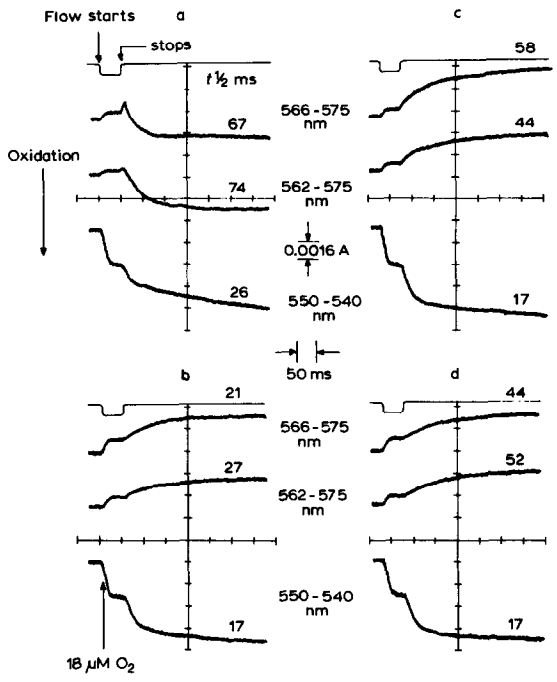

Fig.2. Effect of antimycin and HQNO on the kinetics of rapid aerobic oxidation or reduction of *c* and *b* cytochromes in beef-heart mitochondria. The experimental conditions are those in fig.1 legend. The numbers on the traces refer to the $t_{1/2}$ (ms) of cytochrome *b* oxidation or reduction and cytochrome *c* oxidation.

antimycin, change of the response of *b* cytochromes to oxygen from oxidation to extra-reduction (fig.1,2). The reoxidation of *b* cytochromes, which ensued upon oxygen exhaustion, was in antimycin-treated mitochondria, much slower than re-reduction of *c* cytochromes (fig.1; cf. [8,11]). In the presence of HQNO and HQNO plus antimycin both the rates of cytochrome *b* reoxidation and cytochrome *c* re-reduction were depressed as respect to those measured in the presence of antimycin alone.

The oscilloscope recordings of the initial phase of the redox transitions (fig.2) show that in the absence of inhibitors aerobic oxidation of *b* cytochromes was preceded by a small and rapidly transient reduction (cf. [7,8,12]). The rapid kinetics analysis (fig.2) shows also that reduction of *b* cytochromes proceeded in the presence of HQNO at a rate considerably lower than that recorded in the presence of antimycin.

Furthermore HQNO caused, when added to mitochondria supplemented with antimycin, a marked inhibition of the oxygen-dependent reduction of *b* cytochromes elicited by the latter inhibitor. HQNO and antimycin caused, on the other hand, an equal enhancement of the net rate of aerobic oxidation of *c* cytochromes, and HQNO had no additional effect on the rate and extent of cytochrome *c* oxidation with respect to what produced by antimycin.

Figure 3 shows a titration of the inhibitory effect of HQNO on the antimycin/oxygen-induced reduction of *b* cytochromes. Similar inhibition curves were obtained independently whether the reduction of *b* cytochromes was monitored at 566–575 nm or 562–575 nm. Inhibition of cytochrome *b* reduction increased exponentially with HQNO concentration $\leqslant$30 nmol·mg protein^{-1}, then levelled off giving a maximum inhibition of 60%. Thus there is an HQNO insensitive component in the reduction of *b* cytochromes, this being consistent with the fact that HQNO by itself induces oxygen-linked reduction of *b* cytochromes (see fig.1,2, [5,6]). Half-maximal inhibition of cytochrome *b* reduction occurred at 2.5 nmol HQNO·mg protein^{-1}. This concentration is not much higher than that required for half-maximal inhibition of succinate oxidation [5,6].

Experiments similar to those presented in fig.1

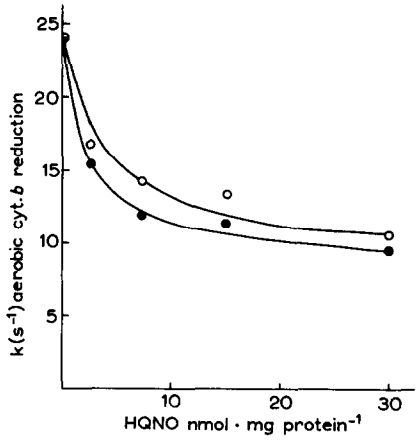

Fig.3. Titration of the inhibitory effect of HQNO on the first-order kinetics constant of aerobic reduction of *b* cytochromes induced by oxygenation of antimycin treated beef-heart mitochondria. For experimental conditions see fig.1 legend. (o–o) 566–575 nm; (•–•) 562–575 nm measurements.

showed that FCCP had no effect on the antimycin/oxygen-induced reduction of *b* cytochromes, neither did it alter the inhibitory pattern produced by HQNO.

Of the various mechanisms proposed to explain the antimycin/oxidant-induced reduction of *b* cytochromes, the evidence at present available (reviewed [2,3,8]) seems to leave two possibilities open:

(i) Reduction of *b* cytochromes by ubiquinol is due to enhancement of their E_m, effected by oxidation of an unknown component, X, situated between the antimycin site and cytochrome c_1 [3,4,13,14].

(ii) Reduction of *b* cytochromes by ubiquinol [8,15] or ubisemiquinone [16,17] is promoted by oxidation of ubisemiquinone or quinol respectively by a reaction which bypasses the antimycin site.

The observation that HQNO depresses the rate of the antimycin/oxygen-induced reduction of *b* cytochromes as well as anaerobic reoxidation shows that this substance exerts additional effect(s) on the respiratory chain with respect to what produced by antimycin (see also [18]). We are faced with two possibilities:

1. That HQNO inhibits the respiratory chain at two sites: at the antimycin site, and at a site involved in the redox equilibration between ubiquinone and *b* cytochromes. Since HQNO causes per se oxygen-induced reduction of *b* cytochromes the inhibitory effect at the antimycin site should prevail on that exerted between ubiquinone and *b* cytochromes.

2. That HQNO inhibits electron flow between the antimycin site and cytochrome c_1. In fact antimycin but not HQNO induces a red shift in the band of ferrocytochrome b_{562} [3,5]. HQNO could inhibit the aerobic oxidation as well as the anaerobic re-reduction of the component X of mechanism (i) thus depressing both reduction and anaerobic reoxidation of *b* cytochromes. Inhibition by HQNO of a redox-step common to electron transfer from *b* cytochromes and from ubisemiquinone (or quinol) to cytochrome c_1, could explain in terms of mechanism (ii) inhibition by HQNO of the antimycin/oxygen-induced reduction of *b* cytochromes. Partial inhibition of electron transfer at this site would promote oxygen-induced reduction of *b* cytochromes (cf. [3,8]). In this case, however an additional inhibitory action of HQNO on redox equilibration between ubiquinone and *b* cytochromes should be involved in inhibition by HQNO of the anaerobic reoxidation of *b* cytochromes.

It should be pointed that the antagonistic effect exerted by antimycin on the binding of HQNO does not allow to discriminate between common [6] or separate binding sites for antimycin and HQNO. In the latter case the antagonism could be due to allosteric interaction between the two binding sites.

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