EFFECT OF CYANIDE ON RESPIRATORY CONTROL OF ELECTRON TRANSPORTING PARTICLES

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SUMMARY: Addition of cyanide to electron transporting particles (ETP) respiring in presence of NADH selectively inhibited uncoupled respiration. The respiratory control index as measured with p-trifluoromethoxy-phenylhydrazone increased from 2 to 10 after addition of cyanide. These results suggest that there are two populations of respiratory chains in electron transporting particles--coupled and uncoupled, and that the lower coupling in electron transporting particles as compared to mitochondria reflects the presence of a proportion of uncoupled chains.

In the best coupled mitochondria, the P/O ratio is near 3 with NADH generating substrates and the RCI* as measured with ADP or uncoupler is above 5. But, after conversion via sonication to electron transporting particles, both the P/O ratio and the RCI of the derivative particles are greatly decreased (1). This decrease in coupling can be a result of two possible causes: (i) all the respiratory chains have decreased coupling efficiency or (ii) some chains become uncoupled and the rest remain coupled. The second possibility requires two distinct classes of chains--coupled (controlled) and uncoupled (uncontrolled). These two extremes of coupling would be easily demonstrable by a respiratory inhibitor that selectively suppresses the activity of chains without respiratory control. Such an inhibitor would increase the RCI to the value found in mitochondria. Since cyanide has been shown by Erecinska, et al. to react much more rapidly with cytochrome oxidase in the uncoupled state (2), it was a likely candidate for an inhibitor of the requisite properties. In this paper, I wish to present evidence that cyanide is a selective inhibitor of uncoupled respiratory chains and that it can dramatically increase the respiratory control index of ETP.

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*Abbreviations: RCI = respiratory control index, ETP = electron transporting particles, FCCP = p-trifluoromethoxy-phenylhydrazone, pms = phenazine methosulfate, and TMPD = tetramethyl-p-phenylenediamine.

MATERIALS AND METHODS

ETP was prepared by the modified method of Linnane and Ziegler (3). For the determination of respiratory control, oxygen uptake was measured using a Beckman oxygen analyzer at 30°. The reaction mixture (4 ml) contained sucrose, 0.25 M; Tris-HCl (pH 7.4), 10 mM; particles (0.5 mg/ml); NADH, 1 mM to start the reaction; and other additions as indicated. Carboxylcyanide p-trifluoromethoxy-phenylhydrazone (FCCP) was a generous gift of Dr. Henry Lardy.

RESULTS AND DISCUSSION

Addition of KCN to ETP respiring with NADH caused a rapid inhibition of oxygen uptake (Figure 1). Addition of the uncoupler FCCP induced a burst of respiration followed by inhibition. Valinomycin plus nigericin, in the presence of K^+, gave a similar response as FCCP. These observations suggest that KCN selectively inhibited the cytochrome oxidase of uncontrolled chains. Addition of an uncoupler or the ionophore combination released the respiratory control in the remaining chains making them in turn KCN sensitive.

Table I gives the results of different KCN concentrations. The RCI increases from 2.2 to a high of 9.6 at a KCN concentration of 5×10^{-6} M. At this KCN concentration, the percentage of chains controlled can be estimated to be 43% by the percentage of NADH oxidase activity (plus FCCP) remaining after KCN treatment. From the experimentally determined percentage of controlled and uncontrolled chains, the RCI of the uncontrolled chains can be calculated. The calculated RCI of uncontrolled chains turned out to be 1.4 which suggests totally uncoupled respiration.

Addition of Mg-ADP to KCN inhibited ETP also produced a burst of respiration (Figure 2). Addition of oligomycin inhibited the Mg-ADP stimulation. This Mg-ADP stimulation appeared to be quite variable. In many preparations of ETP there was little, if any, effect, and never was the stimulation by Mg-ADP large enough to totally eliminate a further stimulation by FCCP. In the experiment shown in Figure 2 even after the Mg-ADP stimulation had leveled off, addition of FCCP still gave a significant stimulation.

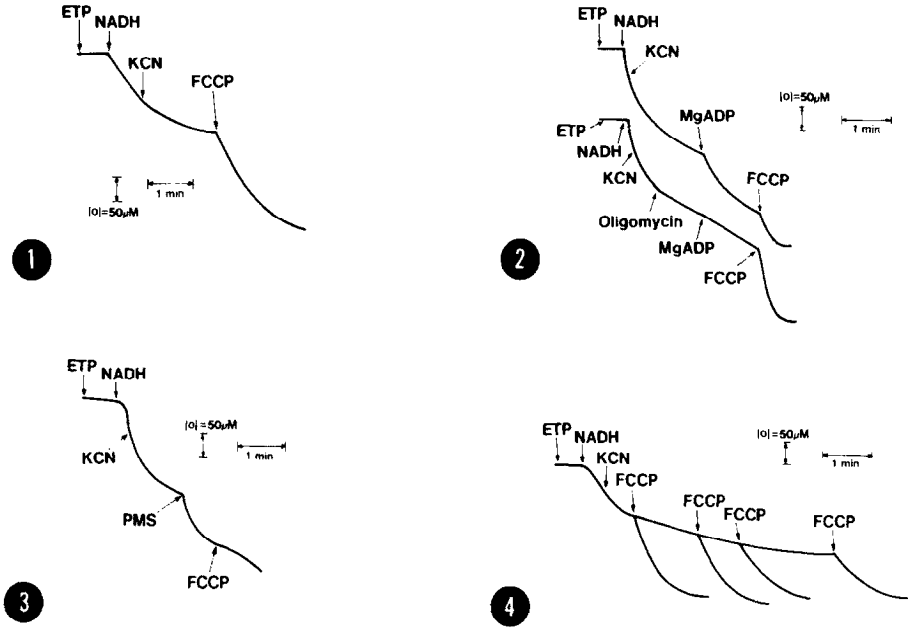

Figure 1: To an ETP suspension (0.5 mg/ml) respiring with NADH in the medium described in methods, KCN (10^{-5} M) and FCCP (10^{-6} M) were added.

Figure 2: To a suspension of ETP (0.75 mg/ml) respiring with NADH in the medium listed in methods containing 2 mM potassium phosphate, was added 2 x 10^{-5} M KCN. The final concentration of ADP and $MgCl_2$ (added together) was 2 mM. Oligomycin was used at a concentration of 0.5 μg/ml and FCCP, at 10^{-6} M.

Figure 3: To a suspension of ETP (0.5 mg/ml) respiring with NADH, KCN (10^{-5} M), Phenazine methosulfate (PMS) (5 x 10^{-6} M) and FCCP (10^{-6} M) were added.

Figure 4: This is a composite of traces obtained from several experiments in which the time interval between the addition of KCN (10^{-5} M) and FCCP (10^{-6} M) was varied. The ETP concentration was 0-5 mg/ml.

Substrates for cytochrome oxidase such as ascorbate plus either cytochrome c, phenazine methosulfate (PMS) or tetramethyl-p-phenylenediamine (TMPD) showed very little respiratory control in either ETP or KCN treated ETP. In fact, addition of either PMS or TMPD produced a burst of respiration in KCN treated ETP that had been respiring with NADH (Figure 3). PMS or TMPD shared with FCCP the capability for releasing the respiration of controlled chains. Ascorbate plus cytochrome c, on the other hand, gave little, if any, stimulatior

TABLE I: Effect of KCN Concentration on Respiratory Control.

(KCN) (μM)	NADH oxidase activity (μmoles/min/mg)	NADH oxidase activity (+ FCCP) (μmoles/min/mg)	RCI
0.0	0.45	0.99	2.2
1.3	0.21	0.68	3.3
2.5	0.12	0.56	4.9
5.0	0.044	0.43	9.6
40.0	0.034	0.22	6.5

To ETP respiring with NADH for 0.5 minutes, KCN was added. Then, after one minute, FCCP (10^{-6} M) was added.

which suggests that external cytochrome c does not interact with controlled chains.

The success of KCN as a selective reagent was only manifested when it was added after NADH. In agreement with Lee et al., addition of KCN to ETP before NADH had no effect on the RCI as measured with FCCP (4). Under non-energized conditions, KCN must inhibit both controlled and uncontrolled chains indiscriminately.

The selectivity of KCN also depended on the KCN concentration. Too low KCN concentration, as shown in Table I, gave incomplete inhibition of the uncontrolled chains. High KCN concentrations, 10^{-4} M or above, gave complete inhibition of all chains. Increasing the time of incubation with KCN likewise caused a decrease in the stimulation by FCCP (Figure 4). By 5 minutes, only about 50% of the FCCP induced oxygen uptake remained. These experiments confirm the conclusion of Erecinska, et al. (2) that selectivity by KCN is a kinetic phenomenom. Controlled chains were being inhibited by KCN at a much slower rate than the uncontrolled or rapidly respiring chains. It is of interest that KCN *inhibition of* controlled chains appeared to require a constant number of turnovers of cytochrome oxidase. The amount of oxygen consumed as shown in

Figure 4 was roughly the same for the different times of FCCP addition.

Of the other respiratory inhibitors tested, antimycin A gave a small increase in the RCI to about 3 and rotenone gave none. If antimycin A gave a selective inhibitory response, it probably would have been missed by the oxygen analyzer. The time difference between the inhibition of the two types of chains by such a rapidly reacting stoichiometric reagent would be too small to measure.

In summary, addition of KCN to ETP respiring with NADH dramatically increased the respiratory control index. KCN appeared to selectively inhibit chains that were rapidly turning over (uncontrolled chains). This would explain the FCCP sensitive biphasic heterogeneity in the KCN induced reduction of cytochromes b and c + c_1 observed by Lee et al. (4). The chains rapidly reduced would be the uncontrolled chains inhibited quickly by KCN and the chains slowly reduced must be the controlled chains only slowly reacting with KCN.

Most important, the selectivity of KCN for uncoupled chains revealed that in ETP there are two classes of respiratory chains--controlled (RCI ≈ 10) and uncontrolled (RCI ≈ 1). Of the controlled chains, only part can be released by Mg-ADP. Furthermore, coupling appears to have a constant efficiency and observed decreases in the respiratory control index (and perhaps even the P/O ratio) are a result of the appearance of uncoupled respiration.

The results in this paper draw attention to another major question which as yet has no answer. What is the difference between coupled and uncoupled respiration? One possibility is that controlled respiration requires the electron to be separated from the proton (charge separation) (5). Complementary movement of the proton with the electron would lead to uncoupled respiration. These proton movements would only influence the particular chain in question and not the whole membrane as proposed by the chemiosmotic model (6).

Recently, our laboratory has shown that lysolecithin, a reagent that causes fragmentation of the membrane, also increased the RCI of ETP similar to

KCN by selectively inhibiting the respiration of uncontrolled chains (7). The basis for the similarity in action of KCN and lysolecithin is not yet understood.

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