Biochemical Pharmacology, 1966, Vol. 15, pp. 759–760. Pergamon Press Ltd., Printed in Great Britain.

Effect of cyanide upon oxidative phosphorylation of lung mitochondria of guinea pig

(*Received* 24 *May* 1965; *accepted* 23 *December* 1965)

EFFECTS of cyanide upon oxidative phosphorylation have been observed or suggested by several authors in vegetal and animal tissues.[1–7] In vegetal tissues,[1–3] inhibition of oxidative phosphorylation could be observed without inhibition of oxygen uptake, when the system was able to use an alternative nonphosphorylative pathway of electron transport. In other tissues,[4–6] cyanide (1 to 10 mM) inhibited both oxidative phosphorylation and oxygen uptake. In mammalian tissues, however, such effects of cyanide upon oxidative phosphorylation have not been reported.

In a previous paper,[8] an uncoupling action on oxidative phosphorylation was suggested to explain a slow and progressive effect of cyanide on the anaphylactic release of histamine from slices of guinea pig lung previously treated with small concentrations of cyanide (0·01 – 0·1 mM).

The present report will show the effect of cyanide upon oxidative phosphorylation and oxygen uptake in mitochondria isolated from the guinea pig lung tissue.

Mitochondria were removed by the Schneider[9] technique with some modifications. The lung was first homogenized in an ice-cold homogenizing medium (0·225 M mannitol, 0·075 M sucrose, 10 mM buffer (Tris pH 7·4), 0·05 mM EDTA) with the ultra-Turrax apparatus (type 48) at 20,000 rev/min for 10 sec and then with the Potter Elvehjem[10] modified homogenizer for 1 min. To remove cell fragments and nuclei the homogenate was centrifuged at 600 *g* for 10 min (Serval superspeed RC 2), great care being taken not to sediment the mitochondria in a loosely packed white pellet above the residue. The mitochondria were then centrifugated at 12,500 *g* for 10 min and resuspended twice. Mitochondria corresponding to 500 mg of initial wet lung weight, suspended in 0·5 ml of suspension medium (0·225 M mannitol, 0·075 M sucrose, 20 mM Tris buffer, pH 7·4) were used for each experiment. Aliquots were taken off for nitrogen assay (semimicro-Kjeldahl method).

Respiration was determined according to Warburg's direct method. The medium contained in the main chamber 0·01 M succinate, 0·01 M phosphate buffer pH 7·35, 0·002 M ATP, 0·005 M SO_4Mg_2, 0·0125 M FNa, and in the side arm 0·0166 M glucose and 3 mg hexokinase (28,000 KM units/g). The total volume of liquid in the flasks was 2·5 ml, plus 0·2 ml of a mixture of cyanide–alkali[11] added to the center well to avoid absorption of the volatile CNH by alkali. Cyanide was added directly to the mitochondria inside the Warburg flasks. The glucose and hexokinase were added from the side arm after 7 min of thermoequilibration, and oxygen uptake then measured for 25 min at 30°. The reaction was stopped at initial and final time by adding 0·3 ml of 50% cold TCA to the flasks placed on ice. The phosphate uptake was determined by the Gomori[12] technique.

Preliminary results[13] showed that the oxidative phosphorylation of lung mitochondria from guinea pig was lowered if these had been isolated from guinea pig lung previously treated (for 2 hr) with a

TABLE 1. EFFECT OF CYANIDE ON OXIDATIVE PHOSPHORYLATION OF GUINEA PIG LUNG MITOCHONDRIA

Experiment	Mitochondria (mg N)	Cyanide (μM)	0 (μ At/mg N)	P:O
1*	0·50	0	9·5	1·3
		25	3·9	0·3
2	0·47	0	9·9	1·65
		25	5·2	1·05
3	0·56	0	8·8	1·9
		25	4·5	0·9

* Three final and three initial flasks, randomly distributed, were used for each experimental value.

concentration of cyanide higher than 1 mM. Similar results were obtained when the mitochondria were previously incubated with cyanide and then washed, before the measurement of P:O ratio.

The results presented in Table 1, show a direct action of cyanide, without preincubation of the tissue or of the isolated mitochondria. When in contact with the lung mitochondria in the Warburg flasks, a concentration of cyanide as low as 25 μM inhibited the oxidative phosphorylation. Indeed, both the oxygen uptake and the P:O ratio were inhibited in the experiments. The effect upon oxidative phosphorylation, thus, did not appear to be purely an uncoupling effect.

However, in mitochondria isolated from commonly used mammalian tissues, cyanide is known to inhibit oxygen uptake without affecting oxidative phosphorylation, even though it has been shown[14–18] that the intermediary reactions of oxidative phosphorylation are affected by relatively high concentrations of cyanide (around 1 mM).

It has been suggested by Hüllsmann, as reported by Howland,[19] that the difference between a respiratory inhibitor and an uncoupler resides only in the affinity between the compound and the target member of the respiratory apparatus. Indeed, Howland[19] showed that antimycin A, known as a specific inhibitor of cytochrome b, is able to uncouple oxidative phosphorylation in some point of the terminal respiratory chain. According to the view of Hüllsmann, cyanide could thus bind strongly at the cytochrome a_3 to produce its well-known inhibition, and loosely at some other point between transport chain and ATP formation to produce the uncoupling. The fact that cyanide had a stronger effect upon phosphorylation than upon oxidation in mitochondria isolated from guinea pig lung, unlike those isolated from other mammalian tissues, might be due to a different structure and organization of the guinea pig lung mitochondria, with a consequent modification of the affinity of binding between cyanide and one or the other target point.

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