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A SLOW AND PROGRESSIVE ACTION OF CYANIDE ON THE ANAPHYLACTIC REACTION OF GUINEA PIG LUNG SLICES

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Abstract—The effect of pretreatment of sensitized guinea pig lung slices with various concentrations of cyanide on the anaphylactic histamine release and the oxygen uptake was studied.

Pretreatment with 1–10 mM cyanide inhibits both histamine release and oxygen uptake, while pretreatment with 0·01–0·1 mM inhibits histamine release without affecting oxygen uptake. The effect of cyanide on histamine release increases with time of contact and is not reversible by washing or by further incubation in cyanide-free medium.

The addition of succinate has no effect on the histamine release of cyanide-treated slices, although it increases the oxygen uptake. Glucose, adenosine, and ribose remove the inhibiting effect of cyanide pretreatment on histamine release.

Uncoupling of oxidative phosphorylation is suggested as an explanation for the mechanism of the effect of cyanide on the anaphylactic reaction.

THE effect of cyanide on the release of histamine from guinea pig lung during the anaphylactic reaction has been studied by several authors, and somewhat different results have been obtained, dependent on the experimental conditions. Mongar and Schild[1] obtained a 46% inhibition of the anaphylactic release of histamine from guinea pig lung with 2 mM cyanide. Austen and Brocklehurst[2] found a 50% inhibition with 1 mM cyanide after preincubation for 10 sec, but a reversal of this inhibition with longer preincubation. Rotschild and Barreto[3] showed that the anaphylactic release of histamine from guinea pig lung was completely blocked by 1 mM cyanide in absence of glucose, and that this effect was significantly reversed by 1 mM glucose. A possible explanation for the different results might be the use by the first authors[1, 2] of the usual glucose containing Tyrode's buffer, as stated by Austen and Humphrey.[4] Indeed, it has been shown that anaerobiosis,[5] specific inhibitors of the respiratory chain such as antimycin A and carbon monoxide,[6] and uncouplers of oxidative phosphorylation[7] inhibit the anaphylactic release of histamine. Metabolites of the Krebs cycle such as succinate,[8] α-ketoglutarate, acetate, and malate[9] increase the histamine release from guinea pig lung slices. Any of the inhibitions can be reversed by addition of glucose,[3, 10, 11] provided that glycolytic activity is present,[10] showing that the anaphylactic reaction depends upon energy furnished either by aerobic or anaerobic metabolism.

The usual type of inhibition of cytochrome oxidase by cyanide is instantaneous and readily reversible. Nevertheless, other inhibitory actions of cyanide have been shown;

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thus, a slow and irreversible inactivation of succinic dehydrogenase has been demonstrated,[12] and some evidence has been presented for an uncoupling action of cyanide on oxidative phosphorylation.[13, 14]

This paper shows that a slow and progressive inhibition of the release of histamine is obtained by pretreatment of guinea pig lung slices with very small concentrations of cyanide for a sufficiently long time. This effect cannot be explained by inhibition of cytochrome oxidase nor by inactivation of succinic dehydrogenase. Uncoupling of oxidative phosphorylation is proposed as a hypothesis to explain the mechanism of that inhibition. A preliminary report has been presented previously.[15]

MATERIALS AND METHODS

Guinea pigs were sensitized against ovalbumin and used at least 3 weeks after sensitization. The Schultz-Dale reaction was used as a test for sensitization. In each experiment one guinea pig lung was sliced and incubated in the presence of different concentrations of cyanide in Ringer–Barron[16] medium inside covered petri dishes, usually for 2 hr at room temperature, Control experiments without cyanide were also made. Excess of cyanide was removed from the incubated slices by washing several times with the Ringer–Barron medium, then the slices were placed in the Warburg flasks in the same medium at 37°. Antigen was kept in the side arm and tipped into the main chamber after 30-min incubation. In some experiments, metabolites such as succinate, glucose, adenosine, or ribose were added to the Ringer–Barron medium. Uptake of oxygen, calculated for the 30 min after addition of antigen to the slices, was expressed as mm^3/mg dry weight per hr.

Histamine of the supernatant fluid was assayed on the guinea pig ileum and expressed as μg/g wet weight of histamine dihydrochloride. Under each experimental condition, the spontaneous release of histamine was measured in the absence of antigen, and correction for this was made; it was not affected, in fact, by the cyanide pretreatment or by the substances added to the Warburg flasks. Nor did the cyanide pretreatment affect the total amount of histamine of the guinea pig lung slices. The influence upon the bioassay procedure of the cyanide added during the pretreatment or of the substances added to the Warburg flasks was also quantitatively tested by adding to the histamine standard an aliquot from the spontaneous supernatant from each control.

The Robbie technique,[17] modified by two of us,[18] was used for estimating the remaining cyanide in the tissue and supernatant fluid at the end of the experiment.

RESULTS

The effect of previous treatment of sensitized guinea pig lung slices with various concentrations of cyanide was studied upon the oxygen uptake and the anaphylactic release of histamine. The completeness of removal of excess cyanide by washing the pretreated tissue was tested. No cyanide was detected either in homogenized tissue or supernatant medium in an experiment in which the lung slices were pretreated by 10 mM cyanide, washed, and incubated in Warburg flasks. These results indicate that less than 5 μg free cyanide remained in the tissue or supernatant medium.

Table 1 shows that pretreatment with 1–10 mM cyanide (2 hr) at room temperature inhibited strongly the histamine release and to a lesser extent the oxygen uptake; 0·01–0·1 mM cyanide pretreatment still inhibited the histamine release by 30–60%,

without significantly inhibiting the oxygen uptake; it may even have increased it slightly.

Table 2 shows that the effect of cyanide on the histamine release is a slow and progressive one, since the release of histamine is depressed only after cyanide has acted upon the tissue for a long time. The effect of cyanide is also observed when the tissue is incubated at 4°.

TABLE 1. EFFECT OF PRETREATMENT WITH CYANIDE ON OXYGEN UPTAKE AND HISTAMINE RELEASE BY SENSITIZED GUINEA PIG LUNG SLICES: INFLUENCE OF THE CONCENTRATION OF CYANIDE

Cyanide* (mM)	Oxygen uptake			Histamine release		
	QO_2	Change (%)	P†	(μg/g wet wt.)	Inhibition (%)	P†
Control	4·24 ± 0·05 (3)‡			1·80 ± 0·23 (3)		
1	3·73 ± 0·24 (3)	−12	<0·1	0·45 ± 0·22 (3)	75	<0·02
5	2·24 ± 0·17 (3)	−47	<0·01	0·30 ± 0·06 (3)	83	<0·01
Control	4·19 ± 0·16 (4)			13·00 ± 1·2 (4)		
1	3·30 ± 0·11 (4)	−21	<0·01	4·90 ± 0·9 (4)	62	<0·01
Control	4·39 ± 0·31 (4)			6·55 ± 0·31 (4)		
1	3·12 ± 0·10 (4)	−29	<0·01	0·95 ± 0·25 (4)	85	<0·01
Control	4·52 ± 0·15 (3)			5·35 ± 0·25 (3)		
0·1	4·94 ± 0·08 (3)	+9	<0·1	2·00 ± 0·58 (3)	62	<0·01
0·5	4·03 ± 0·12 (3)	−11	<0·1	1·80 ± 0·05 (3)	68	<0·01
Control	4·74 ± 0·18 (3)			12·50 ± 1·1 (3)		
0·01	4·98 ± 0·23 (3)	+5	>0·1	8·50 ± 0·58 (3)	32	<0·05
0·1	3·73 ± 0·17 (3)	−21	<0·02	6·00 ± 0·25 (3)	52	<0·01
Control	4·08 ± 0·26 (3)			1·25 ± 0·20 (2)		
0·01	4·58 ± 0·14 (3)	+12	>0·1	1·10 ± 0·17 (3)	12	>0·1
0·1	4·52 ± 0·31 (3)	+11	>0·1	0·6 ± 0·08 (3)	48	<0·05

* Pretreatment, 2 hr.

† Determined by Student's "t" test.

‡ Mean ± S.E.; number of Warburg flasks in parentheses.

Even successive washings of cyanide-pretreated tissue slices on longer time of incubation in cyanide-free Ringer–Barron medium failed to remove the inhibition due to cyanide (Table 3).

The influence of electrolytes was studied (Table 4). A saccharose–mannitol medium containing a calcium-chelating agent such as EDTA was used, either during the previous treatment by cyanide, or during the cyanide removal treatment; antigen was always added to the tissue in the Ringer–Barron medium. The same inhibitory effect of cyanide was observed in the saccharose–mannitol as in the Ringer–Barron medium, showing that electrolytes had no influence on the inhibition of histamine release by the cyanide, even though the control histamine release was potentiated by previous incubation in the electrolyte-free medium.

Tsou showed that succinic dehydrogenase was effectively protected from the action of cyanide by succinate.[12] Figure 1 shows that the presence of 10 mM succinate during

TABLE 2. EFFECT OF PRETREATMENT WITH CYANIDE ON OXYGEN UPTAKE AND HISTAMINE RELEASE BY SENSITIZED GUINEA PIG LUNG SLICES: INFLUENCE OF TIME AND TEMPERATURE

Incubation			Oxygen uptake			Histamine release		
Cyanide (mM)	Time (hr)	Temperature °C	QO_2	Change (%)	P	(μg/g wet wt.)	Change (%)	P
Control	1	room	4·83 ± 0·11 (3)			15·5 ± 0·6 (3)		
0·1	1	room	5·15 ± 0·13 (3)	+7	>0·1	14·0 ± 1·7 (3)	−10	>0·1
Control	3	room	4·59 ± 0·23 (3)			8·6 ± 0·73 (3)		
0·1	3	room	4·29 ± 0·18 (3)	−6*	>0·1*	4·2 ± 0·24 (3)	−51*	<0·01*
Control	2	room	3·95 ± 0·10 (2)			3·6 ± 0·23 (2)		
0·1	2	room	3·94 ± 0·11 (2)	0	>0·1	1·3 ± 0·27 (2)	−64	<0·05
Control	2	4	4·10 ± 0·0 (2)			5·8 ± 0·17 (2)		
0·1	2	room	4·09 ± 0·01 (2)	0†	>0·1†	2·8 ± 0·05 (2)	−51†	<0·01†

Conditions as in Table 1.

* Between this mean and the 3-hr control mean.

† Between this mean and the 4° control mean.

TABLE 3. EFFECT OF CYANIDE PRETREATMENT ON OXYGEN UPTAKE AND HISTAMINE RELEASE BY SENSITIZED GUINEA PIG LUNG SLICES: INFLUENCE OF TREATMENT DESIGNED TO REMOVE CYANIDE

Cyanide (mM)	Removal treatment (washings and incubation)	Oxygen uptake			Histamine release		
		QO_2	Change (%)	P	(μg/g wet wt.)	Inhibition (%)	P
0	none*	4·68 ± 0·09 (3)			3·57 ± 0·10 (3)		
0·1		4·05 ± 0·26 (3)	−13	>0·1	0·44 ± 0·16 (3)	88	<0·01
0	3 hr at room temp.	3·05 ± 0·10 (3)			1·44 ± 0·17 (3)		
0·1		3·13 ± 0·35 (3)	+3†	>0·1†	0·36 ± 0·03 (3)	75†	<0·01†
0	none*	5·35 ± 0·05 (2)			26·2 ± 2·1 (2)		
0·1		4·70 ± 0·3 (2)	−12	>0·1	5·6 ± 1·3 (2)	79	<0·01
0	24 hr at 4°	3·36 ± 0·18 (2)			2·1 ± 0·6 (2)		
0·1		3·0 ± 0·26 (2)	−11‡	>0·1‡	0·3 ± 0·1 (2)	86‡	<0·01‡

Conditions as in Table 1.

* Usual washing treatment.

† Between this mean and the 3-hr control mean.

‡ Between this mean and the 24-hr control mean.

TABLE 4. EFFECT OF PRETREATMENT WITH CYANIDE ON THE OXYGEN UPTAKE AND HISTAMINE RELEASE BY SENSITIZED GUINEA PIG LUNG SLICES: INFLUENCE OF ELECTROLYTE-FREE INCUBATION MEDIUM

Cyanide pretreatment		Cyanide removal treatment		Histamine release		
Cyanide (mM)	Medium	Medium	QO_2	(μg/g wet wt.)	Change (%)	P
0	Ringer–Barron (2 hr)	none*	4·02 ± 0·03 (2)	16·8 ± 1·5		
0·1	Ringer–Barron (2 hr)		3·10 ± 1·0 (2)	4·4 ± 1·00	−74	<0·01
0	Saccharose† (2 hr)	none*	3·50 ± 0·23 (2)	20·1 ± 2·25		
0·1	Saccharose† (2 hr)		3·44 ± 0·16 (2)	6·5 ± 0·42	−68‡	<0·05‡
0	Ringer–Barron (1 hr)	Ringer–Barron (2 hr)	3·86 ± 0·08 (2)	11·2 ± 1·7		
0·1	Ringer–Barron (1 hr)	Ringer–Barron (2 hr)	4·05 ± 0·15 (2)	8·8 ± 0·1	−21	>0·1
0	Ringer–Barron (1 hr)	Saccharose† (2 hr)	3·57 ± 0·13 (2)	21·2 ± 0·0		
0·1	Ringer–Barron (1 hr)	Saccharose† (2 hr)	3·72 ± 0·12 (2)	14·6 ± 2·2	−31‡	<0·1‡

Conditions as in Table 1.

* Usual washing treatment.

† Saccharose–mannitol medium (saccharose 0·0225 M; mannitol, 0·075 M; Tris, 10 mM; EDTA, 0·05 mM).

‡ Between this mean and the saccharose–mannitol control mean.

the cyanide pretreatment afforded no protection against cyanide action upon histmine release.

The influence of some metabolites upon oxygen uptake and histamine release was studied (Table 5). After pretreatment of slices by cyanide, the addition of succinate increased the oxygen uptake; this showed that succinic dehydrogeanse was still active;

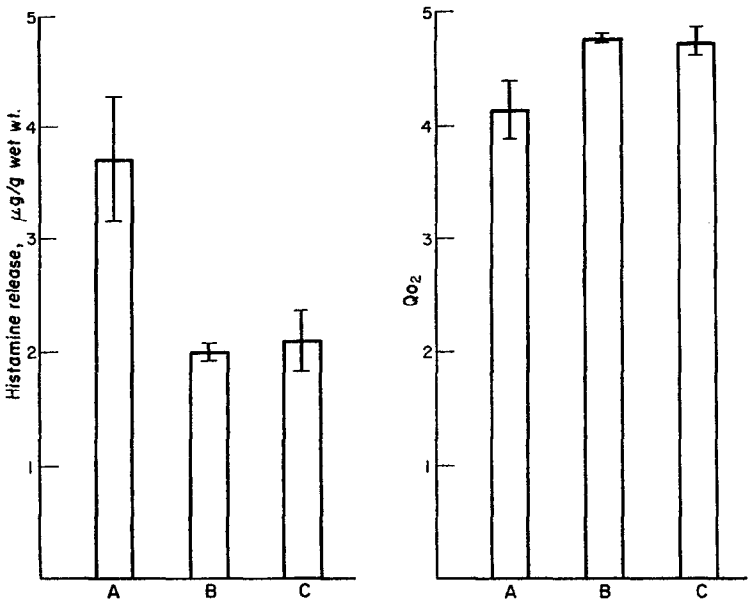

FIG. 1. Effect of the presence of succinate during the pretreatment with cyanide upon the oxygen uptake and histamine release by sensitized guinea pig lung slices. The means of three experiments are shown with standard error.
A = Control; B = preincubated with 0·1 mM cyanide; C = preincubated with cyanide + 10 mM succinate.

however, the addition of succinate did not remove the inhibition of histamine release. Glucose removed the inhibition caused by cyanide pretreatment, as did adenosine and ribose.

DISCUSSION

The present results show that inhibition of histamine release after previous treatment by low concentrations of cyanide cannot be attributed to the influence of cyanide upon cytochrome oxidase, since oxygen uptake was not affected by the concentrations of cyanide that inhibited the release of histamine. Nevertheless, the oxygen uptake measured was that of the whole lung tissue, and it might not be representative of the oxygen uptake of the mast cells from which the histamine is released. The action of cyanide upon cytochrome oxidase, is instantaneous and readily reversible, whereas our results show that the effect upon histamine release was produced only after cyanide has acted upon the tissue for a certain time, and that it was not reversible by further washings even for long periods of time and in different media.

The effect of cyanide cannot be attributed to an inactivation of succinic dehydrogenase,[12] since succinate had no protective action during cyanide incubation, and the

TABLE 5. INFLUENCE OF VARIOUS SUBSTRATES ON THE OXYGEN UPTAKE AND HISTAMINE RELEASE FROM GUINEA PIG LUNG SLICES PREVIOUSLY TREATED WITH CYANIDE

Cyanide pretreatment (mM)	Substrate addition (mM)	Oxygen uptake			Histamine release		
		QO_2	Change (%)	P	(μg/g wet wt.)	Change (%)	P
0	0				4·0 ± 1·3 (2)		
0	Succinate 10				7·3 ± 0·2 (2)	+80	>0·1
0·1	0				1·7 ± 0·4 (2)	−57	>0·1
0·1	Succinate 10				2·0 ± 0·1 (2)	−50	>0·1*
0	0	3·3 ± 1·3 (2)			1·7 ± 0·3 (2)		
0	Succinate 10	4·7 ± 1·3 (2)	+42	>0·1	2·3 ± 0·1 (2)	0	>0·1
1	0	3·5 ± 0·18 (3)	+6	>0·1	0·0 ± 0·03 (3)	−100	<0·01
1	Succinate 10	6·0 ± 0·16 (3)	+82	<0·01*	0·0 ± 0·03 (3)	−100	
0	0	4·8 ± 0·51 (2)			3·8 ± 0·3 (2)		
0	Glucose 10	5·4 ± 0·06 (2)	+12	>0·1	5·0 ± 0·01 (2)	+32	<0·1
0·1	0	5·0 ± 0·63 (2)	+ 4	>0·1	1·8 ± 0·07 (2)	−48	<0·05
0·1	Glucose 10	6·4 ± 0·33 (2)	+33	>0·1*	5·5 ± 1·0 (2)	−45	<0·1*
0	0	4·8 ± 0·11 (3)			0·9 ± 0·06 (3)		
0·1	0	5·5 ± 0·58 (3)	+15	>0·1	0·6 ± 0·20 (3)	−33	>0·1
0·1	Adenosine 1	5·6 ± 0·23 (3)	+16	>0·1*	1·8 ± 0·52 (3)	+100	>0·1*
0	0	4·5 ± 0·27 (3)			4·8 ± 0·38 (3)		
0·1	0	5·0 ± 0·37 (3)	+10	>0·1	1·5 ± 0·10 (3)	−69	<0·01
0·1	Ribose 1	4·6 ± 0·27 (3)	+2	>0·1*	4·8 ± 0·21 (3)	0	<0·01*

Conditions as in Table 1.

* Between this mean and the cyanide-treated control mean.

presence of succinate during the anaphylactic reaction increased oxygen uptake without reducing the inhibition of histamine release. Furthermore, the concentrations of cyanide used by Tsou to obtain inactivation of succinic-dehydrogenase were much higher (5–50 mM) than those able to inhibit histamine release.

Previous work has shown the dependence of the histamine release upon phosphorylated energy-rich compounds, furnished either by oxidative or glycolytic metabolism.[7, 10] The present results, then, suggest that the slow and progressive action of low concentrations of cyanide might be caused by an irreversible uncoupling of oxidative phorphorylation. Indeed, Kubista and Urbankova[13] have given some evidence for the uncoupling action of cyanide upon oxidative phosphorylation in the metathoracic musculature of *Periplaneta americana*. Hackett and Haas[19, 20] showed that low concentrations of cyanide (0·5 mM) can inhibit phosphorylation without affecting the rate of oxygen uptake in mitochondria isolated from aged skunk cabbage spadix and aged potato slices. In mitochondria isolated from various microorganisms, cyanide was also found to uncouple oxidative phosphorylation.[14, 21] Studies currently in progress to investigate the effect of pretreatment of guinea pig lung slices by cyanide on oxidative phosphorylation in mitochondria isolated from these slices, have shown an inhibiting effect on the P/O ratio.[22] The uncoupling action of cyanide should be verified upon oxidative phosphorylation of isolated mast cells.

The inhibitory effect of cyanide was observed equally well in an electrolyte-free medium containing a calcium-chelating agent (EDTA). This indicated that it should not be an indirect effect, due to an increased permeability which favored mitochondrial injury allowing an intracellular accumulation of calcium. The cyanide effect, such as that of "aging",[23] is slow and increases with time, both effects being removed by addition of glucose, adenosine, or ribose. However, while "aging" is known to release a naturally occurring uncoupling factor from the aged mitochondria,[24] there is still no evidence about the mechanism of cyanide effect.

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