Biochemical Pharmacology, 1962, Vol. 11, pp. 603–608. Pergamon Press Ltd., Printed in Great Britain.

INFLUENCE OF INHIBITORS OF THE RESPIRATORY CHAIN ON THE RELEASE OF HISTAMINE DURING THE ANAPHYLACTIC REACTION *IN VITRO*. ACTION OF ANTIMYCIN A AND CARBON MONOXIDE

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(*Received* 9 *February* 1962; *accepted* 23 *February* 1962)

Abstract—Experiments were done to determine the effect of antimycin A and carbon monoxide, two inhibitors of specific steps of the respiratory chain, upon the release of histamine in anaphylaxis.

Antimycin A at concentrations higher than 1·7 μg/g of wet weight tissue, and carbon monoxide at concentrations of 75–88%, significantly inhibit the release of histamine; light inhibits the influence of carbon monoxide.

The results are consistent with the hypothesis that the respiratory chain is involved in the mechanism of liberation of histamine in anaphylaxis, probably by furnishing phosphorylated energy-rich compounds.

PREVIOUSLY it has been shown that several metabolites increase[1, 2] and uncouplers of oxidative phosphorylation inhibit,[3] the histamine released in the anaphylactic reaction *in vitro*. We considered it of interest to investigate the influence of inhibitors of the respiratory chain on the release of histamine in anaphylaxis.

Experiments were carried out with some specific inhibitors of the respiratory chain; the results with antimycin A, an inhibitor of electron transport between cytochrome b and c, and carbon monoxide, an inhibitor of cytochrome oxidase (cytochrome a_3), are given in this paper.

An abstract of these results was presented in 1959.[4] Subsequently, Austen and Brocklehurst[5] also studied the effect of carbon monoxide on the inhibition of histamine release and found results that contradicted ours. These divergences may be explained by the different experimental conditions, and they will be discussed later.

MATERIALS AND METHODS

Lungs of guinea pigs, sensitized against horse serum, were sliced and incubated in the main chamber of Warburg flasks containing 2·5 ml of Ringer–Barron[6] suspension medium, at 37 °C. Oxygen or carbon monoxide was used as gas phase, and the oxygen uptake measured every 15 or 30 min for 1 or 2 hr after thermoequlibration.

Antimycin A (200 μg/ml in 95% ethyl alcohol) in the required amount was incubated with the tissues; the same volume of 95% alcohol was added to the control flasks. Antimycin A was kept in contact with the lung slices 1 hr before the addition of antigen. Horse serum (0·05 ml) was kept in the side arm of the Warburg flasks and

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tipped into the main chamber at the appropriate time. In some of the experiments succinate or α-ketoglutarate was added to the suspension fluid together with antimycin A. Uptake of oxygen was calculated as mm^3/mg dry weight/hr after tissue slices were dried. Each figure was the mean of no fewer than two Warburg flask determinations.

Histamine of the supernatant fluid was assayed on the guinea-pig ileum and calculated as μg/g wet weight of histamine dihydrochloride. Neither antimycin A nor metabolites influenced significantly the spontaneous release of histamine in the controls, these figures being subtracted from the antigen-release values. The Schultz–Dale reaction was used as test for sensitization.

Carbon monoxide was prepared in the laboratory by the Döbereiner method (reaction between formic acid and concentrated sulfuric acid) and after purification was mixed with oxygen and nitrogen; the concentration was measured in an Orsat apparatus with cuprous chloride for the carbon monoxide and pyrogallol for the oxygen.

Photochemical decomposition of the carbon monoxide-binding and photochemical reversal of the inhibitory effect of carbon monoxide was tested by illuminating half of the Warburg flasks used and wrapping the other half with a dark cloth.

TABLE 1. ACTION OF ANTIMYCIN A ON THE UPTAKE OF OXYGEN AND RELEASE OF HISTAMINE BY ANTIGEN FROM SENSITIZED LUNG SLICES OF GUINEA PIG

Experimental* conditions (% Alcohol)	Antimycin conc. (μg/g tissue)	QO_2†	Histamine release	
			(μg/g wet wt)	(% of control)
Control (1·0)		6·09 ± 0·25§	3·3 ± 0·3	100
Antimycin A (2 μg/ml)	60	0·94 ± 0·12**	0·0 ± 0·3**	0
Control (0·5)		6·53 ± 0·49	3·7 ± 0·2	100
Antimycin A (1 μg/ml)	38	0·71 ± 0·14**	0·2 ± 0·1**	5
Control (0·1)		4·43 ± 0·43	3·5 ± 0·1	100
Antimycin A (0·2 μg/ml)	7·5	1·54 ± 0·56**	0·1 ± 0·3**	3
Control (0·1)		5·50 ± 0·31	2·2 ± 0·0	100
Antimycin A (0·2 μg/ml)	6·4	2·72 ± 0·18**	0·1 ± 0·1**	5
Control (0·1)		5·92 ± 0·20	3·9 ± 0·2	100
Antimycin A (0·2 μg/ml)	4·9	3·34 ± 0·09**	0·5 ± 0·1**	13
Control (0·05)		5·95 ± 0·22	12·0 ± 0·8	100
Antimycin A (0·1 μg/ml)	2·8	4·65 ± 0·30**	5·0 ± 0·2**	42
Control (0·025)		5·50 ± 0·35	12·4 ± 0·8	100
Antimycin A (0·05 μg/ml)	1·7	4·30 ± 0·19**	5·9 ± 0·1	48
Control (without alcohol)		5·97 ± 0·30	2·9 ± 0·2	100
Antimycin A (0·1 μg/ml)	4·1	4·70 ± 0·08**	0·9 ± 0·2**	31
Antimycin A (0·05 μg/ml)	1·7	5·33 ± 0·16††	0·9 ± 0·2**	31
Control (0·05)		5·29 ± 0·12	2·1 ± 0·2	100
Antimycin (0·01 μg/ml)	0·3	5·57 ± 0·40	2·0 ± 0·1	95

* Addition 1 hr before the antigen was tipped.
† Figures taken 30 min after antigen addition.
‡ Antimycin in the same alcohol concentration as control.
§ Mean of 3 Warburg flasks with standard deviation.
** $P < 0·01$.
†† $P < 0·05$.

RESULTS

Inhibition by antimycin A of the histamine released from lung-slices of sensitized guinea-pig; influence of succinate and α-ketoglutarate

Antimycin A is almost insoluble in water but is soluble in ethyl alcohol. Control experiments showed that no decrease in release of histamine was observed at concentrations of 0·1% ethyl alcohol.

Table 1 shows the uptake of oxygen and release of histamine by sensitized lung tissues after the addition of antimycin A and antigen. Each experimental series includes control flasks containing ethyl alcohol at the same concentration as that in the flasks with antimycin A. This factor was taken into account in calculating the statistical significances.

Antimycin A did not significantly inhibit the release of histamine and the uptake of oxygen until the amount of 0·05 μg/ml (1·7 μg/g of wet weight) was reached. It can be seen that at low concentrations of antimycin A, such as 0·2 μg/ml, when the ethyl alcohol concentration scarcely interferes, inhibition of histamine release was practically complete.

Table 2 shows the effects of succinate and α-ketoglutarate as substrates. Both substrates increased the histamine released, and antimycin A inhibited this action.

TABLE 2. ACTION OF ANTIMYCIN A ON THE OXYGEN UPTAKE AND HISTAMINE-RELEASE BY ANTIGEN FROM SENSITIZED LUNG SLICES OF GUINEA PIG, IN THE PRESENCE OF METABOLITES

Experimental conditions*	QO_2†	Histamine release	
		(μg/g wet wt)	(% of control)
Control‡	5·64 ± 0·06§ (2)	7·2 ± 0·6§ (2)	100
Succinate (0·01 M)	9·27 ± 0·27 (3)††	16·0 ± 1·1 (3)††	222
Antimycin A (6 μg/g tissue)	3·00 ± 0·32 (2)††	1·0 ± 0·1 (2)††	14
Succinate + antimycin A	3·38 ± 0·40 (3)††	2·8 ± 0·3 (3)††	40
Control**	6·03 ± 0·17 (2)	2·6 ± 0·2 (2)	100
Succinate (0·01 M)	9·01 ± 0·30 (3)††	4·5 ± 0·2 (3)††	173
Antimycin A (14·2 μg/g tissue)	2·10 ± 0·08 (2)††	0·2 ± 0·1 (2)††	8
Succinate + antimycin A	2·42 ± 0·12 (3)††	0·2 ± 0·1 (3)††	8
Control**	6·75 ± 0·0 (2)	3·5 ± 0·2 (2)	100
α-Ketoglutarate (0·01 M)	7·44 ± 0·19 (3)§§	7·5 ± 0·4 (3)††	183
Antimycin A (10·7 μg/g tissue)	2·69 ± 0·24 (2)††	0·4 ± 0·0 (2)††	11
α-Ketoglutarate + antimycin A	2·36 ± 0·14 (3)††	0·4 ± 0·0 (3)††	11

* Compounds were added 1 hr before antigen was tipped.
† Figures taken 30 min after antigen tipping.
‡ Same alcohol concentration as in metabolite and antimycin flasks: 0·1%.
§ Mean with standard deviation; number of flasks in parentheses.
** Same alcohol concentration as in metabolite and antimycin flasks: 0·25%.
†† $P < 0·01$, by "t" test between this mean and the control mean.
§§ $P < 0·05$.

Inhibition by carbon monoxide of the histamine released from lung-slices of sensitized guinea pig; effect of removing light

Table 3 shows that carbon monoxide at concentrations higher than 75% inhibits significantly the release of histamine as well as the uptake of oxygen by the tissue. The

values of oxygen uptake in the control flasks for the experiments with carbon monoxide were lower than those for antimycin A, since air or lower tensions of oxygen were used in the gas phase.

Light removed the inhibition of histamine release and cell respiration caused by carbon monoxide. The values obtained in the light were significantly higher than those obtained in the dark (Student's '*t*' test).

TABLE 3. ACTION IN THE DARK AND IN THE LIGHT OF CARBON MONOXIDE ON OXYGEN UPTAKE AND HISTAMINE RELEASE IN THE ANAPHYLACTIC REACTION *in vitro*

Experimental conditions Gases (mixture in %)				QO_2	Histamine released (μg/g wet wt)	% of controls	
CO	O_2	N_2				QO_2	Histamine released
	Air			3·51 ± 0·09	5·1 ± 0·2	100	100
75	20	5	dark	3·36 ± 0·10	3·4 ± 0·2*	96	67
75	20	5	light	3·46 ± 0·19	4·2 ± 0·2†	98	83
0	11·8	88		2·49 ± 0·12	2·7 ± 0·25	100	100
82·4	11·6	6	dark	1·89 ± 0·13*	0·9 ± 0·1	76	33
82·4	11·6	6	light	2·36 ± 0·02‡	1·8 ± 0·0‡	95	67
0	11	89		2·32 ± 0·10	11·6 ± 0·5	100	100
83	11	6	dark	1·57 ± 0·03*	2·3 ± 0·4*	68	20
83	11	6	light	2·59 ± 0·02‡	8·0 ± 1·5‡	111	69
0	11	89		2·67 ± 0·13	3·1 ± 0·1	100	100
83	11	6	dark	1·80 ± 0·14*	0·7 ± 0·1	67	22
83	11	6	light	2·32 ± 0·08‡	1·9 ± 0·5	87	61
0	11	89		3·05 ± 0·34	9·2 ± 0·75	100	100
88	11	1	dark	1·87 ± 0·24	1·8 ± 0·15*	61	20
88	11	1	light	2·64 ± 0·15‡	4·6 ± 0·3‡	87	50

The sensitized lung slices of guinea-pig were incubated 50 min in the gas mixture before the addition of antigen. Every figure represents the mean of a triplicate test, with standard deviation.
* $P < 0·01$ by "*t*" test between this mean and the control mean without CO.
† $P < 0·05$ and ‡ $P < 0·01$ by "*t*" test between the light and dark means.

DISCUSSION

We have shown in several papers[1, 2] that some metabolites such as succinate, α-ketoglutarate, acetate, and malate,[7] or such inhibitors of oxidative phosphorylation[3] as 2,4-dinitrophenol and pentochlorophenol, can influence the release of histamine by anaphylaxis *in vitro*. This suggested a close relationship between release of histamine and cellular metabolism, especially a utilization of energy-rich compounds in the mechanism of histamine release. In the present paper it has been shown that antimycin A and carbon monoxide, two inhibitors of specific steps of the respiratory chain, can inhibit *in vitro* the release of histamine, by the antigen–antibody reaction, from slices of sensitized guinea-pig lung.

Several authors[8, 9] have shown that the antibiotic, antimycin A, is a specific inhibitor of electron transport by means of an action on a component of the respiratory chain referred to as the "Slater factor", which acts between cytochrome *b* and c_1 in the succinoxidase system and between diaphorase and cytochrome c_1 in the DPN system. It was shown that the ratio between the amount of antimycin A and the amount of tissue for QO_2 inhibition was very small, indicating that antimycin A must react

very selectively with this component. In our experiments an amount of antimycin A as low as 1·7 μg/g of tissue was enough to inhibit more than 50% of the histamine release, a figure very similar to that obtained in oxidative systems by other authors, which suggests a similar mode of action.

Such metabolites as succinate and α-ketoglutarate, which increase the amount of histamine released in anaphylaxis, were not effective after antimycin A action. Since antimycin A inhibits specifically a factor in electron transport necessary for the oxidation of several substrates, including succinate and α-ketoglutarate, in the phosphorylative oxidation system of the Krebs cycle[9] (see Table 2), the inhibited release of histamine, observed in our experiments, might be expected.

Warburg demonstrated that carbon monoxide inhibits the respiration of living cells in the dark and that this inhibition can be abolished by light.[10] High concentrations of carbon monoxide are employed to inhibit the cellular respiration, net inhibition of respiration being obtained with a concentration of about 80%. Later studies showed that carbon monoxide forms a complex with the iron of the prosthetic group of the enzyme, cytochrome oxidase.

In our experiments an 80% inhibition of the release of histamine was obtained only when concentrations as high as 75 to 88% of carbon monoxide were used. Light inhibits the influence of CO. No greater concentrations of carbon monoxide were used because concentrations lower than 10% of oxygen inhibit release of the histamine by anoxic interference.[11] These experiments indicate that cytochrome oxidase was involved in the mechanism of histamine release, since this enzyme is very sensitive to carbon monoxide.

Austen and Brocklehurst[5] recently failed to inhibit by carbon monoxide the release of histamine in chopped or perfused guinea-pig lung, and concluded that cytochrome-mediated aerobic metabolism is not required for release of histamine in anaphylaxis. We have shown lately[12] that the inhibition by anaerobic conditions of the anaphylactic release of histamine by guinea-pig and rat lung can be removed by glucose. We found that inhibitors of glycolysis, such as sodium monoiodoacetate and sodium fluoride, inhibit completely the anaerobic release of histamine in the presence of glucose, while 2,4-dinitrophenol, uncoupler of oxidative phosphorylation but not of anaerobic phosphorylation, has no effect.[12] Other authors[14–17] have also shown in several histamine-release systems that glucose removed the inhibition of histamine release by anoxia, dinitrophenol, and sodium cyanide.

These results suggest that, when the furnishing of energy-rich compounds from the cytochrome-mediated aerobic metabolism is blocked, other energy-rich compounds, arising from glycolytic anaerobic metabolism upon addition of glucose, become a source of energy available for the histamine-releasing process. The results of Austen and Brocklehurst might possibly be explained by their use of Tyrode's solution as an incubating medium, since such a solution usually contains glucose.

In conclusion, there appears to be good evidence that the respiratory chain is normally involved in the release of histamine in anaphylactic studies *in vitro*, probably by furnishing phosphorylated energy-rich compounds for a reaction that requires energy.

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