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THE EFFECT OF ETHANOLAMINE ON CHANGES IN LUNG LIPIDS INDUCED BY ANAPHYLAXIS

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Abstract—It has recently been reported from this laboratory that ethanolamine inhibits the release of SRS-A (slow-reacting substance of anaphylaxis) in sensitized guinea pigs lungs injected with specific antigen. The known effects of ethanolamine on phospholipid synthesis and turnover thus prompted an investigation of the effects of anaphylaxis on the lipid content of guinea pig lungs and the influence thereon of anti-anaphylactic doses of ethanolamine. Anaphylaxis caused alterations in the lipid content of sensitized lungs, especially the triglyceride, cephalin, lecithin and sphingomyelin fractions. Lungs from animals which had been pretreated with ethanolamine and which had liberated no SRS-A during anaphylactic shock showed smaller alterations in their lipid content.

PILGERAM and his co-workers[1] studied the metabolic fate of ^{14}C and ^{15}N labelled ethanolamine in rats using intraperitoneal dosage of the order of 10 mg/kg and concluded that approximately 10 per cent of the dose administered was metabolized to carbon dioxide and ammonia. The remainder was incorporated into glycerophosphatide either unchanged or after conversion to choline or serine. Smith[2] was able to potentiate the anti-anaphylactic activity of mepyramine and promethazine in sensitized guinea pigs exposed to an aerosol of specific antigen using intramuscular dosage of the same order. This effect was considered to be due to the ability of ethanolamine to inhibit the release of SRS-A during anaphylaxis, and evidence was obtained under *in vitro* conditions of anaphylaxis showing that ethanolamine possessed such a property. If it is assumed that the metabolic fate of ethanolamine in the guinea pig is the same as that in the rat, these findings imply that during anaphylaxis the inhibited release of SRS-A (which is a lipid-soluble acid[3, 4]) might be brought about by its prior incorporation into glycerophosphatide together with ethanolamine, or choline or serine derived from ethanolamine *in vivo*. The following preliminary investigation was therefore undertaken to examine the lung lipids of the guinea pig for any changes induced by anaphylaxis and the effects thereon of anti-anaphylactic doses of ethanolamine.

EXPERIMENTAL AND RESULTS

Pharmacological

Anaphylactic shock was induced in intact guinea pig lungs undergoing perfusion through the pulmonary artery with Tyrode solution at 37 °C as described by Brocklehurst.[5] The perfusate was collected for 30 min after antigen administration, centrifuged to remove blood cells and then examined for histamine and SRS-A using the techniques

of Brocklehurst.[5] Three groups of three animals were used. One group (I) was subjected to anaphylactic shock as described above. One group (II) was perfused with Tyrode solution but was injected with normal saline instead antigen. A third group (III) was pretreated with single intramuscular injections of ethanolamine hydrochloride in distilled water equivalent to 200 mg of ethanolamine per kilogram on each of the 2 days preceding the day of anaphylactic shock and with a third dose 1 hr before excision of the lungs. All animals used in the experiment had been sensitized to egg albumin by the subcutaneous injection of 100 mg as a 5 per cent solution in distilled water 28 days before experiment. The results of the pharmacological analysis of the lung perfusates are shown in Table 1. It can be observed that there was no spontaneous release of either histamine or SRS-A during perfusion alone; that both

TABLE 1. PHARMACOLOGICAL ANALYSIS OF PERFUSATES FROM GUINEA PIG LUNGS UNDERGOING ANAPHYLAXIS

	Perfused antigen injected		Ethanolamine treated perfused antigen injected		Perfused	
	Group I		Group III		Group II	
	Histamine (μg)	SRS-A (units)	Histamine (μg)	SRS-A (units)	Histamine (μg)	SRS-A (units)
	26·5	530	28·0	nil	nil	nil
	20·4	510	36·0	nil	nil	nil
	12·8	640	12·6	nil	nil	nil
Mean	19·9	560	25·5	nil	nil	nil
s.d.	6·9	20	12·9			

s.d. = standard deviation; nil indicates no response to undiluted perfusate.

histamine and SRS-A were released after antigen injection; and that pretreatment with ethanolamine inhibited the anaphylactic release of SRS-A but not histamine.

Biochemical

Immediately after collecting the perfusate from each lung, the tissue was chopped into small pieces on blotting paper, which absorbed the oedema fluid present, and then freeze dried overnight in a suitably sized stoppered test tube. Each freeze dried lung was then stored in nitrogen at − 20 °C until required for lipid analysis. The tissue was ground to powder in a small glass mortar and then subjected to three 24 hr extractions with twenty times its own weight of chloroform: methanol combined in 2:1 proportions. The extracts obtained from each experimental group of animals were then combined and washed with ten times their volume of normal saline. Each washed extract was then chromatographed on silicic acid and separated into thirteen major lipid fractions which were characterized and estimated by suitable gravimetric and chemical procedures as described by Turner and his co-workers.[6] Since the control lungs for the pharmacological investigations (group II) had all been perfused for 30 min, with Tyrode solution, three unperfused sensitized guinea pig lungs were washed free of blood,[7] chopped, freeze dried, and subjected to the same procedure.

The results of the lipid analyses are shown in Table 2. Anaphylaxis caused appreciable falls in most of the lipid fractions examined, and these changes were less pronounced in lungs taken from animals which had been pretreated with ethanolamine.

DISCUSSION

The results obtained in this investigation suggest that lipid is lost from lung tissue during anaphylaxis. The only exceptions in the thirteen fractions examined were the

TABLE 2. LIPID ANALYSES OF GUINEA PIG LUNGS AFTER ANAPHYLAXIS

	Group II	Group I	Group III	
	Perfused	Perfused antigen injected	Ethanolamine treated perfused antigen injected	Chopped
Cholesterol ester	14·7	11·6	12·7	10·3
Triglyceride	46·8	31·2	44·7	52·8
Cholesterol	20 0	22·8	13·6	12·1
Free fatty acid	7·0	8·2	7·8	7·6
Diglyceride	14·4	8·8	9·2	3·7
Monoglyceride	9·3	5·0	5·3	14·1
Phosphatidic acid	9·6	6·6	4·9	7·3
Phosphatidylglycerol	19·5	2·6	6·2	8·9
Unidentified phospholipid	15·6	13·6	9·9	3·9
Cephalin	34·8	16·4	25·4	22·0
Lecithin	37·8	20·4	51·1	29·1
Sphingomyelin	66·6	73·6	37·2	12·3
Lysolecithin	10·2	2·8	5·8	2·1

All results are expressed as milligrams per gram of freeze dried tissue.

free cholesterol and free fatty acid fractions where anaphylaxis caused no change, and the sphingomyelin fraction in which there was an increase following anaphylaxis. Pretreatment with ethanolamine which prevented the release of SRS-A during subsequent anaphylaxis appeared to prevent a number but not all of these changes in the lung lipids. These conclusions should as yet be accepted with some reservation since there are differences between the lipid analysis of chopped guinea pig lungs and those perfused with Tyrode solution for 30 min. The *in vitro* conditions of anaphylaxis used in the present study may thus have introduced artifacts which are difficult to assess. Nevertheless, the possibility that hypersensitivity reactions might lead to appreciable changes in the lipid content of the tissue involved is one worthy of further experimental study, since if such changes occur during hypersensitivity reactions provoked *in vivo* they have important implications.

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