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The anti-anaphylactic activity of theophylline and some related xanthine derivatives

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Theophylline and theobromine, but not caffeine and xanthine, protect sensitised guinea-pigs against anaphylactic shock induced by aerosolised antigen. There is an inhibition of release of slow reacting substance of anaphylaxis and some reduction in histamine release. The lung lipid changes associated with anaphylactic shock are prevented by pre-treatment with theophylline.

THE effectiveness of theophylline in the treatment of severe bronchial asthma was first reported by Hermann & Aynesworth (1937) although previously Efron (1936) was able to arrest severe attacks with aminophylline injected intravenously. Numerous authors have since confirmed that the intravenous injection of aminophylline gave prompt, effective, reliable and safe relief from allergic bronchospasm (Brown, 1938; Rowe, 1938; Huber, Kahn, Maytum, Ratner & Piness, 1939; Carr, 1940; Rackemann, 1940).

It is still commonly assumed, as by Urbach & Gottlieb (1946), that the beneficial effect of theophylline is due to an antispasmodic or bronchodilator action. Young & Gilbert (1941), by direct microscopical observations of freshly isolated bronchi and bronchioles, demonstrated a protective action of aminophylline against the bronchoconstrictor action of histamine.

Sollman & Gilbert (1937) and Gilbert & Goldman (1940) have shown aminophylline to be an effective dilator of bronchiolar sections which had been contracted by histamine. Osgood & Ehret (1943) suggested that the principal action of aminophylline in relieving asthma is by increasing the blood flow through the pulmonary circulation by vasodilatation and that its bronchodilating effect is of secondary importance. We set out to assess the anti-anaphylactic activity of theophylline and related xanthine derivatives and to further elucidate the underlying mechanisms of their actions.

Experimental

ANAPHYLAXIS *in vivo*

Actively sensitised guinea-pigs were exposed to aerosolised antigen using the technique of Herxheimer (1952) as described by Smith (1961). Three weeks after sensitisation, groups of nine guinea-pigs were exposed to an aerosol of 1% antigen (egg albumin) and the time to onset of dyspnoea and cough noted. This procedure was repeated at weekly intervals for 3 weeks during which it was found that the time to onset of dyspnoea and cough became relatively constant for each animal. This was termed the "normal collapse time" (Smith, 1961). Fifteen min before the fourth weekly exposure to antigen, each animal was injected intraperitoneally with a solution of drug in Water for Injection, B.P. The "treated collapse time" of each animal was expressed as a ratio to its "normal collapse time" and

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the figure termed the "protection ratio" for each animal. Animals recording a protection ratio of 20 were considered to be fully protected.

DETERMINATION OF ANTIHISTAMINE EFFECT *in vivo*

This was carried out in the same manner described in the section on anaphylaxis *in vivo* except that the aerosoliser contained a 5% solution of histamine acid phosphate instead of 1% egg albumin. Protection ratios were calculated in the same way as in the anaphylaxis experiments.

ANAPHYLAXIS *in vitro*

Anaphylactic shock was induced in intact sensitised guinea-pig lungs undergoing perfusion through the pulmonary artery with Tyrode solution at 37° by the technique of Brocklehurst (1960). The perfusate (60 ml) was collected over 30 min and then centrifuged to remove blood cells. The released histamine and slow reacting substance of anaphylaxis (SRS-A) were assayed on guinea-pig ileum as described by Goadby & Smith (1963a).

EFFECT OF THEOPHYLLINE ON CHANGES IN LUNG LIPIDS AFTER ANAPHYLAXIS

The procedure was identical with that of Goadby & Smith (1962), except that instead of hydrocortisone being administered 18 hr before exposure to antigen, theophylline was administered intraperitoneally at a dose level of 80 mg/kg 15 min before exposure.

Results

ANTI-ANAPHYLACTIC EFFECT OF XANTHINE DERIVATIVES

The results obtained with these four compounds are given in Table 1. All the compounds are insoluble in water but can be solubilised by the addition of suitable amounts of ethylenediamine or sodium salicylate.

TABLE 1. THE ANTI-ANAPHYLACTIC ACTIVITY OF XANTHINE AND ITS METHYL DERIVATIVES

Drug	Dose mg/kg	No. of animals	No. fully protected	Mean protection ratio*	Standard deviation
Theophylline with ethylenediamine ..	40	9	1	2·02	1·26
	80	9	6	10·74	
Theobromine Na salicylate	40	9	Nil	1·25	0·53
	80	9	9		
Caffeine Na salicylate.	40	9	Nil	1·55	0·23
	80	9	1	2·55	0·97
Xanthine Na salicylate	80	9	Nil	1·02	0·25
Na salicylate	80	9	Nil	1·00	0·09
Ethylenediamine	15	9	Nil	1·33	0·27

* Of animals not fully protected.
All doses in terms of base. All drugs were administered intraperitoneally 15 min before exposure to antigen.

Control observations were made with both these solubilising agents at the maximum dose levels used. Both were without anti-anaphylactic effect. Of the xanthine derivatives examined, both theophylline and theobromine had marked anti-anaphylactic activity at a dose level of 80 mg/kg. Caffeine and xanthine were without significant activity at the same dose

level (except caffeine in one animal only). Thus the anti-anaphylactic effect observed with both xanthines having common dimethyl groups is absent in trimethylxanthine (caffeine) and also the parent ring structure (xanthine). The last observation was confirmed in further experiments using aqueous suspensions of xanthine and hypoxanthine. A group of 9 animals which had received xanthine in aqueous suspension at 80 mg/kg 30 min before exposure to antigen, recorded a mean protection ratio of 1·49 with a standard deviation of 0·33. A corresponding group of animals that had received the same dose of hypoxanthine recorded a protection ratio of 1·41 with a standard deviation of 0·28.

ANTIHISTAMINE EFFECT OF THEOPHYLLINE, THEOBROMINE AND CAFFEINE

The antihistamine effect induced after a dose of 80 mg/kg of these compounds is shown in Table 2. When compared with the effect of

TABLE 2. ANTIHISTAMINE ACTIVITY *in vivo* OF XANTHINE DERIVATIVES IN GUINEA-PIGS EXPOSED TO AEROSOLISED HISTAMINE SOLUTION

Drug	Dose mg/kg	No. of animals	No. fully protected	Mean protection ratio*	Standard deviation
Theophylline with ethylenediamine ..	80	9	Nil	3·28	0·91
Theobromine Na salicylate	80	8	Nil	1·51	0·48
Caffeine Na salicylate..	80	8	Nil	1·56	0·23
Mepyramine maleate	1	8	8		

* Of animals not fully protected.
All doses in terms of base. All drugs were administered intraperitoneally 15 min before exposure to antigen except for mepyramine which was given intramuscularly 1 hr before exposure.

1 mg/kg of mepyramine, all the compounds exhibited negligible amounts of histamine antagonism at the dose level used. Thus the anti-anaphylactic activity of theophylline and theobromine is probably not due to pharmacological antagonism of the histamine released during anaphylactic shock.

EFFECTS OF THEOPHYLLINE, THEOBROMINE AND CAFFEINE ON RELEASE OF HISTAMINE AND SRS-A DURING ANAPHYLAXIS

The amounts of histamine and SRS-A released by anaphylaxis *in vitro* from the lungs of animals pretreated with 80 mg/kg of a xanthine derivative by the intraperitoneal route 15 min before being killed were compared with the amounts released from the lungs of control animals not so pretreated. The results are in Table 3. The amounts of SRS-A released

TABLE 3. EFFECT OF XANTHINE DERIVATIVES ON THE RELEASE OF HISTAMINE AND SRS-A DURING ANAPHYLAXIS IN GUINEA-PIG LUNG

Drug	No. of animals	Histamine μg/ml	Standard deviation	No. of animals	SRS-A units/ml	Standard deviation
Nil	6	0·93	1·42	4	20·06	5·26
Theophylline with ethylenediamine	6	0·775	1·32	6	13·02*	4·24
Theobromine Na salicylate ..	6	0·438	0·105	6	14·37†	4·207
Caffeine Na salicylate	6	0·968	1·296	6	22·35	12·37

* Statistically significant from control value in Student's *t* test at P = 0·95
† Statistically significant from control value in Student's *t* test at P = 0·90

after pretreatment with theophylline or theobromine are significantly smaller than the amounts released by the control animals. Whilst the mean

histamine release in these groups of animals is less than the mean histamine release from the controls, the differences are not statistically significant. Histamine and SRS-A were released in substantially the same amounts from the lungs of animals pretreated with caffeine as from the controls.

EFFECT OF PRETREATMENT WITH THEOPHYLLINE ON THE LIPID CONTENT OF GUINEA-PIG LUNGS SUBJECTED TO ANAPHYLAXIS

The changes in lung lipids of animals exposed to both aerosolised distilled water and aerosolised antigen were determined 15, 30 and 60 min after exposure to aerosol. They are summarised in Table 4. The most

TABLE 4. LIPID CONTENT (MG/G) OF FREEZE-DRIED LUNGS FROM SENSITISED GUINEA-PIGS EXPOSED TO AN AEROSOL OF DISTILLED WATER OR AEROSOLISED ANTIGEN (EGG ALBUMIN) EXPRESSED AS MEAN ± STANDARD DEVIATION

	Distilled Water			Antigen		
	15 min	30 min	1 hr	15 min	30 min	1 hr
Phospholipid	117 ± 14·75	103 ± 17·25	82 ± 12·25	119 ± 41·25	108 ± 4·25	83 ± 10·75
Cholesterol	27·98 ± 9·86	18·81 ± 1·67	26·43 ± 6·82	21·21 ± 12·32	16·95 ± 1·1	18·7 ± 1·25
Glyceride	19·1 ± 4·43	77·4 ± 10·44	22·9 ± 7·92	23·8 ± 4·55	64·8 ± 29·78	19·75 ± 9·32

prominent changes are a fall in phospholipid content and an increase in glyceride content which is pronounced 30 min after exposure to aerosol. One hr after exposure to aerosol, the glyceride content had returned to a normal level, but the amount of phospholipid appeared to be still falling.

The administration of theophylline itself produced no substantial change in the lipid content of guinea-pig lungs as shown in Table 5. In this

TABLE 5. LIPID CONTENT (MG/G) OF FREEZE-DRIED LUNGS FROM CONTROL ANIMALS COMPARED WITH THAT OF LUNGS FROM ANIMALS PRETREATED WITH THEOPHYLLINE EXPRESSED AS MEAN ± STANDARD DEVIATION

	Controls	Theophylline-treated
Phospholipid	117 ± 8·25	111·05 ± 5·4
Cholesterol	20·25 ± 1·52	18·11 ± 1·38
Glyceride	19·0 ± 5·0	15·62 ± 5·96

table the lipid content of a group of animals pretreated with an intraperitoneal injection of 80 mg/kg theophylline (solubilised with ethylenediamine) is compared with the control group of animals.

The changes in lung lipids in animals pretreated with theophylline and then subsequently exposed to aerosolised distilled water or antigen were determined at 15, 30 and 60 min after exposure to aerosol (Table 6).

TABLE 6. LIPID CONTENT (MG/G) OF FREEZE-DRIED LUNGS FROM ANIMALS EXPOSED TO AN AEROSOL OF DISTILLED WATER OR AEROSOLISED ANTIGEN 15 MIN AFTER PRETREATMENT WITH THEOPHYLLINE EXPRESSED AS MEAN ± STANDARD DEVIATION

	Distilled water			Antigen		
	15 min	30 min	1 hr	15 min	30 min	1 hr
Phospholipid	116·5 ± 14·35	108·8 ± 4·32	107·6 ± 1·98	101·15 ± 1·95	99·0 ± 2·2	99·37 ± 18·35
Cholesterol	16·25 ± 0·77	15·17 ± 1·92	20·9 ± 1·47	17·28 ± 0·67	16·83 ± 0·46	18·38 ± 0·79
Glyceride	34·3 ± 17·8	26·02 ± 16·2	32·08 ± 19·8	9·22 ± 3·74	19·94 ± 12·0	16·23 ± 4·15

A comparison of Tables 5 and 6 shows that animals pretreated with theophylline do not exhibit any marked change in their lung lipids when subsequently exposed to an aerosol of distilled water or antigen. The changes in lung lipids associated with anaphylactic shock are not observed in animals pretreated with an anti-anaphylactic dose of theophylline.

Discussion

The results have an interesting similarity to earlier observations obtained in this laboratory using anti-inflammatory steroids (Goadby & Smith, 1962, 1964) and ethanolamine (Smith, 1961). Theophylline, hydrocortisone and ethanolamine have all been shown to inhibit the release of SRS-A during an anaphylactic reaction in sensitised guinea-pig lung tissue, and theophylline and hydrocortisone have prevented changes in the lipid content of sensitised guinea-pig lungs associated with anaphylactic shock *in vivo*. *In vitro* studies have shown a similar effect with ethanolamine.

Although all three substances inhibit the release of SRS-A and prevent the changes in lipid metabolism invoked by anaphylaxis, the times required for these effects to become manifest differ markedly. These effects are induced by theophylline 15 min after administration. With ethanolamine the effects are maximal 2 hr after administration (Goadby & Smith, 1963b); whereas with hydrocortisone, 18 hr must elapse between dose and maximal anti-anaphylactic effect (Goadby & Smith, 1964).

The exact role of SRS-A as a chemical mediator of allergic bronchospasm in both guinea-pigs and the human asthmatic has yet to be elucidated. Nevertheless there is evidence pointing to its involvement in this condition in both guinea-pigs and man (Brocklehurst, 1956, 1960, 1962, 1963). Thus the beneficial effect of theophylline, hydrocortisone and ethanolamine in the treatment of allergic asthmatics is most probably due to their ability to reduce the SRS-A induced component of bronchospasm initiated by an antigen-antibody reaction. There is no evidence of other pharmacological activities possessed by these molecules which would themselves account for their anti-anaphylactic activity. For instance, in guinea-pigs, none of these substances has bronchodilator, anti-SRS-A or antihistamine properties (Smith, 1961; Goadby & Smith, 1964).

The observation that all three substances prevent the changes in lipid metabolism normally induced in anaphylaxis in sensitised guinea-pig lung, is itself one of profound significance. Since these agents protect the tissue from changes in lipid metabolism which in unprotected animals represent a marked distortion of the normal intermediary metabolism (Smith, 1964), their effects are beneficial at a biochemical level as well as the purely pharmacological level of bronchospasm.

Since inhibition of SRS-A release and the prevention of changes in lipid metabolism invoked by anaphylaxis are effects which follow pretreatment with theophylline, hydrocortisone or ethanolamine, it is possible that these two events are related. The differences in times that must elapse between administration and maximal anti-anaphylactic effect can be interpreted as manifestations of a biochemical mechanism of action and it is profitable to

speculate that these two events have some common biochemical cause. If this is so, the effects are not caused by histamine release or the release of SRS-A (Marquis & Smith, 1963). It is more likely that protection is achieved by blocking a biochemical event common to both lipid metabolism and the metabolism of SRS-A.

Besides incurring substantial losses of phospholipid as a result of anaphylactic shock (Goadby & Smith, 1962), guinea-pig lung also loses large amounts of neuraminic acid (Anderson, Goadby & Smith, 1963). Up till the present time, there is no chemical evidence contradictory to the suggestion that SRS-A is a mixture of neuraminyl glycosides (Smith, 1962). Thus a metabolic reaction common to the metabolism of SRS-A and lipid metabolism might be one situated in the intermediary metabolism of the tissue somewhere between the reactions of mucopolysaccharide synthesis on the one hand and the reactions of lipid synthesis on the other.

Neuraminic acid has recently been shown to be an important constituent of cell membranes (Wallach & Eylar, 1961). It seems probable that any biochemical effect capable of modifying the ability of cells to withstand the biochemical disorganisation attendant upon the formation of an antigen-antibody complex on or within the surface structure of their cytoplasm may inhibit the release of SRS-A and prevent the loss of other pharmacologically inert neuraminyl glycosides and phospholipid. The latter is also richly distributed in membrane structures.

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