Br. J. Pharmac. (1976), 57, 399–408

THE PHARMACOLOGY OF ANAPHYLAXIS IN THE CHICKEN INTESTINE

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1 The Schultz-Dale phenomenon has been demonstrated in several circular smooth muscle strips of oesophagus, crop, duodenum, jejunum and ileum taken from young and adult domestic fowl sensitized actively to crystalline bovine albumin or horse plasma.

2 The ileal strips contract to acetylcholine, histamine, 5-hydroxytryptamine (5-HT), prostaglandins E_1, E_2, $F_{2\alpha}$, bradykinin and bovine slow reacting substance of anaphylaxis (SRS-A). Marked seasonal and individual variations in the responsiveness of gut tissues to these exogenous agonists were noted.

3 Antagonism of contractions to histamine by mepyramine suggests the existence of H_1-histamine receptors in chicken ileum. Blockade of 5-HT-induced contractions by methysergide shows the preponderance of 'D'-musculotropic tryptamine receptors.

4 Failure of selective receptor antagonists of acetylcholine, histamine and 5-HT to modify the Schultz-Dale reaction suggests the nonparticipation of aminergic mechanisms in this reaction.

5 Partial to complete blockade of the Schultz-Dale reaction by a prostaglandin receptor antagonist (polyphloretin phosphate, PPP); prostaglandin synthetase inhibitors (sodium meclofenamate and phenylbutazone); inhibitors of synthesis and release of histamine and SRS-A (PR-D-92-EA, M&B 22948, diethylcarbamazine citrate, and PPP) and an inhibitor of proteinases (aprotinin) strongly suggests the involvement of vasoactive lipids and polypeptides in the anaphylactic response of chicken ileum to specific antigen.

Introduction

In 1910, Schultz reported contraction of the isolated intestinal strip from a sensitized guinea-pig on exposure to the specific antigen. Three years later, Dale (1913) demonstrated a similar response of the uterus. Since then, antigen-induced smooth muscle contraction *in vitro* has become a standard technique for demonstrating anaphylactic hypersensitivity. Subsequently this 'Schultz-Dale' effect has been described in the oesophagus (Califano & Scapagnini, 1968) and ileum (Dale, 1965; Dale & Okpako, 1969; Okpako, 1970) of guinea-pig; jejunum (Aitken, Deline & Eyre, 1975), pulmonary (Eyre, 1971; Aitken *et al.*, 1975), mesenteric and hepatic (Holroyde & Eyre, 1975) blood vessels of calf. However, Hirata & Cambell (1961) failed to demonstrate a Schultz-Dale reaction in the intestine of passively sensitized adult domestic fowl.

Forceful fluid defaecation (suggestive of increased gastrointestinal motility and secretions) during acute anaphylaxis in the chicken has been documented repeatedly (Lecomte & Beaumariage, 1958; Pedersoli, 1973; Chand & Eyre, unpublished). This prompted us to study the Schultz-Dale phenomenon in several parts of the gastrointestinal tract of actively sensitized chickens. In order to illustrate the possible nature of the principal chemical mediator(s) participating in anaphylaxis in chicken intestine, known specific antagonists, together with non-steroidal anti-inflammatory agents and some new anti-anaphylactic drugs were included in the study.

Methods

Seventy-nine adult and ten one week old chickens (White or Brown Leghorn) were used. The birds were sensitized either to bovine albumin (ICN Pharmaceuticals, Cleveland, Ohio) (80 mg kg^{-1}, i.v.) (Makinodan, Wolfe, Goodman & Ruth, 1952) or horse plasma (1 ml kg^{-1}, i.v.). On the seventh day of sensitization, overnight-fasted chickens were killed by intravenous injection of pentobarbitone. Sections of intestine were immediately removed and transferred to cold aerated Krebs-Henseleit solution (Everett, 1966; Everett & Mann, 1967). Segments (approximately 2 cm) of duodenum, jejunum and ileum were removed and their contents washed out with Krebs solution. Each segment of intestine was cut spirally and the resultant single strip then bisected longitudinally to produce two identical strips (containing pre-

dominantly circular smooth muscle). This technique allowed comparisons of responses of 'twin' strips to exogenous agonists and antigen in the presence and absence of antagonists; one strip of each pair always serving as a control (untreated by antagonist) in each experiment (Eyre, 1971). A transverse crop strip was carefully dissected without stretching the tissue (Everett, 1966). An oesophageal strip was prepared by longitudinally cutting a 2 to 4 mm wide segment of oesphagus. Each tissue was suspended in a 30 ml isolated organ bath (Phipps & Bird, Richmond, Va.) containing Krebs solution at 37°C, gassed with 5% CO_2 in O_2 mixture and was allowed to equilibrate for at least 1 h under a resting tension of 3 grams.

Responses of the strips were recorded with an E & M isotonic myograph transducer (Narco Instruments, Houston, Tex.) connected to an E & M (Narco) Desk Model, 4-Channel Physiograph (DMP-4B) pen recorder. All the strips were exposed to each dose of agonist for 1 to 5 min followed by 5 to 15 min recovery period. Three-point dose-response curves to each agonist were established. It was noted that both strips usually produced approximately equal responses to agonists. However, any pairs which exhibited marked differences in sensitivity or showed excessive spontaneous activity were discarded.

After establishing dose-response curves to randomly injected agonists, a concentration of each agonist producing approximately equivalent reproducible responses (about 50% maximum) was chosen. A predetermined concentration of an antagonist was added to one of the tissues and left in contact for 30 minutes. Each agonist was then randomly retested in both tissues as before to establish new dose-response curves. The effectiveness and specificity of the antagonist was determined by measuring the 'dose-ratio' i.e. the ratio of the doses of agonist which give equal responses in the presence and absence of antagonist (Gaddum, Hameed, Hathway & Stephens, 1955). Both the strips were 'challenged' with crystalline bovine albumin (25 $\mu g\ ml^{-1}$) or horse plasma (0.3 ml in 30 ml) to produce a Schultz-Dale reaction. The Schultz-Dale contraction of each muscle strip was measured and the degree of inhibition caused by the antagonist was expressed as a percentage reduction of the unantagonized response.

Drugs

Histamine diphosphate, bradykinin triacetate, 5-hydroxytryptamine (serotonin) creatinine sulphate and atropine sulphate were purchased from Nutritional Biochemical Corp., Cleveland, Ohio; acetylcholine chloride from Sigma Chemical Co., St. Louis, Mo.; phenylbutazone sodium (Butazone) from Stevenson, Turner and Boyce, London, Ontario. Slow-reacting substance of anaphylaxis (SRS-A) was obtained from calf lung and assayed on guinea-pig ileum (Burka & Eyre, 1975).

The following drugs were obtained as gifts: mepyramine maleate from Poulenc Ltd., Montreal, Quebec; methysergide bimaleate from Sandoz, Basle, Switzerland; diethylcarbamazine citrate (Franocid, DECC) from Burroughs Wellcome Ltd., London, England; sodium meclofenamate from Parke, Davis and Co., Detroit, Mich.; prostaglandins E_1, E_2 and $F_{2\alpha}$ from Upjohn Co., Kalamazoo, Mich.; polyphloretin phosphate (PPP, Leo 101K) from Leo, Helsingborg, Sweden; 2-9-propoxyphenyl-8-azapurin-6-one (M&B 22948) from May and Baker Ltd.; FPL 55712 from Fison Limited; aprotinin (Trasylol) from Boehringer Ingelheim (Canada) Ltd., Montreal, Quebec; 5,5-dimethyl-11-oxo-5H,11H-(2)-benzopyrano(4,3-g) (1)benzopyran-9-carboxylic acid ethanolamine salt (PR-D-92-EA) from Pharma Research, Pointe-Claire, Quebec. All the drugs were dissolved in Krebs solution and the concentrations expressed as salts. FPL 55712 was dissolved initially in distilled water and diluted further in Krebs solution.

Results

Threshold doses of exogenous agonists and antigens on several intestinal strips are presented in Table 1. Histamine (1 nM to 0.1 μM) sometimes produced a slight relaxation but as the doses of histamine were increased, only contractile responses were recorded. In general, acetylcholine (ACh) was 100 to 1000 times more active than histamine and 5-hydroxytryptamine (5-HT) on most tissues. It was noted that tissues were measurably more sensitive to agonists in the spring and summer compared with the winter. Crop strips in summer were extremely sensitive to 5-HT. Responses to histamine and 5-HT were slow in onset and recovery (1 to 5 min) depending upon the dose used and sensitivity of the tissues.

Threshold doses and effects of bradykinin, prostaglandins and bovine SRS-A were variable. However, in summer months, it was noted that prostaglandins E_1, E_2 (0.1 to 10 $ng\ ml^{-1}$) and $F_{2\alpha}$ (1 to 100 $ng\ ml^{-1}$) exhibited strong consistent dose-dependent contractions of ileal strips ($n=6$), whereas in winter, sensitivity to prostaglandins was so low that frequently it was not possible to elicit any contractile response ($n=5$).

The Schultz-Dale reaction was characterized by a contraction associated with increased 'spontaneous' activity after 1 to 5 min of antigen exposure to the tissues. The absolute intensity of contraction and spontaneity was highly variable depending upon the organ used; the sensitizing and challenging dose of antigen, type of antigen (bovine albumin or horse plasma), and season. The tissue reaction was highly consistent between 'twin' strips taken from the same organ on the same occasion.

A number of crystallized albumins and globulins

(dog, bovine, pig, rabbit, sheep, goat) (1 to 100 μg ml^{-1}) and horse or bovine plasma (0.01 to 0.3 ml in 30 ml) were devoid of any visible effect on intestinal strips from five nonsensitized chickens.

Intestinal strips (2/6) from chickens sensitized to bovine albumin exhibited cross (nonspecific) Schultz-Dale reaction to horse and pig albumins but not to globulins. However, tissues ($n=6$) from birds sensitized to horse plasma did not show any cross reaction to albumins and globulins of other species. Furthermore, a few gut strips (7/36) from bovine albumin-sensitized birds did not contract to challenge with bovine albumin. Alimentary tissues from one week old chicks were about 100 to 1000 times more sensitive to exogenous agonists but the *in vitro* anaphylactic responses were similar to those of adult chickens.

Ileal strips ($n=4$) passively sensitized to chicken anti-horse sera (diluted with aerated Krebs solution, 1:10, at 4°C for 2 h) also showed strong Schultz-Dale contractions to horse plasma. The nonsensitized second strip of each pair did not respond to antigen challenge.

Antagonists

The inhibitory activity of the specific antagonists on ACh, histamine, 5-HT and the Schultz-Dale anaphylactic response are shown in Table 2.

Atropine At lower concentrations (0.05 and 0.5 μg ml^{-1}), atropine selectively antagonized ACh. At higher doses (1 to 10 μg ml^{-1}) atropine also inhibited histamine (dose-ratio 5 to 60) and 5-HT (dose-ratio 10 to 100) without modifying the Schultz-Dale reaction in any way (not included in Table 2).

Mepyramine (0.5 to 1.0 μl mg^{-1}) specifically inhibited histamine without inhibiting the Schultz-Dale effect. Larger doses (2 to 10 μg ml^{-1}) of mepyramine partially contracted the intestinal strips and inhibited 5-HT (dose-ratio 15 to 50), also reducing the Schultz-Dale response an average 40% ($n=3$) (not included in the Table). In some (3/7) ileal strips, mepyramine reversed the histamine-induced contraction (Figure 1a) into a relaxant effect.

Methysergide (0.2 and 2.0 μg ml^{-1}) selectively antagonized 5-HT without modifying the responses to histamine, ACh or antigen.

In some additional experiments ($n=5$) (not included in the Table), combined addition of atropine, mepyramine and methysergide (1 μg ml^{-1} of each) also failed to alter the anaphylactic Schultz-Dale reaction. Furthermore, tachyphylaxis to 5-HT induced by repeated injection of 5-HT ($n=3$) also failed to inhibit the Schultz-Dale phenomenon.

Sodium meclofenamate (1 to 10 μg ml^{-1}) did not inhibit contractions to ACh, histamine, 5-HT and antigen. Larger doses of meclofenamate (20 and 50 μg ml^{-1}) exhibited varying degrees of nonspecific blockade of histamine, ACh, 5-HT and strongly inhibited the Schultz-Dale anaphylactic response (60 and 85% block).

Phenylbutazone (10 to 500 μg ml^{-1}) antagonized ACh, histamine and 5-HT, but inhibited the Schultz-Dale response only at the highest dose (500 μg ml^{-1}).

Diethylcarbamazine citrate (DECC) (10 to 100 μg ml^{-1}) inhibited exogenous agonists slightly (dose-ratio 2 to 10) (not included in the Table). However, large doses strongly antagonized histamine, ACh, 5-HT and Schultz-Dale contraction. DECC itself contracted gut strips and in three preparations, it also reversed the SRS-A-induced contractions.

Table 1 Threshold dose ranges of some spasmogens on certain circular smooth muscle strips of gastrointestinal tract of adult domestic fowl

	Agonists (M)				*Antigens*	
Source of the tissues	*5-HT*	*Histamine*	*Actyl-choline*	*Carbachol*	*BA* μg ml^{-1}	*HP ml/30 ml*
Anterior and posterior oesophagus	10^{-9} to10^{-8}	10^{-7} to 10^{-6}	10^{-9} to 10^{-7}	10^{-10} to 10^{-8}	0.1 to 2	0.01 to 0.02
Crop	10^{-10} to 10^{-8}	10^{-7} to 10^{-6}	10^{-9} to 10^{-8}	10^{-10} to 10^{-9}	0.1 to 2	0.01 to 0.02
Small intestine (duodenum, jejunum, ileum)	10^{-7} to 10^{-4}	10^{-7} to 10^{-4}	10^{-9} to 10^{-7}	10^{-10} to 10^{-8}	1 to 10	0.02 to 1
Caecum	—	10^{-7} to 10^{-6}	10^{-10} to 10^{-9}	—	—	—

BA=bovine albumin; HP=horse plasma.

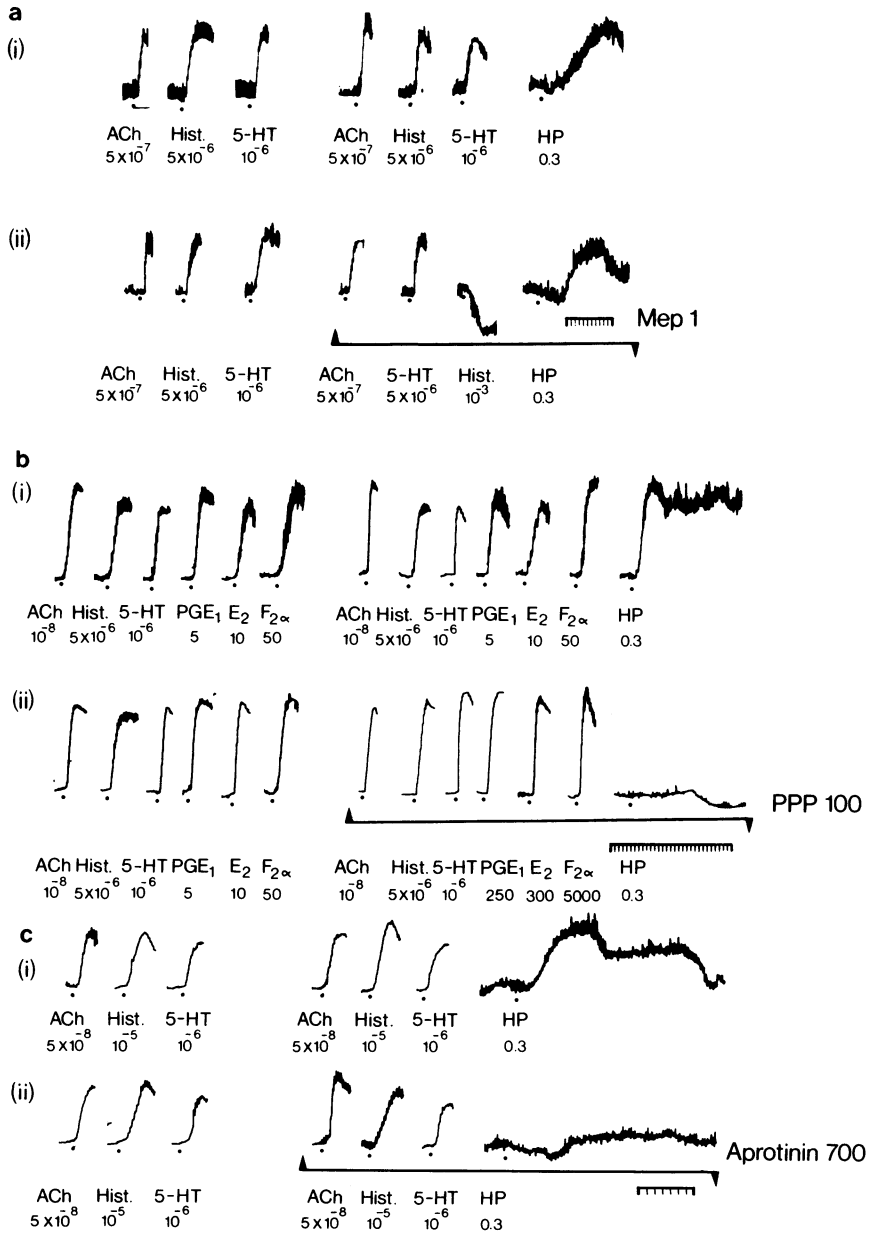

Figure 1 Three pairs (a), (b) and (c) of isolated spiral strips of ileum taken from three adult domestic fowl sensitized to horse plasma (1 ml kg^{-1}, i.v.). All strips contracted to acetylcholine (ACh), histamine (Hist), 5-hydroxytryptamine (5-HT) and horse plasma (HP) in 30 ml Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2 at 37°C. Both strips of the second pair (b) also showed contractions to prostaglandins (PG E_1, E_2 and $F_{2\alpha}$). In the second (ii) strip of each pair an antagonist was present in the bath fluid between the arrows shown: (a, ii) mepyramine maleate (Mep) reversed histamine contraction, reduced 5-HT-induced response but did not inhibit ACh and Schultz-Dale reaction. (b, ii) Polyphloretin phosphate (PPP) inhibited responses to prostaglandins E_1, E_2 and $F_{2\alpha}$ (ng ml^{-1}) and Schultz-Dale response. (c, iii) Aprotinin (KIU ml^{-1}) did not affect responses to histamine, ACh and 5-HT but blocked Schultz-Dale phenomenon. Doses of agonists (histamine, ACh, 5-HT) are in Molar concentration; antagonists in μg ml^{-1} and horse plasma in ml. Time marker indicates minutes.

Polyphloretin phosphate (PPP) (100 μg ml^{-1}) reduced or blocked the anaphylactic response and reversed or inhibited contractions to prostaglandins E_1, E_2, and $F_{2\alpha}$ ($n=4$) (Figure 1b) without antagonizing ACh, histamine or 5-HT.

FPL 55712 at all doses (0.1 to 100 μg ml^{-1}) did not inhibit contractions to histamine, ACh, 5-HT and antigen. However, addition of compound FPL (1 to 100 μg ml^{-1}) before or after antigen-challenge invariably inhibited antigen-induced spontaneous activity of the intestine, but did not block actual contractions.

PRD-92-EA (10 to 200 μg ml^{-1}) antagonized ACh, histamine, 5-HT slightly and reduced antigen-induced spontaneity only. Large doses strongly inhibited exogenous agonists and completely inhibited the Schultz-Dale anaphylactic responses.

M&B 22948 (10 to 60 μg ml^{-1}) did not possess any inhibitory activity towards histamine, ACh, 5-HT and antigen-induced contractions. At a higher dose (200 μg ml^{-1}), it had little or no inhibitory effect upon exogenous agonists, but blocked the Schultz-Dale response completely.

Table 2 Dose-ratios of histamine, 5-hydroxytryptamine (5-HT) and acetylcholine, and percent inhibition of Schultz-Dale contractions in chicken ileum *in vitro*, in the presence of antagonists

Antagonist	*μg ml^{-1}*	*Dose-ratios of agonists*			*% Inhibition of Schultz-Dale reaction (Mean ± s.d.)*
		Histamine	*5-HT*	*Acetylcholine*	
Atropine	0.05	1,1,1,1	1,1,1.5,1	300,900,500, 1000	0 (4)
	0.5	1,2,1.5,1	1,1,1,2.5	3050,6000, 6050,2000	0 (4)
Mepyramine	0.05	10,5,10	1,1,1	1,1,1	0 (3)
	0.1	30,120,80	1.5,1,1	1,1,1	0 (3)
	1.0	500,300,165, 250	2,1,5,2	1,2,1.5,1	0 (4)
Methysergide	0.2	1,2,1,1	20,15,10,4	1,1,2,1	0 (4)
	2.0	1,1,1,3	40,100,40,35	1,1,1,2	0 (4)
Sodium meclofenamate	1.0	1,1,1	1,1,1	1,1,1	0 (3)
	10.0	3,1,1,1	10,1,2,1	1,1,1,1	0 (4)
	20.0	5,2,1,1,1	10,30,2,5,2	5,2,1,2,1	60 ± 25 (5)
	50.0	5,20,1,2	25,10,30,10	5,2,2,5	85 ± 25 (4)
Phenylbutazone	10	1,2,1.5	1,2,1.5	2,1.5,2	0 (3)
	100	3,10,2.5,6,3,5	2,22,5,10,2	1,2,5,3,6,2.5	0 (6)
	500	6,5,10,60,5	5,30,2,10,6	5,20,6.5,100, 15	100 (5)
DECC	500	3,6,25	12,25,60	2,1,1	0 (3)
	4000	100,250,150 irreversible	300,250, irreversible,450	2,10,15,100	90 ± 15 (4)
PPP	100	1,1,1,1,1	1,1,1,1,1	1,1,1,1,1	60 ± 25 (5)
FPL 55712	0.1	1,1,1	1,1,1	1,1,1	0 (3)
	1.0	1,1,1	1,1,1	1,1,1	0 (3)
	10.0	1,1,1	1,1,1	1,1,1	0 (3)
	100.0	1,1,3	1,2,1	1,1,1	0 (3)
PRD-92-EA	200	1,3,2,1	1,10,2,1	3,2,1,10	0 (4)
	500	25,100,15,10	10,2,50,110	20,3,1.5,2	100 (4)
M&B 22948	30	1,1,1,1	1,1,1,1	1,1,1,1	0 (4)
	60	1,1,1	1,1,1.5	1,1,1	0 (3)
	200	2,1,1,1	2,1,1.5,2	1,2,1,3	100 (4)
Aprotinin	100 KIU ml^{-1}	1,1,1	1,1,1	1,1,1	0 (3)
	700 KIU ml^{-1}	1,1,1	1,1,1	1,1,1	75 ± 25 (4)
	1000 KIU ml^{-1}	1,1,1,1.5	1.5,1,1,2	1,1,1.5,1	80 ± 20 (4)

Aprotinin (700 to 1000 kallikrein inactivating units (KIU) ml^{-1}) blocked the Schultz-Dale response without interfering with the responses of histamine, 5-HT or ACh (Figure 1c).

Discussion

Many intestinal strips (oesophagus, crop, duodenum, jejunum and sometimes ileum) exhibited marked spontaneous activity which was resistant to atropine, methysergide and mepyramine. Earlier, Everett (1966) had reported similar observations which she ascribed to intrinsic myogenic activity. This spontaneity was inhibited by the addition of catecholamines (noradrenaline and isoprenaline) which also produced dose-related reduction in basic tone of tissues. Furthermore, reduction of spontaneous activity of these smooth muscle strips could be achieved by halving the amount of Ca^{2+} in the Krebs solution and by lowering the temperature (37°C to 25°C). This resulted in significant (50 to 75%) reduction in sensitivity to exogenous spasmogens and antigens. These findings are in general agreement with the fact that for the anaphylactic release of histamine (and other vasoactive substances like SRS-A) responsible for the antigen-induced contraction of smooth muscles, an appropriate concentration of Ca^{2+} ions and optimum temperature of tissue bathing fluid is essential (Mongar & Schild, 1957; Grosman & Diamant, 1974). It is generally assumed that calcium participates in intracellular reactions of the release process.

The seasonal and individual variability in the responsiveness of intestinal tissues to exogenous agonists, particularly insensitivity to histamine and 5-HT in winter, supports the similar earlier finding on the crop (Everett, 1966). The delay of 1 to 3 min in the initiation of contraction of intestinal strips of adult chickens to 5-HT and histamine may be due to the presence of dense connective tissue barriers retarding the diffusion of these drugs to sites of action (Everett, 1966). Similar reasons may explain the delay in recovery from contractions.

In spite of this individual and seasonal variability to exogenous agonists, the only marked difference in the character of the Schultz-Dale anaphylactic reactions was a longer latent period (2 to 10 min) in the initiation of contraction followed by greater spontaneity in winter compared to shorter latent period (1 to 5 min) and relative absence of increased spontaneity in summer. No obvious correlation could be established between the responsiveness of tissues to exogenous agonists and anaphylactic Schultz-Dale contraction.

Schultz-Dale anaphylactic responses were observed in the following decreasing order of intensity in the crop, oesophagus, jejunum, duodenum and ileum strips. The Schultz-Dale reaction could not be demonstrated in caecal strips. This variability in the intensity of this *in vitro* anaphylactic reaction may be attributed to differences in mast cell numbers (Wight, 1970; Carlson & Hacking, 1972), smooth muscle sensitivity, their avidity for cytotropic antibody, or their histamine (or other vasoactive spasmogen) contents. Demonstration of Schultz-Dale reactions in the intestine of actively sensitized chickens suggest the presence of tissue-fixed antibodies, capable of interacting with specific sensitizing antigens on challenge, resulting in the release of pharmacological vasoactive substances (most probably from mast cells) and thus producing contraction of smooth muscles. Reaginic antibodies mediating passive cutaneous anaphylaxis (PCA) in chickens have been found to belong to γ_1, a subclass of IgG immunoglobulins (Faith & Clem, 1973).

Desensitization of tissues to subsequent antigenic challenge was a common feature of this reaction. Desensitization to antigen(s) has been widely reported (Dale, 1965; Dale & Okpako, 1969; Okpako, 1970; Eyre, 1971).

It is pertinent to mention that antigen-induced contractions and increased spontaneity were maintained for 30 min to 2 h in spite of repeated washings in many preparations. This observation suggests continuous release of vasoactive substances from sensitized tissues once the immunological reaction has been triggered by specific antigen.

The cross (non-specific) Schultz-Dale reaction observed on some strips may be due to the existence of some common antigenic determinants on albumin molecules of different species (cow, horse and pig).

Reduction or blockade of histamine and 5-HT-induced responses by large doses of atropine may be due to its well known antihistamine (Schild, 1947; Chand & Eyre, 1976 and antitryptamine ('D' musculotropic) effects (Gaddum & Picarelli, 1957; Chand & Eyre, 1976). Lack of antianaphylactic effect of atropine suggests a lack of involvement of cholinergic mechanisms in intestinal anaphylaxis in chickens.

Slight relaxant effects to low doses of histamine on chicken small intestine may be attributed to catecholamine-releasing action from the intestine (Everett & Mann, 1967). However, the possibility of the presence of H_2-histamine receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972) sensitive to low doses of histamine and mediating slight relaxant effects cannot be ruled out. Rather, this possibility is further substantiated by the observations that complete blockade of H_1-receptors (mediating contractile responses) by mepyramine (Ash & Schild, 1966) reversed the contractile responses to a relaxant effect. This may indicate an unmasking of H_2-receptor activity after complete H_1-blockade. The small contractile responses to larger doses of mepyramine

may be due to its known histamine-releasing properties (Mota & DaSilva, 1960).

It has been reported that classical H_1-receptor antagonists inhibit anaphylaxis in domestic fowl (Lecomte & Beaumariage, 1958) but this has not been confirmed (Pedersoli, 1973). Furthermore, in the present study, mepyramine (a preferential H_1-histamine receptor antagonist) (Chand & Eyre, 1975) is ineffective in blocking antigen-induced contractions. This findings seems to support the earlier suggestion that in chicken anaphylaxis, histamine may be playing a minor role (Pedersoli, 1973).

5-HT antagonists have been reported to be ineffective in protecting chickens from anaphylactic shock (Lecomte & Beaumariage, 1958; Pedersoli, 1973). Resistance of the Schultz-Dale reactions to methysergide appears to strengthen this earlier view and 5-HT may not be an important mediator of anaphylaxis in domestic fowl (Lecomte & Beaumariage, 1958). Furthermore, intestinal strips (insensitive to higher doses of histamine and 5-HT) or tissue strips (rendered tachyphylactic to 5-HT by frequent injection of 5-HT) showed equal sensitivity to anaphylactic Schultz-Dale contraction. These observations, together with the ineffectiveness of atropine, mepyramine and methysergide in blocking Schultz-Dale contractions strongly suggest that cholinergic, histaminergic or tryptaminergic mechanisms are of low significance in this anaphylactic reaction.

Sodium meclofenamate and phenylbutazone are non-steroidal anti-inflammatory agents, possessing strong prostaglandin synthetase inhibitory activity (Flower, Gryglewski, Herbaczynska-Cedro & Vane, 1972). Sodium meclofenamate inhibits certain actions of kinin, SRS-A, prostaglandin $F_{2\alpha}$ and anaphylactic bronchoconstriction in the guinea-pig (Collier & Shorley, 1963; Berry & Collier, 1964; Collier & Sweatman, 1968). At low concentrations, neither antagonist had any inhibitory activity against exogenous agonists. These findings are in agreement with an earlier report (Burka & Eyre, 1974). At higher doses, both the drugs showed marked inhibitory activity against exogenous agonists and the Schultz-Dale reaction. Thus, the non-steroidal anti-inflammatory agents are nonspecific, possessing a variety of modes of action, both at the sites of the mediators synthesis and at receptor sites. The inhibition of the Schultz-Dale response by prostaglandin-synthetase inhibitors (meclofenamates and phenylbutazone) suggests a role for prostaglandins in the reaction. Specific inhibition of anaphylaxis by PPP further supports a role for prostaglandins.

DECC has been reported to release histamine (Deline, Eyre & Wells, 1973) which may account for the contractile responses observed. Subsequent blockade of histamine responses by DECC may be attributed to its known antihistaminic activity (Orange, Valentine & Austen, 1968). It has earlier been reported that DECC inhibits responses to histamine and 5-HT (Burka & Eyre, 1974) and the Schultz-Dale reaction (Eyre, 1971). High doses of DECC have been reported to inhibit immunological release of SRS-A (Orange *et al.*, 1968; Burka & Eyre, 1975) and the inhibition of the Schultz-Dale phenomenon by large doses of DECC appears to suggest the formation and release of SRS-A from intestinal tissues but lack of specificity of DECC on a variety of exogenous agonists makes such a conclusion tenuous. Furthermore, antigen-induced release of SRS-A and histamine from chicken lung and intestine *in vitro* could not be demonstrated (Chand & Eyre, unpublished).

Alimentary tissues of several species have the ability to synthesize, release and metabolize prostaglandins. The pharmacological actions (relaxation or contraction) vary greatly depending upon the species, strain, region of gastrointestinal tract, and type of smooth muscles (circular or longitudinal) involved. It has been suggested that generation of prostaglandins appears to maintain the tone of the gut (Wilson, 1974). In general prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ produce contraction of longitudinal muscles in several species, whereas circular smooth muscles are relaxed by prostaglandins E_1, E_2 and contracted by prostaglandin $F_{2\alpha}$ (Wilson, 1974). In contrast, prostaglandins E_1, E_2 and $F_{2\alpha}$ contract circular smooth strip of ileum of chicken. Thus it may be suggested that prostaglandins are involved in the maintenance of tone of circular smooth muscles of chicken gut. The inhibitory activity of PPP on Schultz-Dale reaction may be attributed to the inhibition of formation and release of SRS-A and histamine (Strandberg, 1973; Foucard & Strandberg, 1975; Burka & Eyre, 1975) as well as to the blockade of prostaglandin receptor sites *per se* (Eakins, Karim & Miller, 1970).

FPL 55712 has been introduced as a potent specific SRS-A receptor antagonist (Augstein, Farmer, Lee, Sheard & Tattersall, 1973). This compound does not inhibit Schultz-Dale contraction in chicken intestine (this paper) or in guinea-pig ileum (Eyre, unpublished). However, inhibition of antigen-induced spontaneity associated with Schultz-Dale contraction by FPL 55712 possibly suggests that released SRS-A may be playing a role in antigen-induced spontaneous activity but not in antigen-induced contractions.

PR-D-92-EA is a new antianaphylactic agent chemically related to disodium cromoglycate (Stewart, Devlin & Freter, 1974). The inhibition of the Schultz-Dale response by this agent may be attributed to the complex inhibitory effects on the immunological release of histamine (Stewart *et al.*, 1974), SRS-A (Burka & Eyre, 1975), degranulation of mast cells (Stewart, personal communication), and antagonistic

activity towards bradykinin, 5-HT, prostaglandins E_2, $F_{2\alpha}$ and SRS-A (Possanza, Bauen & Stewart, 1974).

M&B 22948 is a derivative of methylxanthine and is another new inhibitor of reagin-mediated anaphylaxis (Broughton, Chaplen, Knowles, Lunt, Pain, Wooldridge, Ford, Marshall, Walker & Maxwell, 1974). The inhibition of the Schultz-Dale reaction by this compound may be due to the inhibition of formation and release of histamine and SRS-A (Broughton *et al.*, 1974; Burka & Eyre, 1975) by virtue of blocking cyclic 3',5'-adenosine monophosphate phosphodiesterase.

Bradykinin is known to contract many intestinal smooth muscles, e.g. rat fundus strip, rabbit duodenum and large intestine and to relax rat duodenum and hen rectal caecum (Rocha e Silva, 1970). The significance of avian kinin (ornithokinins) in pathophysiological processes is not known (Eisen, 1971). The effect of bradykinin on chicken ileum was variable, i.e. no effect or slight to marked contractions at high doses. This needs further study. The reduction or blockade of Schultz-Dale anaphylactic reaction by aprotinin may be attributed to the inhibition of intestinal kallikreins (Zeitlin, 1971) and/or other intestinal proteases (Webster, 1970).

Prostaglandin-synthetase inhibitors (sodium meclofenamate, phenylbutazone, indomethacin), prostaglandin-receptor antagonist (PPP), inhibitors of formation and release of histamine and SRS-A (PPP, PR-D-92-EA, M&B 22948, DECC, disodium cromoglycate) and the kallikrein inhibitor aprotinin have been found to inhibit *in vivo* systemic anaphylaxis significantly. These agents are 100% effective in inhibiting the antigen-induced bradycardia and increased central venous pressor and are 50 to 90% effective in blocking the arterial hypotension in systemic anaphylaxis of chiken (Chand & Eyre, unpublished).

This study suggests that cholinergic, histaminergic and tryptaminergic mechanisms are of low importance in intestinal anaphylaxis of chickens. Results with non-steroidal anti-inflammatory drugs and the so-called anti-anaphylactic agents implicate the vasoactive lipid substances. More specifically since FPL 55712, a proposed SRS-A blocking agent (Augstein *et al.*, 1973) does not inhibit anaphylactic smooth muscle contraction, whereas PPP, a prostaglandin-receptor antagonist (Eakin *et al.*, 1970) strongly inhibits anaphylaxis; it may be tentatively suggested that prostaglandins are more important than SRS-A. On the other hand FPL 55712 and DECC both antagonized the increased 'spontaneity' of the alimentary tract during anaphylaxis, which might implicate SRS-A in the rhythmic low-amplitude movements, rather than in the contracture *per se*. It is pertinent to mention that bovine SRS-A exogenously applied to the chicken ileum, caused a small contraction but a large increase in spontaneous movement. The anti-anaphylactic action of aprotinin, a potent kallikrein inhibitor, also implicates kinins in intestinal Schultz-Dale reactions of chickens.

It would not necessarily be valid to extend these observations to explain systemic anaphylaxis of chickens in which many other organs participate and where the relative significance of chemical mediators may be quite different. It would be reasonable to suggest, however, that Schultz-Dale anaphylaxis may contribute to increased intestinal motility ('forced defaecation') which is a consistent feature of systemic anaphylaxis in chickens. It is a matter for speculation, but certainly a possibility, that local anaphylaxis may contribute to the aetiology of important diseases of the avian digestive tract.

The authors are especially grateful to Professor B.S. Reinhart for generous supplies of chickens; to Mr T.R. Deline for technical assistance and to Dr H.G. Downie for facilities. Thanks are also extended to the numerous pharmaceutical companies (listed above) for generous gifts of drugs. The study was made possible by financial support from the Ontario Ministry of Agriculture and Food.

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(Received December 8, 1975.)