Br. J. Pharmac. (1971), **43**, 302–311.

Pharmacology of bovine pulmonary vein anaphylaxis *in vitro*

P. EYRE

Pharmacology Laboratory, Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada

Summary

1. The bovine pulmonary vein contracts in response to acetylcholine, histamine, 5-hydroxytryptamine and bradykinin. The tissue is particularly sensitive to 5-hydroxytryptamine (>0·1 ng/ml). Specific Schultz-Dale reactions were elicited in the pulmonary vein in response to horse plasma.

2. The Schultz-Dale reaction is inhibited both by antihistaminics and by the anti-5-hydroxytryptamine agent methysergide, but not by atropine.

3. Sodium meclofenamate inhibited anaphylactic contraction but showed a strong tendency to antagonize many agonists indiscriminately.

4. Disodium cromoglycate (DSCG: 100 μg/ml) which inhibits some immunological reactions of mast cells, and diethylcarbamazine citrate (DECC: 50 μg/ml) which inhibits slow-reacting substance of anaphylaxis (SRS–A) elaboration, each inhibited anaphylaxis incompletely (50% or less). However, a combination of DSCG and DECC virtually abolished the Schultz-Dale reaction in this preparation. It is tentatively suggested that the component of the anaphylactic contraction which is resistant to cromoglycate but sensitive to diethylcarbamazine could be due to SRS–A.

5. The bovine pulmonary Schultz-Dale reaction appears to be a complex interaction of histamine, 5-hydroxytryptamine, SRS–A and possible other agents including kinins.

Introduction

Schultz (1910) and Dale (1913) demonstrated that the ileum and uterus respectively, from the antigenically sensitized guinea-pig, contracted *in vitro* on exposure to the same antigen. This Schultz-Dale phenomenon has formed a basis for the study of antigens and antibodies and of the mechanisms of anaphylaxis *per se*, but so far the reaction has been described in only a few tissues from a few species. Most of the information to date has been obtained using the isolated ileum or uterus of the guinea-pig.

In recent years, some attention has been focussed on allergic and anaphylactic reactions of ruminants, particularly cattle and sheep, to foreign antigens, partly because of the economic importance of hypersensitivity conditions such as 'atypical pneumonia' and 'emphysema' of cattle.

The production of skin-(cell)-sensitizing or homocytotropic antibody in the calf has been demonstrated by passive cutaneous anaphylaxis (Wells & Eyre, 1970) and the 'gross' responses of both the anaesthetized and unanaesthetized calf *in vivo* to anaphylactic challenge have been described as dyspnoea, pulmonary arterial hypertension and peripheral hypotension with oedema formation (Aitken & Sanford,

1968, 1969a). In addition, Eyre (1970a) showed that the Schultz-Dale reaction was readily induced in the pulmonary artery and vein and was occasionally elicited in the isolated bronchus of protein sensitized calves. The pulmonary vein was the most sensitive tissue tested. It is clear that the lung is an important anaphylactic shock organ in the bovine species.

During anaphylaxis at least four major pharmacologically active mediators are released and are responsible in some measure for smooth muscle contractions and other phenomena. These agents are histamine, 5-hydroxytryptamine, slow-reacting substance and bradykinin. In the guinea-pig, histamine appears to be the most important agent. However, in species such as the mouse and rat, 5-hydroxytryptamine may be of greater significance than histamine (Parratt & West, 1957a, b; Halpern, Neveu & Spector, 1963). The relative importance of each mediator or the possible presence of other active substances in bovine anaphylaxis has not been described.

It seemed important, therefore, as part of a wider pharmacological investigation of anaphylaxis in cattle and the liberation of biogenic mediators, to study more fully the Schultz-Dale reaction in the calf pulmonary vein and its modification by known pharmacological antagonists.

Methods

Tissue preparation

Lungs were obtained from healthy male Friesian, Ayrshire or Guernsey calves, 1–4 months old. The calves had been sensitized for homocytotropic antibody production by several methods modified from Freund & MacDermott (1942). The methods have been described previously by Eyre (1970b) and Wells & Eyre (1970). The animals were killed with pentobarbitone and one whole lung was removed within 5 min of death, placed in ice-cold Krebs-Henseleit solution (1932) and immediately transported to the laboratory for dissection.

The principal pulmonary vein was carefully removed and placed in cold Krebs solution (normally within 20–30 min of death). The vein was cut spirally into a single strip which was then bisected longitudinally to produce 'twin' strips of vein which were approximately equal width and weight. It was feasible to create several such pairs of vein strips from the same animal and this technique proved useful in affording comparison of responses of analogous strips with and without the presence of antagonist agents.

All muscle strips were set up individually under 500 mg tension in similar 20 ml organ baths containing Krebs-Henseleit solution at 35° C, gassed with 5% CO_2 in oxygen, and were allowed to equilibrate for at least 1 h before use.

All tissues were exposed to agonists for 2 or 3 min every 10 or 15 min and three or four point dose-response curves to histamine, 5-hydroxytryptamine, acetylcholine or bradykinin were established in each strip. It was normally observed that both strips of a pair had approximately the same sensitivity to the agonists. Muscle pairs which showed marked discrepancy in sensitivity were discarded.

The first of each pair of tissues received no antagonist, whereas the second tissue was exposed to a predetermined concentration of antagonist after its dose-response sensitivity to agonists had been established. The activity of an antagonist was

determined by measuring the ratio of doses of agonist which gave equal responses in the presence and absence of antagonist in the second muscle strip. This is the dose ratio (Gaddum, Hameed, Hathaway & Stephens, 1955).

At this point both muscle strips were 'challenged' with the specific antigen, that is 50–100 μg ovalbumin or 0·1–0·2 ml horse plasma. The antigen-induced contraction of each strip was measured and the degree of inhibition caused by the antagonist was expressed as a percentage reduction of the unantagonized response.

Drugs

The agonists used were histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate and acetylcholine chloride expressed as base, and bradykinin as the triacetate.

The antagonists included two antihistamines, mepyramine maleate and tripelennamine hydrochloride; the anti5-HT agent methysergide bimaleate; atropine sulphate; sodium meclofenamate; disodium cromoglycate and diethyl carbamazine citrate. The concentrations of inhibitors referred to are expressed as salts.

Results

Tissue responses

The bovine pulmonary vein is particularly sensitive to 5-hydroxytryptamine. Some preparations contracted to as little as 0·1 ng 5-hydroxytryptamine per ml, as reported elsewhere (Eyre, 1970b). The threshold dose of histamine was approxi-

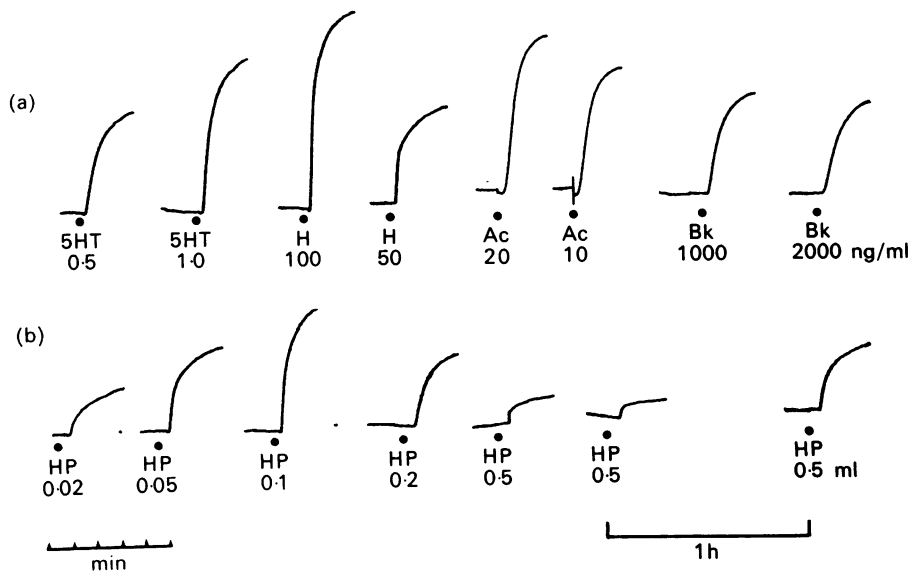

FIG. 1. Isolated spiral strips of pulmonary vein taken from a 6 week old Guernsey calf sensitized with horse serum (0·2 ml/kg). Preparation (a) is contracting to 5-hydroxytryptamine (5HT), histamine (H), acetylcholine (Ac) and bradykinin (Bk), in 20 ml Krebs-Henseleit solution mixed with 5% CO_2 in oxygen at 35° C. Preparation (b) is contracting to increasing concentrations of horse plasma (HP) and shows desensitization with partial recovery after resting 1 hour. Drug concentrations in ng/ml; horse plasma doses in ml.

mately 1–20 ng/ml ; of acetylcholine 1–20 ng/ml and of bradykinin 50–1,000 ng/ml, as shown in Fig. 1a. Bradykinin showed tachyphylaxis.

Figure 1b shows Schultz-Dale reactions on addition of horse plasma. Contractions occurred after a latency of approx. 0·5–1 min and were specific for the particular antigen, no reaction being caused by other proteins such as egg albumin in pulmonary veins of animals which had been sensitized specifically with horse serum.

The threshold dose was of the order of 0·01 ml horse plasma in a 20 ml organ bath. Using the same sensitized vein it was possible to show an antigen dose-response relationship between 0·01 and 0·20 ml horse serum. Each dose of horse serum was left in contact for 2 minutes. Washing out was repeated until the muscle had fully relaxed. This usually occurred within 20 min, at which time the next higher dose of plasma was added. Contractions increased in size with increasing horse plasma concentration up to approximately 0·2 ml plasma, above which the contractions diminished rapidly. On leaving the vein strip untreated apart from occasional 'washing' with Krebs, over a period of 1–2 h, it was noted that the tissue recovered part of its sensitivity to the same doses of horse plasma (Fig. 1b). A similar observation was made by Dale & Okpako (1969).

Antihistaminics

Mepyramine (0·1–1·0 μg/ml) specifically antagonized the actions of histamine and strongly inhibited the Schultz-Dale reaction: 66% and 73% respectively (Table 1: Fig. 2a). Tripelennamine (1·0 μg/ml) also selectively antagonized histamine and inhibited the anaphylactic reaction by 80%.

TABLE 1. *Dose ratios of histamine, 5HT, acetylcholine and bradykinin, and percentage inhibition of Schultz-Dale reaction in calf pulmonary vein* in vitro, *in the presence of antagonists*

Antagonist	(μg/ml)	Dose ratios of agonists				% Inhibition of Schultz-Dale reaction
		Histamine	5HT	Acetylcholine	Bradykinin	
Mepyramine	0·1	250 (4)	1·1 (4)	—	—	66 (4)
	1·0	3010 (2)	4·1 (2)	6·1 (2)	—	73 (2)
Tripelennamine	1·0	150 (2)	1·0 (2)	—	—	80 (2)
Methysergide	0·1	1·0 (4)	7·7 (4)	—	—	82 (4)
	0·2	1·1 (3)	23·0 (3)	1·0 (3)	1·0 (2)	85 (3)
Atropine	0·1	1·5 (2)	1·0 (2)	100·0 (2)	—	0 (2)
Sodium meclofenamate	1·0	1·0 (2)	1·0 (2)	—	—	0 (2)
	5·0	1·0 (2)	1·2 (2)	—	21·0 (2)	58 (2)
	20·0	12·0 (2)	20·2 (2)	18·0	55·0 (2)	70 (2)
Diethylcarbamazine citrate (DECC)	25·0	4·0 (3)	2·2 (3)	—	—	38 (3)
	50·0	3·1 (2)	2·5 (2)	2·1 (2)	2·0 (2)	51 (2)
Disodium cromoglycate (DSCG)	10·0	1·0 (4)	1·1 (4)	—	—	0 (4)
	20·0	1·1 (3)	1·2 (3)	—	1·0 (2)	21 (3)
	50·0	2·1 (2)	1·3 (2)	1·4 (2)	—	32 (2)
	100·0	3·6 (2)	3·0 (2)	—	—	38 (2)
DSCG plus DECC	50·0 50·0	3·5 (2)	2·4 (2)	—	—	61 (2)
DSCG plus DECC	100·0 100·0	9·5 (2)	5·0 (2)	—	—	90 (2)

Figures are means and number of observations are in parentheses.

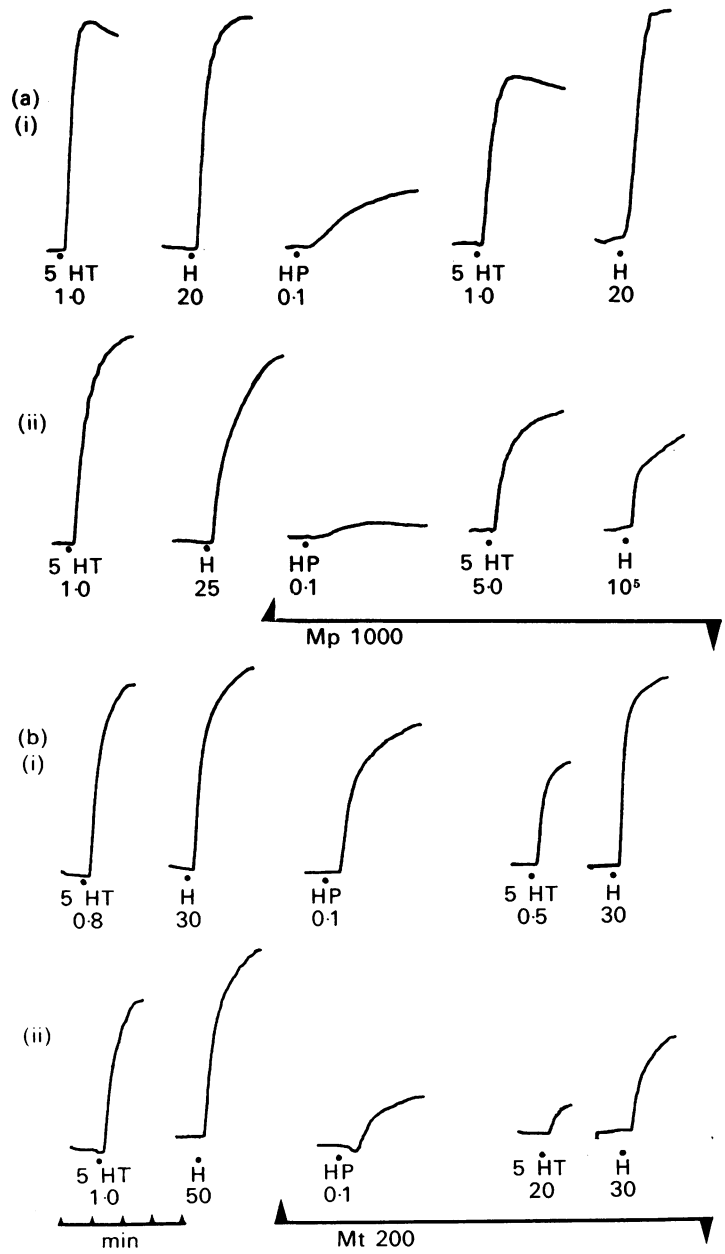

FIG. 2. Two pairs (a) and (b) of isolated spiral strips of pulmonary veins taken from an 8 week old Guernsey calf sensitized to horse serum (0·2 ml/kg). Both strips of each pair were obtained from the same vein. All strips contract to 5-hydroxytryptamine (5HT), histamine (H), and horse plasma (HP) in 20 ml Krebs-Henseleit solution mixed with 95% O_2 in 5% CO_2 mixture at 35° C. In the second strip (ii) of each pair an antagonist was present in the bath fluid between the arrows shown: (a, ii) mepyramine maleate (Mp) antagonizes histamine and inhibits the Schultz-Dale anaphylactic response; (b, ii) methysergide (Mt) inhibits 5HT and the anaphylactic response. Drug concentrations in ng/ml; horse plasma doses in ml.

Anti-5-hydroxytryptamine

Methysergide (up to 0·1 μg/ml) weakly inhibited 5-hydroxytryptamine (dose ratio 7·7). Methysergide (0·2 μg/ml) antagonized 5-hydroxytryptamine more effectively (dose ratio 23·0) and inhibited the anaphylactic reaction by approximately 80%. Despite this low level of 5-hydroxytryptamine antagonism, methysergide was a specific antagonist: the contractions due to other agonists, that is acetylcholine, histamine and bradykinin, remained unaffected (Fig. 2b).

Methysergide (>0·1 μg/ml) raised the tone of the venous muscle strip. Concentrations of 0·1–0·2 μg/ml elicited only a small contraction which did not interfere markedly with subsequent testing of the drug. However, concentrations of methysergide greater than 0·2 μg/ml strongly contracted the strip and prevented further investigation of increasing concentrations of this antagonist.

Sodium meclofenamate

Meclofenamic acid derivatives inhibit bradykinin, slow-reacting substance and antigenic bronchospasm in the guinea-pig (Berry & Collier, 1964 ; Collier & James, 1967 ; Collier, James & Piper, 1968).

Sodium meclofenamate (1 μg/ml) did not show any inhibitory actions in this preparation. Five microgrammes per millilitre inhibited the anaphylactic response by 58% and at 20 μg/ml the Schultz-Dale response was reduced 70%. However, the highest concentration of meclofenamate tended to antagonize several agonists in the non-specific way. The inhibition of bradykinin, though not marked even at 20 μg meclofenamate per ml, was approximately 3 times greater than that of any other agonist tested.

Diethylcarbamazine citrate

Diethylcarbamazine is said to block the elaboration of slow-reacting substance of anaphylaxis (Orange, Valentine & Austen, 1968) and thus inhibits hypersensitivity reactions in which SRS–A occurs (Orr, Gwilliam & Cox, 1970). Diethylcarbamazine may thus be useful in the pharmacological investigations of anaphylactic reactions.

It can be seen in Table 1 that diethylcarbamazine (50 μg/ml) inhibits the anaphylactic reaction of the muscle by approximately 50%. This drug, however, showed a small non-specific inhibitory effect on acetylcholine, histamine, 5-hydroxytryptamine and bradykinin.

Disodium cromoglycate

This agent has been reported to inhibit ' reaginic ' hypersensitivity both *in vivo* and *in vitro* (Cox, 1967 ; Sheard & Blair, 1970).

Disodium cromoglycate (10 μg/ml) did not inhibit the bovine pulmonary Schultz-Dale reaction. Twenty microgrammes per millilitre produced 21% inhibition of anaphylaxis without inhibiting histamine, 5-hydroxytryptamine or bradykinin, whereas cromoglycate (50 μg/ml) inhibited anaphylaxis by 32%. The response of the vein strip to antigenic challenge was reduced by 38% by cromoglycate (100 μg/ml), but this concentration of the inhibitor also had some antagonistic action on histamine and 5-hydroxytryptamine (Fig. 3a).

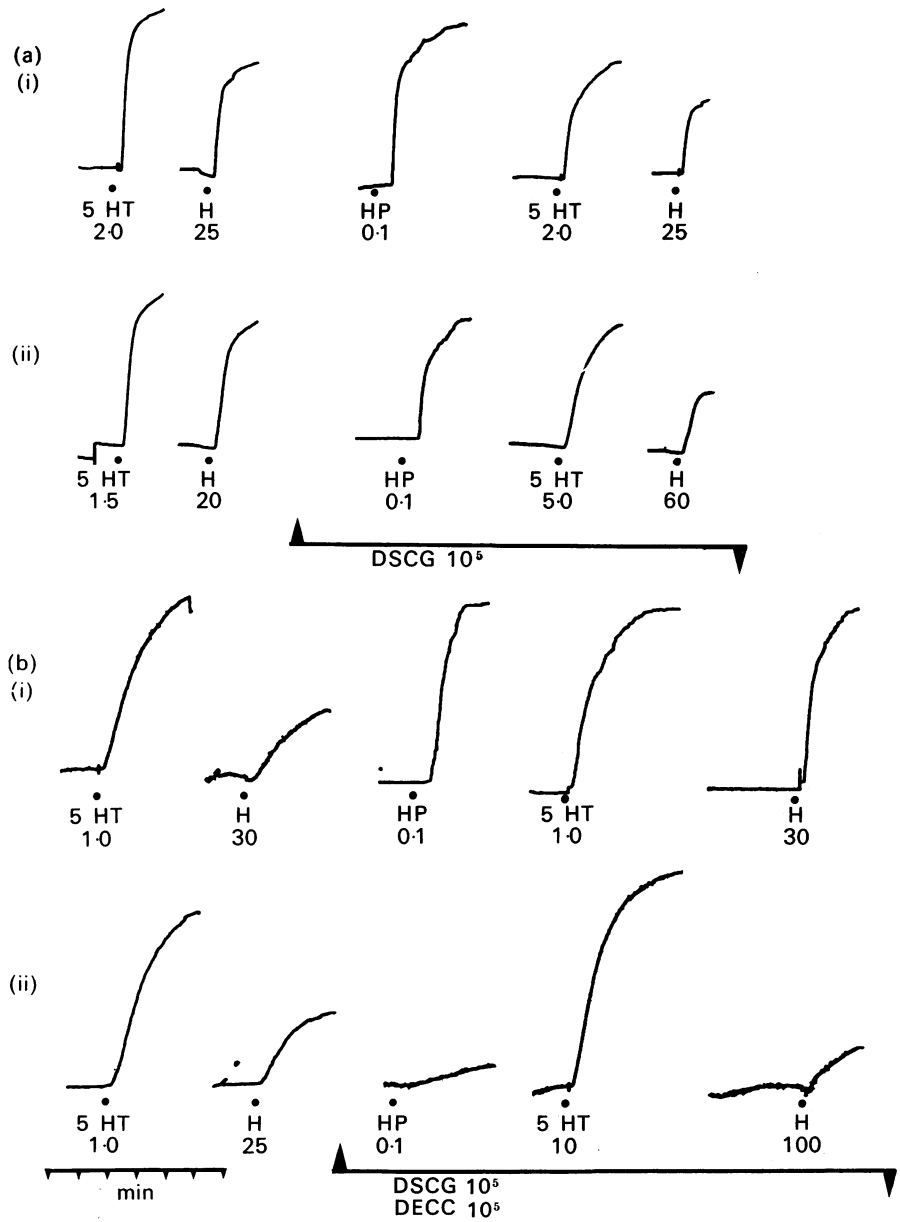

FIG. 3. Pairs of isolated spiral strips of pulmonary veins taken (a) from a 6 week old Friesian calf and (b) from a 7 week old Guernsey calf, both animals having been sensitized to horse serum (0·2 ml/kg). Both strips of each pair were obtained from the same vein. All tissues contract to 5-HT, histamine (H) and horse plasma (HP) in 20 ml Krebs-Henseleit solution gassed with 95% O_2:5% CO_2 mixture. In the second strip of each pair (ii) antagonists were present between the arrows shown. (a, ii) Disodium cromoglycate (DSCG) weakly inhibited the anaphylactic response and showed a small, non-specific inhibition of 5-HT and histamine, whereas (b, ii) DSCG plus diethylcarbamazine (DECC) inhibited the anaphylactic response almost completely. Drug concentrations in ng/ml, horse plasma doses in ml.

A combination of disodium cromoglycate (50 μg/ml) and diethylcarbamazine (50 μg/ml) caused 61% inhibition of anaphylactic reaction ; and disodium cromoglycate (100 μg/ml) combined with diethylcarbamazine (100 μg/ml) inhibited the Schultz-Dale reaction by 90% (Fig. 3b). These combinations of inhibitors non-specifically reduced the responses to several agonists tested.

Discussion

The results clearly show that the pulmonary vein strip of the calf is sensitive to compounds such as acetylcholine, histamine, bradykinin and 5-hydroxytryptamine (particularly the last). Furthermore, this tissue is now added to the short list of smooth muscle preparations in which the Schultz-Dale phenomenon has been observed and studied pharmacologically. This report is also in agreement with the concept that the lung is an important anaphylactic shock organ of cattle and focusses some attention on the pulmonary vein in addition to the artery and bronchus (Eyre, 1970a) which have been discussed previously (Aitken & Sanford, 1969a ; Alexander, Eyre, Head & Sanford, 1970).

The pulmonary vein of the calf is particularly sensitive to 5-hydroxytryptamine and it is possible to use this preparation for bioassay. Vein strips frequently responded to as little as 0·1 ng 5-hydroxytryptamine/ml. This high sensitivity to serotonin caused an initial problem because horse serum contains in the order of 1·0 μg 5-hydroxytryptamine/ml. Thus horse serum may cause on occasion, a contraction of the calf pulmonary blood vessel unrelated to the Schultz-Dale effect. This 'unspecific' action was eliminated when horse plasma was used as the challenging antigen.

The antihistaminic drugs efficiently inhibited the Schultz-Dale effect. That this blockade was a specific one is shown by the selective antagonism of histamine itself in the same tissue, and suggests that histamine participates in the Schultz-Dale phenomenon.

Methysergide caused specific 5-hydroxytryptamine blockage which was of unexpectedly low intensity (dose ratio of 23 at 200 ng methysergide/ml) in contrast to the high level of Schultz-Dale inhibition (85% at 200 ng methysergide/ml). It was interesting that methysergide caused contraction of the bovine pulmonary vein and thus it was impossible to test concentrations greater than 200 ng/ml. This vascular spasmogenic action of methysergide has been reported by Halmagyi & Colebatch (1961) and Sutter (1965).

Sodium meclofenamate inhibits some of the actions of kinins, SRS–A and anaphylactic bronchospasm in the guinea-pig (Berry & Collier, 1964 ; Collier & James, 1967 ; Collier, James & Piper, 1968) ; but these authors indicate that the drug is less effective on blood vessels than on bronchial muscle. Aitken & Sanford (1969b), Eyre (1970b), and Alexander *et al.* (1970) reported that sodium meclofenamate was an effective antagonist of anaphylaxis of cattle and sheep. In these experiments sodium meclofenamate (5 μg/ml) inhibited bovine pulmonary anaphylaxis *in vitro* by some 50%. Doses greater than 5 μg/ml (for example 20 μg/ml) had a more efficient anti-anaphylactic effect, but also showed a marked tendency to inhibit several agonists in a non-specific way. Sodium meclofenamate thus seems to be less efficient than antihistaminics or methysergide.

Diethylcarbamazine citrate (DECC: 'Hetrazan', 'Franocid')—primarily known as a nematocidal anthelmintic—has recently been shown to inhibit the elaboration and/or release of SRS–A. DECC (50 μg/ml) inhibited the Schultz-Dale effect by 51%.

Disodium cromoglycate (DSCG 'Intal'), which protects the mast cells of some species against immunological damage was seen to be an incomplete inhibitor of the bovine pulmonary anaphylactic reaction: even at the extremely high concentration, DSCG (100 μg/ml) caused only 38% inhibition. It was interesting that the cromoglycate-resistant contraction of the vein to antigen appeared to be a slower contraction which had a longer latency than in the untreated tissue. This component of the anaphylactic contraction which is resistant to cromoglycate could be due to SRS–A according to the evidence of Stechschulte, Austen & Bloch (1967). These authors showed that in the rat, the release of amines appears to be immunologically distinct from the mechanism controlling production of SRS–A. Pretreatment of rats with disodium cromoglycate inhibits amine release but not that of SRS–A. In rats, SRS–A release is mediated through a heat stable IgG antibody whereas amine release depends upon a heat labile reagin (Stechschulte *et al.*, 1967). However, species differences may be very important because in human lung no such immunological distinction has been shown. Sheard & Blair (1970) believe that in man both release mechanisms depend on the same antigen-antibody system and showed that both are inhibited by cromoglycate. No similar study has yet been completed in ruminants. However, in view of the evidence given here that diethylcarbamazine strongly inhibits the bovine Schultz-Dale reaction, and particularly that carbamazine blocks the cromoglycate-resistant anaphylactic response, it is tempting to suggest that SRS–A is participating in pulmonary anaphylaxis of cattle. We are currently attempting to identify SRS–A released from bovine lung.

It is safe to conclude that the Schultz-Dale phenomenon in bovine pulmonary vessels is complex and cannot be ascribed to any one mechanism in particular: the total effect probably being an interaction of histamine, 5-HT, SRS–A with the possible addition of kinins and other undetected substances. In any event it may not be valid to extend the conclusions to other sites in the body or to general systemic anaphylaxis where the relative significance of mediators could well be quite different.

Disodium cromoglycate and diethylcarbamazine will be worthy of further study in the attempt to elucidate bovine anaphylaxis. It is further possible that either or both of these drugs may be useful in treating clinical respiratory hypersensitivity of ruminants. This possibility will be investigated.

I am indebted to Professor H. C. Downie for facilities and encouragement, also to Mr. D. Sandals for technical and other assistance during his tenure of a Summer Studentship. Thanks are due to Parke-Davis of Hounslow, England, for meclofenamic acid and to Fisons of Loughborough, England, for disodium cromoglycate 'Intal'. The work was supported by the Ontario Department of Agriculture and Food, and by grant A-5937 of the National Research Council of Canada, to whom I am grateful.

REFERENCES

AITKEN, M. M. & SANFORD, J. (1968). Experimentally induced anaphylaxis in cattle. *Vet. Rec.*, **82**, 418–419.

AITKEN, M. M. & SANFORD, J. (1969a). Experimental anaphylaxis in cattle. *J. Comp. Path.*, **79**, 131–139.

AITKEN, M. M. & SANFORD, J. (1969b). Protection of cattle against experimentally-induced anaphylaxis. *Nature, Lond.*, **223**, 314–316.

ALEXANDER, F., EYRE, P., HEAD, K. W. & SANFORD, J. (1970). Effects of anaphylaxis and chemical histamine liberators in sheep. *J. Comp. Path.*, **80**, 19–30.

BERRY, P. A. & COLLIER, H. O. J. (1964). Bronchoconstrictor action and antagonism of a slow-reacting substance of anaphylaxis from guinea-pig isolated lung. *Br. J. Pharmac. Chemother.*, **23**, 201–216.

COLLIER, H. O. J. & JAMES, G. W. L. (1967). Humoral factors affecting pulmonary inflation during acute anaphylaxis in the guinea-pig *in vivo*. *Br. J. Pharmac. Chemother.*, **30**, 283–301.

COLLIER, H. O. J., JAMES, G. W. L. & PIPER, P. J. (1968). Antagonism by fenamates and like-acting drugs of bronchoconstriction induced by bradykinin and antigen in the guinea-pig. *Br. J. Pharmac. Chemother.*, **34**, 76–87.

COX, J. S. G. (1967). Disodium cromoglycate (FPL 670) 'Intal': a specific inhibitor of reaginic antibody antigen mechanisms. *Nature, Lond.*, **216**, 1328–1329.

DALE, H. H. (1913). The anaphylactic reaction of plain muscle in the guinea-pig. *J. Pharmac. exp. Ther.*, **4**, 167–223.

DALE, MAUREEN M. & OKPAKO, D. T. (1969). Recovery of anaphylactic sensitivity in the guinea-pig ileum after desensitization. *Immunology*, **17**, 653–663.

EYRE, P. (1970a). The Schultz-Dale reaction in bovine pulmonary smooth muscles. *Br. J. Pharmac.*, **40**, 166–167*P*.

EYRE, P. (1970b). Cutaneous vascular permeability factors (histamine, 5-hydroxytryptamine, bradykinin) and passive cutaneous anaphylaxis in sheep. *J. Pharm. Pharmac.*, **22**, 104–109.

FREUND, J. & MACDERMOTT, K. (1942). Sensitization to horse serum by means of adjuvants. *Proc. Soc. exp. Biol. N.Y.*, **49**, 548–552.

GADDUM, J. H., HAMEED, K. A., HATHAWAY, D. E. & STEPHENS, F. F. (1955). Quantitative studies of antagonists for 5-hydroxytryptamine. *Q. Jl exp. Physiol.*, **40**, 49–74.

HALMAGYI, D. F. J. & COLEBATCH, H. J. H. (1961). Serotonin-like cardiorespiratory effect of a serotonin antagonist. *J. Pharmac. exp. Ther.*, **134**, 47–52.

HALPERN, B. N., NEVEU, T. & SPECTOR, S. (1963). On the nature of chemical mediators involved in anaphylactic reactions in mice. *Br. J. Pharmac. Chemother.*, **20**, 389–398.

KREBS, H. A. & HENSELEIT, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seylers Z. physiol. Chem.*, **210**, 33–66.

ORANGE, R. P., VALENTINE, M. D. & AUSTEN, K. F. (1968). Inhibition of SRS-A in the rat by diethylcarbamazine. *Proc. Soc. exp. Biol. Med.*, **127**, 127–132.

ORR, T. S. C., GWILLIAM, J. & COX, J. S. G. (1970). Studies on passive cutaneous anaphylaxis in the rat with disodium cromoglycate. I. Cutaneous reactions induced by an anti-DNP $7S\gamma_1$ antibody. *Immunology*, **19**, 469–479.

PARRATT, J. R. & WEST, G. B. (1957a).5- Hydroxytryptamine and tissue mast cells. *J. Physiol., Lond.*, **137**, 169–178.

PARRATT, J. R. & WEST, G. B. (1957b). Release of 5-hydroxytryptamine from tissues of the rat. *J. Physiol., Lond.*, **137**, 179–192.

SCHULTZ, W. H. (1910). Physiological studies in anaphylaxis. I. The reaction of smooth muscle of the guinea-pig sensitized to horse serum. *J. Pharmac. exp. Ther.*, **1**, 549–567.

SHEARD, P. & BLAIR, A. M. J. N. (1970). Disodium cromoglycate. Activity in three *in vitro* models of the immediate hypersensitivity reaction in lung. *Int. Archs Allergy*, **38**, 217–224.

STECHSCHULTE, D. J., AUSTEN, K. F. & BLOCH, K. J. (1967). Antibodies involved in antigen-induced release of SRS-A in the guinea-pig and rat. *J. exp. Med.*, **125**, 127–147.

SUTTER, M. C. (1965). The pharmacology of isolated veins. *Br. J. Pharmac. Chemother.*, **24**, 742–751.

WELLS, P. W. & EYRE, P. (1970). Homocytotropic antibodies demonstrated by passive cutaneous anaphylaxis in calves. *Vet. Rec.*, **86**, 173–175.

(*Received April* 21, 1971)