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Acute systemic anaphylaxis in the calf

P. EYRE, A. J. LEWIS* AND P. W. WELLS†

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

Summary

1. Acute systemic anaphylaxis in calves was characterized by marked systemic hypotension ; hypertension in the pulmonary arteries and abdominal vena cava, and transient apnoea. Calves responded with a second reaction to antigen, but a third anaphylactic response could not be evoked.

2. Suppression of systemic anaphylaxis could not be effected with mepyramine, whereas methysergide or diethylcarbamazine each suppressed anaphylaxis by 50%. Disodium cromoglycate alone did not inhibit anaphylaxis: however, when disodium cromoglycate was combined with diethylcarbamazine almost total suppression (85%) was achieved. Sodium meclofenamate also was a powerful inhibitor of anaphylaxis (80%). It is tentatively suggested that slow reacting substance (SRS-A) may be an important mediator of bovine anaphylaxis.

3. Bilateral vagotomy did not modify the circulatory responses to injected histamine, 5-hydroxytryptamine (5-HT) or antigen, whereas the effects of these agents on ventilation (apnoea) were abolished by vagotomy.

4. Plasma histamine concentration was increased during anaphylaxis, whereas plasma 5-HT was not. Whole blood histamine concentration fell sharply and remained depressed during 20 min of anaphylactic shock. Reduced whole blood histamine levels probably reflect the severe leucopoenia in the calves.

5. Histamine concentrations in six tissues taken from calves subjected to anaphylaxis were not different from those in control calves ; mast cells were of similar numbers to controls, but showed some swelling, granular spilling and metachromasia.

6. Histamine, 5-HT, bradykinin and antigen caused increased pulmonary artery perfusion pressure and ventilation resistance in isolated lungs from sensitized calves. However, there was no difference in histamine and 5-HT concentration in perfusates obtained during antigen infusion of sensitized and control lungs.

7. Systemic anaphylaxis of calves may result from the interaction of histamine, 5-HT and SRS-A. The present data implicate (by indirect measurement) SRS-A as an important mediator of anaphylaxis in this species.

* Present address: Yale University, Lung Research Center, 333 Cedar St., New Haven, Conn. 06510, U.S.A.

† Present address: Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh EH9 1QH, Scotland.

Introduction

The implication that hypersensitivity may be involved in certain disease processes of cattle (e.g. acute pulmonary emphysema (Hyslop, 1969 ; Dungworth, 1965), milk allergy (Campbell, 1970) and laminitis (Nilsson, 1963)), has stimulated interest in the nature of the mediators of anaphylaxis in the bovine species (Aitken & Sanford, 1968; Wells & Eyre, 1972; Eyre & Lewis, 1972; Eyre, 1970, 1971a, b, c).

Features of the acute experimental anaphylactic reaction include dyspnoea, pulmonary hypertension and systemic hypotension (Aitken & Sanford, 1969a; Eyre & Lewis, 1972). At necropsy, major pathological changes such as oedema, congestion and emphysema were recognized in the respiratory tract (Aitken & Sanford, 1969a ; Wray & Thomlinson, 1969). Such findings suggest that the lung is a major anaphylactic shock organ in cattle. The demonstration of Schultz–Dale responses in isolated strips of the pulmonary blood vessels of sensitized calves and release of amines from isolated calf lung lends further support to this contention (Eyre, 1970, 1971a, b, c).

Earlier studies employing antagonists (Aitken & Sanford, 1969b) indicated that antihistaminics and anti-5-hydroxytryptamine agents were not effective in reducing the clinical signs of experimentally-induced anaphylaxis in calves. Cardio-respiratory recordings were not made in this study. Minor roles in bovine anaphylaxis were proposed for histamine and 5-hydroxytryptamine, but the possible involvement of kinins and slow-reacting substance (SRS-A) was suggested from the finding that sodium meclofenamate effectively protected conscious calves against the symptoms of anaphylactic shock (Aitken & Sanford, 1969b). The release of kinins during systemic anaphylaxis has now been established in this species (Eyre & Lewis, 1972). The release of histamine and 5-hydroxytryptamine from sensitized bovine pulmonary tissue *in vitro* by antigen challenge, has also been demonstrated (Eyre, 1971a).

In this study an attempt to observe whether comparable changes occurred in perfused isolated lung is described, together with measurement of concentrations of these amines in the blood during anaphylaxis. The effects of the following known pharmacological inhibitors of putative mediators of anaphylaxis were examined: mepyramine, which inhibits histamine: methysergide, a 5-hydroxtryptamine antagonist: sodium meclofenamate which inhibits kinins and SRS-A: diethylcarbamazine, an SRS-A inhibitor (Orange, Valentine & Austen, 1968) and disodium cromoglycate (Cox, 1967) an inhibitor of the anaphylactic liberation of vasoactive mediators. The possible role of vagal reflexes in the mediation of anaphylaxis was also investigated in view of observations in other species (Auer & Lewis, 1910; Karczewski & Widdicombe, 1969).

Methods

Thirty male calves of the Jersey and Guernsey breeds (1–2 months old, 30–60 kg) were sensitized to whole horse serum. Following intravenous injection of horse serum (0·2 ml/kg), a similar dose of horse serum emulsified in an equal volume of Freund's complete adjuvant was given subcutaneously. The subcutaneous injection was repeated after seven days. After a further 14–21 days, the calves were anaesthetized with pentobarbitone sodium (20 mg/kg). Blood pressures were recorded in the femoral artery, pulmonary artery and abdominal vena cava as previously described by Eyre & Lewis (1972). Respiratory ventilation volume was recorded with

a Statham volumetric transducer and the heart rate was measured with an electrocardiogram using a Grass polygraph with standard limb lead II.

Responses to histamine, 5-hydroxytryptamine (5-HT) and bradykinin were obtained in four sensitized calves and the animals were then challenged with horse serum (0·2 ml/kg, given i.v. over a 2 min period) to induce anaphylaxis.

The effects of five inhibitor drugs or drug combinations on systemic anaphylaxis were measured in fourteen calves sensitized to horse serum. They were divided into seven pairs which were treated respectively as follows: (i) mepyramine maleate (2 mg/kg); (ii) methysergide bimaleate (2 mg/kg); (iii) sodium meclofenamate (2 mg/kg) given approximately 30 min prior to challenge; (iv) diethylcarbamazine citrate (20 mg/kg) given alone; (v) disodium cromoglycate (10 mg/kg) given alone, or (vi) diethylcarbamazine plus disodium cromoglycate given simultaneously, were administered within two to three min before antigen challenge. In the seventh pair, diethylcarbamazine was given 10–15 min and disodium cromoglycate two min before antigen challenge.

Dose-response relationships based on several doses of each agonist (histamine, 5-HT and bradykinin) were established before and after each antagonist. An estimate of the effectiveness and specificity of antagonists was measured by determining the ratio of doses of agonist which gave equal carotid blood pressure responses in the presence and absence of antagonist. This is the concept of 'dose-ratio' (Gaddum, Hameed, Hathway & Stephens, 1955).

In three calves sensitized to horse serum, both vagi were exposed in the cervical region and cut individually. Minimum effective doses of histamine, 5-HT and bradykinin were recorded before and after vagotomy and any changes were noted. Fifteen to 30 min after vagotomy these calves were challenged with horse serum to induce anaphylaxis as previously described.

In a further four sensitized calves, carotid arterial blood samples were taken 15–30 min before antigen challenge, at challenge and 0·5, 1·0, 2, 3, 4, 6, 8, 10, 15, 20 and 25 min afterwards. Plasma histamine concentration (Noah & Brand, 1961) and whole blood histamine (Shore, Burkhalter & Cohn, 1959) were measured. Plasma 5-HT concentrations were estimated by the method of Andén & Magnusson (1967).

Calves were killed with pentobarbitone at the end of the experiment and subjected to post-mortem examination. Portions of lung (pleura and parenchyma), subcutaneous tissue, liver (capsule and parenchyma) and small intestine were removed for determination of histamine concentration (Shore *et al.*, 1959). Tissue spreads of pleura, subcutaneous tissue, omentum and liver capsule were fixed in 80% ethanol, stained with Toluidine blue (Riley, 1953) and examined histologically for mast cell numbers and morphology.

Four unsensitized (control) calves were anaesthetized in the same way as described above and injected with horse serum (0·2 ml/kg, i.v.). Haemodynamic and respiratory changes were monitored and serial carotid blood samples were assayed for histamine and 5-HT as described above. Similar tissue spreads and portions of organs were taken at post-mortem and examined in the same way as in the anaphylactic group, for mast cell changes and amine concentrations.

Two calves that had been sensitized to horse serum but had not been subjected to any other experimentation were killed with pentobarbitone sodium and their lungs removed immediately. One whole lung from each animal was perfused

through a cannula in the pulmonary artery with Krebs–Henseleit solution at 37° C which had been thoroughly gassed with 95% oxygen/5% CO_2 mixture. The perfusion rate was maintained at 10 ± 1 ml per min with a peristaltic pump. The whole system was maintained at 37° C by means of water jackets. Perfusion pressure was measured by means of a T-tube in the arterial cannula attached to a Statham strain gauge transducer recording on an E & M Physiograph. Venous effluent was collected from the pulmonary veins. The lung was ventilated by a Bird animal respirator pump and inflationary resistance was estimated by the method described by Konzett & Rössler (1940).

Small doses of histamine, 5-hydroxytryptamine or bradykinin were injected into the arterial cannula and responses recorded. Five ml horse serum was then injected and changes in perfusion pressure and inflationary resistance were recorded. Con-

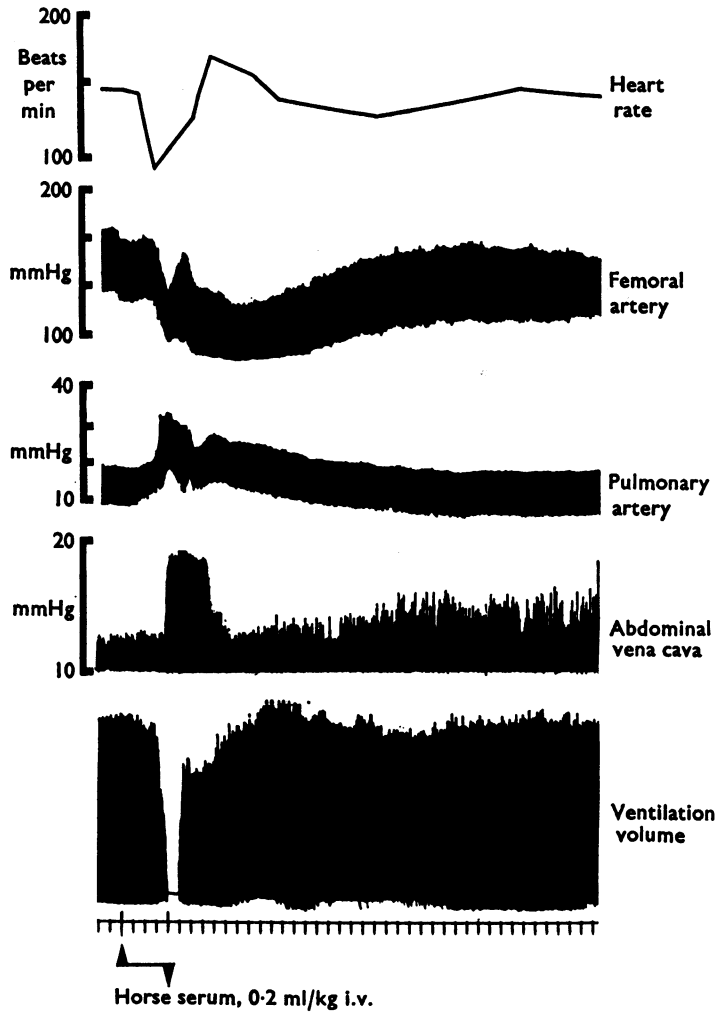

FIG. 1. Calf sensitized with horse serum and Freund's complete adjuvant: anaesthetized with pentobarbitone sodium. Top graph represents heart rate (beats/min). Pen records from above downwards are (i) femoral artery pressure; (ii) pulmonary artery pressure; (iii) abdominal vena cava pressure; (iv) ventilation volume. Effects of horse serum (0·2 ml/kg, i.v.) given over a two min period (indicated by arrows). Time marker indicates 30 s.

centrations of histamine and 5-HT in the venous effluent were measured as previously described. Lungs from two unsensitized (control) calves were similarly perfused, ' challenged ', and amine levels measured.

Histamine diphosphate, 5-hydroxytryptamine creatinine sulphate, methysergide bimaleate and bradykinin triacetate were purchased from Nutritional Biochemicals Co., Cleveland, Ohio. The following drugs were gifts: mepyramine maleate (Poulenc Ltd., Montreal, Canada); methysergide bimaleate (Sandoz, Basle, Switzerland); sodium meclofenamate (Parke, Davis & Co., Detroit, Michigan); diethylcarbamazine citrate (Burroughs Wellcome) and disodium cromoglycate (Fisons).

Results

Characteristics of acute anaphylaxis

Actions of histamine, 5-HT and bradykinin in calves have been described previously (Eyre & Lewis, 1972; Lewis & Eyre, 1972).

Anaphylaxis was characterized by marked changes in recorded parameters in all of the sensitized calves which had not been treated with inhibitors (Fig. 1). The systemic arterial pressure fell sharply, initially. This was followed by a transient rise, and a further hypotension before returning to normal. Pulmonary arterial pressure increased approximately at the same time as the primary fall in systemic pressure. The pulmonary hypertension was reduced, then rose for a second time before slowly returning to the normal value. Increased pressure in the vena cava was delayed some 20 s in onset, and coincided with a period of apnoea as monitored by ventilation volume. The bradycardia which was observed 60 s after the beginning of challenge, was followed by a marked tachycardia four min later (Fig. 1). On three occasions a calf was challenged a second time with the same volume of horse serum, 30–60 min after the primary challenge. Each calf responded with a

TABLE 1. *Changes in mean femoral arterial blood pressure in eight pairs of calves subjected to systemic anaphylactic shock, and in two controls. Effects of pretreatment with inhibitors*

Calf No.	Treatment	Mean systemic arterial B.P. (mmHg) Pre-shock	Shock	Percentage change in systemic arterial pressure in anaphylaxis	Effect of inhibitors: Dose ratio Histamine	5-HT	Bradykinin	Per cent inhibition of anaphylactic response
1	Unsensitized control	160	160	0	–	–	–	–
2	No inhibitor	130	140	+ 8	–	–	–	–
3	Sensitized	160	50	−69	–	–	–	–
4	No inhibitor	140	60	−57	–	–	–	–
5	Mepyramine maleate	100	30	−70	10	1	1	−11
6	(2 mg/kg)	120	80	−33	20	1	1	48
7	Methysergide bimaleate	125	80	−36	2	10	1	43
8	(2 mg/kg)	150	110	−27	2	8	1	59
9	Sodium meclofenamate	150	135	−10	1	1	1	84
10	(2 mg/kg)	130	120	−15	1	1	2	76
11	Diethylcarbamazine	140	90	−36	–	–	–	43
12	(DECC: 20 mg/kg)	110	80	−27	–	–	–	57
13	Disodium cromoglycate	150	90	−40	–	–	–	37
14	(DSCG: 10 mg/kg)	140	30	−79	–	–	–	−25
15	DECC (20 mg/kg) and	130	120	− 8	–	–	–	87
16	DSCG (10 mg/kg) simultaneously	110	100	− 9	–	–	–	86
17	DECC (20 mg/kg) and	160	50	−69	–	–	–	−10
18	DSCG (10 mg/kg) separately	130	60	−54	–	–	–	14

typical anaphylactic response as described above, although all parameters recorded were reduced in amplitude. A third anaphylactic reaction could not be evoked 15–30 min after the secondary challenge, in any of the calves examined.

Effects of inhibitors

It was noted that suppression of systemic anaphylaxis was proportionately similar for all three blood pressure parameters measured and for respiration. For the purpose of measuring drug-induced inhibition of anaphylaxis, only the mean systemic arterial blood pressure was used (Table 1).

Of the seven drug treatments employed, sodium meclofenamate or a combined injection of disodium cromoglycate and diethylcarbamazine were most effective in

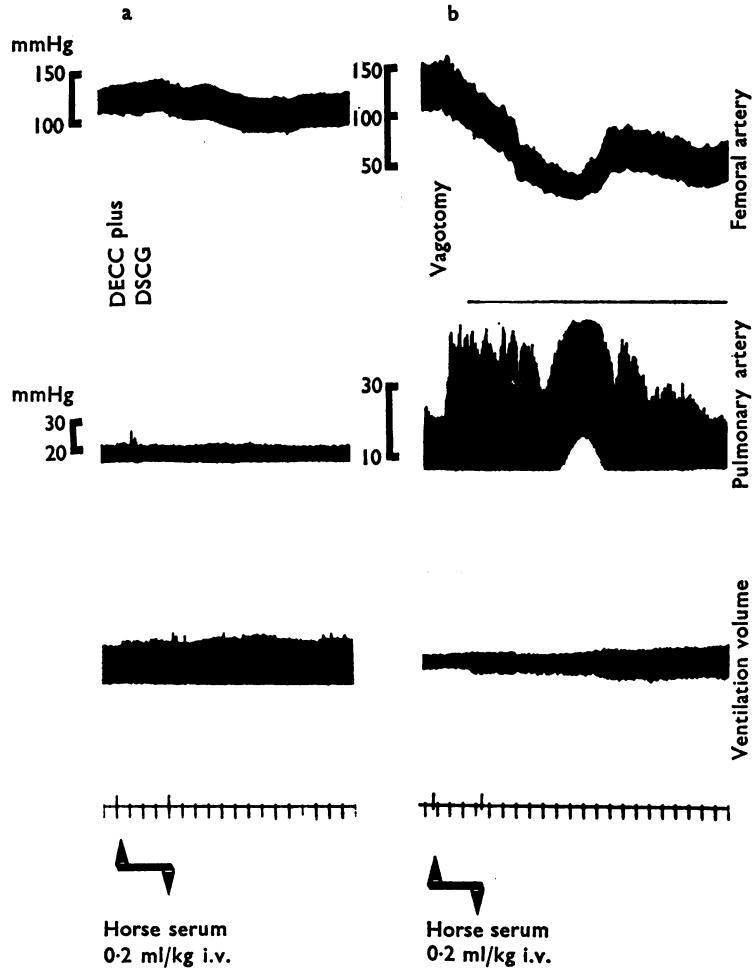

FIG. 2. Two calves a and b, sensitized with horse serum and Freund's complete adjuvant: anaesthetized with pentobarbitone sodium. Pen records from above downwards are (i) femoral artery pressure; (ii) pulmonary artery pressure; (iii) ventilation volume. Effects of horse serum (0·2 ml/kg, i.v.) given over a two min period (indicated by arrows). Time marker indicates 30 s. *Calf a*—pretreated with diethylcarbamazine (DECC, 20 mg/kg, i.v.) and disodium cromoglycate (DSCG, 10 mg/kg, i.v.) immediately before horse serum. Suppression of changes in all parameters is recorded. *Calf b*—subjected to bilateral vagotomy 15–30 min before horse serum 'challenge'. Abolition of apnoea is recorded.

suppressing systemic anaphylactic responses (approximately 80 and 85% respectively: Table 1). Mepyramine showed a very small inhibitory action, but methysergide reduced systemic shock by some 50 per cent. Mepyramine and methysergide each showed specific antagonism for the appropriate agonist: i.e. mepyramine antagonized histamine (dose-ratio 15) without inhibiting 5-HT or bradykinin and methysergide strongly antagonized 5-HT (dose-ratio 10) without significantly affecting the actions of histamine or bradykinin. On the other hand, sodium meclofenamate which almost abolished the systemic anaphylactic shock, showed no measurable inhibition of histamine, 5-HT or bradykinin.

Diethylcarbamazine resulted in 50% inhibition of anaphylaxis, whereas no inhibition was evident when disodium cromoglycate was given alone. However the most effective anti-anaphylactic action of all (85%) was achieved when diethylcarbamazine and disodium cromoglycate were administered simultaneously by intravenous infusion immediately before challenge (Fig. 2a). In contrast, when diethylcarbamazine was given 10–15 min before disodium cromoglycate infusion, which was given immediately prior to antigen, no effective suppression of anaphylaxis was observed.

Mepyramine alone produced hypotension when given intravenously. Blood pressure had returned to normal within five to ten min of injection of the inhibitor: i.e. had recovered prior to antigen challenge. Methysergide decreased the systemic and increased the pulmonary arterial and the venous pressures. Diethylcarbamazine reduced the systemic arterial pressure and raised the pulmonary artery pressure.

Effects of bilateral vagotomy

Bilateral vagotomy produced a slight reduction in systemic blood pressure and a transient small increase in pulmonary arterial tension. There was a very brief apnoea. Minimal effective doses of histamine, 5-HT and bradykinin on circulatory parameters were not changed significantly by vagotomy. In contrast, the reduced ventilation volume produced by all three agonists was abolished by vagotomy. In the same way, circulatory responses due to antigen were unaltered, whereas antigen-induced apnoea or dyspnoea did not occur in animals in which both vagi had been sectioned (Fig. 2b).

Blood histamine and 5-hydroxytryptamine

Figure 3 illustrates that during circulatory shock induced by antigen, a dramatic ten-fold increase in plasma histamine concentrations was observed during the first 30 s to one min after horse serum injection. Histamine concentrations were reduced during the next five to six min, then showed a brief small secondary increase before falling to control levels at approximately 20–25 minutes. Infusion of horse serum into unsensitized controls caused no measurable rise in circulating plasma histamine concentrations. Control values for bovine plasma histamine were of the order of 1·0 ng/ml or smaller, which was approximately the lower limit of sensitivity of the method.

Whole blood histamine concentrations were reduced sharply during the first two min of anaphylaxis and remained depressed during the 20 min recording period (Fig. 3). Whole blood histamine values were highly variable. The maximum decrease during anaphylaxis was approximately 20 per cent.

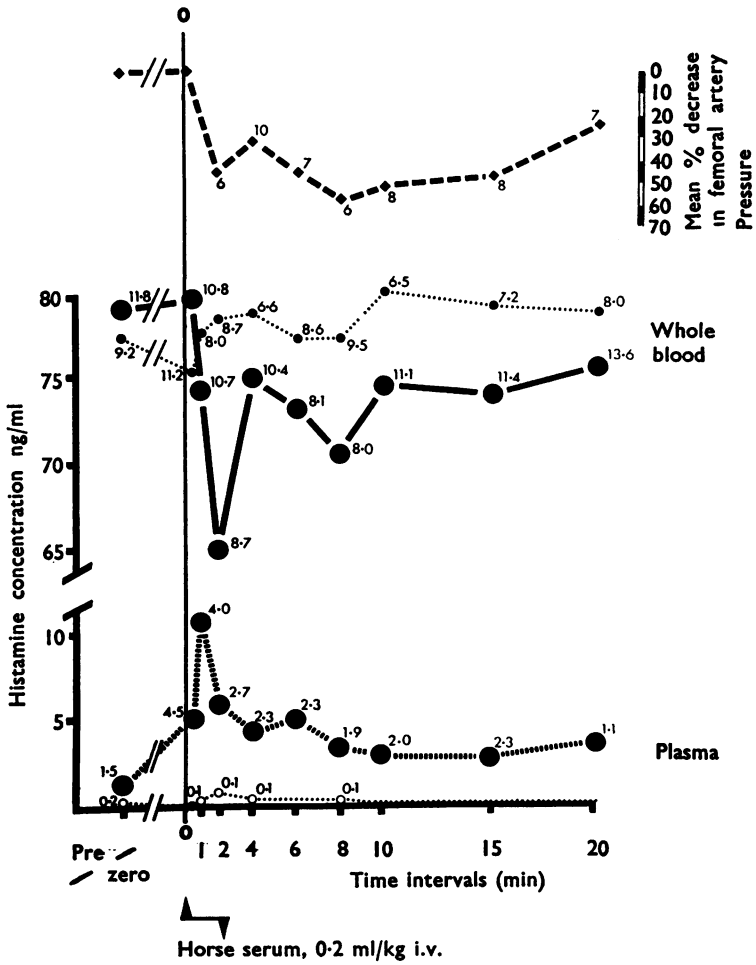

FIG. 3. Femoral artery blood pressures, and whole blood and plasma histamine concentrations during acute systemic anaphylaxis induced by horse serum in calves sensitized with horse serum and Freund's complete adjuvant. Five graphs from above downwards: (i) mean percentage decrease in femoral artery pressure in six calves during anaphylactic shock ◆---◆; (ii) whole blood histamine concentrations in four *unsensitized* controls ●····●; (iii) whole blood histamine concentrations in six *sensitized* calves during anaphylactic shock ●——●; (iv) plasma histamine concentrations in four *sensitized* calves during anaphylaxis ●----●; (v) plasma histamine concentrations in four *unsensitized* controls ○·····○. All calves were anaesthetized with pentobarbitone sodium and received horse serum (0·2 ml/kg, i.v.) over a 2 min period (shown by the arrows). Pre-zero readings are measurements after anaesthesia and cannulation, 5–10 min prior to antigen injection. Histamine concentrations were measured spectrofluorimetrically and are expressed as ng/ml. Time intervals are minutes. Numbers on the graphs represent standard errors of the means.

Plasma concentrations of 5-HT were also highly variable. Although mean plasma 5-HT values tended to decrease slightly during anaphylaxis, the changes were not significant.

Tissue histamine concentrations

Table 2 shows tissue histamine concentrations in four sensitized calves after challenge with horse serum i.v., compared with three similarly-treated (control) unsensitized animals. There was no significant difference in histamine concentrations in any of six tissues examined in the two groups of calves ($P>0·05$).

TABLE 2. *Total histamine concentrations of tissues from control unsensitized calves and sensitized calves; both groups were challenged with 2 ml/kg horse serum i.v.*

Tissue	Histamine concentration μg/g after 'challenge' with horse serum Control unsensitized*	Sensitized†
Lung pleura	13·4±0·9	12·5±1·2
Lung parenchyma	14·9±0·8	15·4±0·9
Subcutaneous tissue	7·0±0·8	8·2±1·0
Liver capsule	5·8±0·4	7·1±0·9
Liver parenchyma	5·4±0·7	6·3±0·5
Small intestine	12·9±0·9	10·9±0·8

Each value is the mean from 5 animals ± S.E. (*) or 6 animals ± S.E. (†).

Mast cells

Mast cells in pleura, liver capsule and omentum of calves that had undergone anaphylaxis were similar in numbers to the control animals. However, following anaphylaxis, the mast cells were less densely-staining and the granules showed more pink (metachromatic) reaction. There was some dispersal of mast cell granules in tissue spreads taken from calves after anaphylactic shock.

Isolated perfused lung

Histamine (minimum effective dose 10–20 μg), 5-hydroxytryptamine (minimum effective dose 15–25 μg) and bradykinin (minimum effective dose 5–10 μg) caused increase in pulmonary artery perfusion pressure and increased ventilation resistance, in all four Krebs-perfused lungs. Horse serum (5 ml infused over 30 s) produced no observable changes in the two unsensitized lungs, whereas in the lungs taken from two immunized calves, horse serum caused a slowly-rising prolonged increase in pulmonary artery perfusion pressure and a protracted increase in ventilation resistance.

A second 'shock' could be evoked when the same dose of horse serum was repeated after 20–25 min when recorded parameters had returned to normal. The second anaphylactic response was qualitatively similar but much smaller than the first.

Measurement of the concentration of histamine and 5-HT in the perfusates obtained during horse serum infusion showed no significant differences between sensitized and control lungs.

Discussion

Our findings in respect of cardiorespiratory changes of anaphylaxis are in general similar to those of Aitken & Sanford (1969a) and we have confirmed their detection of an initial bradycardia, but in addition report a subsequent tachycardia which these authors did not describe in the single animal in which heart rate was measured.

It is interesting to note that we were able to produce typical though smaller changes in the cardio-respiratory parameters on a second, but not a third, challenge with specific antigen in the intact animal and in isolated perfused lung. This observation contradicts previous reported findings (Aitken & Sanford, 1969a). However, it should be emphasized that in the work of Aitken & Sanford, a different sensitization procedure was employed (i.e. egg albumen without adjuvants) and their

second challenge was performed much earlier after the first challenge (i.e. approximately four to five min compared with 30–60 min in our experiments).

It has already been suggested that histamine and 5-HT may have low significance in anaphylaxis of ruminants such as cattle and sheep because antihistaminics and anti-5-HT agents do not effectively suppress the cardio-respiratory features of anaphylactic shock (Aitken & Sanford, 1969a ; Alexander, Eyre, Head & Sanford, 1970). On the other hand kinins and SRS-A may be more important in view of the strong inhibition of the clinical signs of bovine anaphylaxis by sodium meclofenamate. In the previous bovine study (Aitken & Sanford, 1969a), no estimate was made of the specificity of each inhibitor used. In our present investigations (Table 1) the potency and specificity of the inhibitors used was estimated prior to induction of anaphylaxis in the same animals. The data are based on duplicate measurements at one dose level only for each antagonist, and while caution is necessary in drawing definite conclusions, our findings have some general agreement with the clinical observations of Aitken & Sanford. Important differences are evident, however. Our results have shown that specific 5-HT antagonism had an inhibitory action in anaphylaxis which was greater than that shown by a comparable level of specific histamine blockade. This suggests that although neither amine would appear to be a primary mediator of bovine anaphylaxis, 5-HT probably makes some contribution to the overall effect.

A further important observation was that although meclofenamate effectively abolished anaphylaxis in the calf, this inhibitor failed to antagonize bradykinin, histamine or 5-HT. Thus, although we have reported that considerable quantities of kinins are generated during acute bovine anaphylaxis (Eyre & Lewis, 1972), these peptides may not be as significant as we and others have implied. It would appear that the strong anti-anaphylactic properties of meclofenamate do not necessarily indicate a major role for kinins in anaphylaxis.

Meclofenamate may inhibit bovine anaphylaxis to some extent by antagonizing SRS-A, a property it has in common with several other non-steroidal anti-inflammatory drugs (Berry & Collier, 1964 ; Collier & James, 1967). SRS-A participation is further supported by the fact that diethylcarbamazine (a specific SRS-A inhibitor: Orange *et al.*, 1968) strongly reduces anaphylaxis in calves. Prostaglandin release seems to be an important additional factor in anaphylaxis in other species. Anti-inflammatory acids are known to inhibit production of prostaglandins and rabbit aorta-contracting substance (Piper & Vane, 1969 ; Collier, 1971), and this property may be part of the mode of action of meclofenamate in bovine anaphylaxis. Thus meclofenamate may be inhibiting the release as well as the effects of putative mediators (transmitters).

Recently we have reported similar effects of meclofenamate, diethylcarbamazine and disodium cromoglycate in the Schultz-Dale reaction in calf pulmonary vessels (Eyre, 1971a) and of diethylcarbamazine and disodium cromoglycate in passive cutaneous anaphylaxis (Wells & Eyre, 1972). In the latter study meclofenamate did not suppress cutaneous anaphylaxis and showed no antagonism to bradykinin. Mepyramine, however, strongly inhibited both passive cutaneous anaphylaxis and the effects of histamine. Thus it seems that histamine may be a more significant mediator of cutaneous anaphylaxis than of systemic (cardio-pulmonary) anaphylaxis. Such a difference between the two experimental models may reflect a difference at the antigen-antibody level, since passive cutaneous anaphylaxis tested at 48–72

hours probably depends only on cell-fixed (reaginic) antibodies, whereas the systemic anaphylactic reaction may result from antigen combination both with cell-fixed and circulating antibodies. It is known in some other species (e.g. rat), that SRS-A release is mediated through a heat stable IgG antibody, whereas amine release depends more on the heat labile skin (mast cell) sensitizing reaginic antibody (Stechsculte, Austen & Bloch, 1967). Furthermore, pretreatment of rats with disodium cromoglycate inhibits amine release but not that of SRS-A. This study and previous reports (Eyre, 1971c ; Wells & Eyre, 1972) have shown clearly that disodium cromoglycate does not protect against anaphylaxis in calves, yet the disodium cromoglycate-resistant reaction is effectively blocked when diethylcarbamazine is also injected. This apparent synergism between disodium cromoglycate and diethylcarbamazine does not seem to have been reported previously.

In addition to active release of chemical mediators which act directly on receptors in target tissues such as smooth muscle, nervous reflex involvement in anaphylaxis has been implicated in the guinea-pig and rabbit (Karczewski, 1962 ; Karczewski & Widdicombe, 1969 ; Szereda-Przestaszewska, 1971a, b). From the present study it seems that anaphylactic shock in the calf is modified by vagotomy. The period of apnoea which had been observed in shocked calves with both vagi intact was abolished in vagotomized calves although the haemodynamic changes of anaphylaxis were not noticeably affected. This is in accordance with the findings that vagotomy lessens anaphylactic respiratory effects in the guinea-pig (Auer & Lewis, 1910) and rabbit (Karczewski & Widdicombe, 1969) and that the chemical mediators of anaphylaxis increase nervous discharge in both efferent and afferent vagal fibres in these species (Karczewski, 1962 ; Karczewski & Widdicombe, 1969).

Although considerable increases in plasma histamine concentrations were recorded, whole blood histamine levels were reduced in anaphylaxis ; a finding first described by Code & Hester (1939). It is likely that most of the histamine present will be, as in other species, in the formed elements ; principally the leucocytes (Greaves & Mongar, 1968). It has been observed that calves undergoing anaphylactic shock exhibit a leucopoenia (Dungworth, 1965 ; Wray & Thomlinson, 1969). We have confirmed that leucopoenia and thrombocytopoenia occurred during anaphylaxis in the calf and that the most significant change was in the numbers of polymorphonuclear neutrophils which in some individual calves were reduced to nil in peripheral venous blood during anaphylactic shock. Such a drastic leucopoenia probably explains the reduction in whole blood circulating histamine during bovine anaphylaxis. (Haematological changes in bovine anaphylaxis will be published in full elsewhere.)

The failure to observe significant changes in tissue histamine concentrations or marked histological changes in mast cells could quite readily be due to variation in tissue samples examined. Owing to the great size of the organs of cattle, sampling cannot be entirely consistent and amine concentration is not uniform through the organ (unpublished observations). Thus, actual alterations in histamine concentration or mast cell morphology could be masked entirely by sample variations. Furthermore it is possible that optimal sampling sites were missed or indeed that whole organs or tissues in which changes had occurred, had not been sampled at all. A compensatory increase in histamine-forming capacity of tissues has been described in guinea-pig anaphylaxis (Kahlson & Rosengren, 1968) and this may be another possible reason for the failure to observe large changes in calf tissue

histamine concentration. Aviado & Sadavongvivad (1970) reported that concentrations of both histamine and 5-HT were increased in the lungs of rabbits after anaphylaxis. We have not observed any such increase in calves.

We are unable to offer any proper explanation of the failure to observe liberation of histamine and 5-HT from isolated perfused lungs, particularly in view of our previous published findings that both substances are released from sensitized bovine chopped lung (Eyre, 1971a, 1972). It is however, possible that released amine had been taken up in the lung tissue or had been destroyed by increased enzymic activity. Changes in perfusion pressure and ventilation resistance in the isolated lung preparation could be explained on the basis of release of other mediators—particularly SRS-A.

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