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Cutaneous vascular permeability factors (histamine, 5-hydroxytryptamine, bradykinin) and passive cutaneous anaphylaxis in sheep

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The cutaneous blood vessels of sheep are more sensitive to histamine than those of laboratory rodents. The threshold dose in sheep was 0·0025 μg histamine. The ovine vessels are 70 to 100 times less sensitive to 5-hydroxytryptamine (5-HT) than to histamine, but only four times less sensitive to bradykinin than to histamine. The effects of compound 48/80 are antagonized both by the antihistamine agent mepyramine and by the anti-5-HT agent methysergide, which suggests that compound 48/80 may release 5-HT in addition to histamine in sheep. The capillary-damaging effects of passive cutaneous anaphylaxis in sheep are antagonized by methysergide and by sodium meclofenamate—an agent which antagonizes kinins and slow-reacting substance. The antihistamine agent mepyramine has a small anti-anaphylactic effect, whereas promethazine—a less specific antihistamine—offers more protection to the blood vessels against local anaphylaxis. It is concluded that in the complex interaction of chemical mediators of anaphylaxis in the cutaneous blood vessels of sheep, 5-HT and kinin (and/or SRS-A) may be more important than histamine.

The role of endogenous chemical mediators of anaphylactic reactions in "laboratory" animals and in man has been investigated and documented for many years. Histamine seems to be an important factor in anaphylaxis in the dog, guinea-pig and man (Code, 1937; Halpern, 1958; Humphrey & Mota, 1959). On the other hand in certain other species, histamine is thought to play a smaller part, and 5-hydroxytryptamine (5-HT) may be more important. For example, histamine antagonists have a small effect on anaphylaxis in the rat (Halpern, Liacopoulos & Perez Del Castillo 1955) and rabbit (Reuse, 1949). In mice, anaphylaxis may result in simultaneous release of histamine and 5-HT and each substance may have an approximately equal significance (Halpern, Neveu & Spector, 1963). There is little available data relating to anaphylactic reactions in the large domesticated ungulates. However, a number of disease-processes in these animals may have an anaphylactic basis; notably laminitis which occurs in the feet of all ungulates (Nilsson, 1963; McLean, 1965) "fog fever" or atypical pneumonia of cattle (Moore, 1952; Sweet 1949) bowel-oedema disease of swine (Thomlinson & Buxton, 1963) and pulmonary emphysema of horses (Andberg, 1941). Code & Hester (1939) were unable to detect histamine in the blood of horses, calves, sheep or goats during anaphylactic shock, and Alexander, Eyre & others (1969) reported that antihistamine drugs had no inhibitory effect on experimentally induced systemic anaphylaxis in sheep.

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The aim of these investigations was to study the nature of the mediators of increased capillary permeability during passive cutaneous anaphylaxis in the ungulate species using various pharmacological antagonists. This paper presents results in sheep.

EXPERIMENTAL

Materials and methods

Sheep. Either females or castrated males of the Scottish blackface and South Country Cheviot breeds were used.

Antigen. Whole dried hen egg albumin was used for sensitization, and for "challenge".

Antisera were prepared in a group of four Scottish blackface ewes using a modification of a method evolved in rabbits, described by Brocklehurst (1960). 250 mg whole ovalbumin dissolved in 5 ml isotonic saline, mixed with an equal volume of (Difco) Complete Freund's adjuvant, was injected, half subcutaneously and half intramuscularly. This was repeated after 7 days. After 6 weeks, six graded doses, 5, 10, 20, 20, 50 and 100 mg respectively, of aluminium hydroxide-absorbed ovalbumin (Colquhoun, 1964) were injected intravenously at 2-day intervals. Seven days after the last injection, 200 ml of blood was collected from each animal by venepuncture and the serum was separated, centrifuged and stored at −20°, until used.

By the P.C.A. test (see below) the most active of the four sera was determined and this was used as a standard antiserum for all tests. The antibody content was not determined.

Passive cutaneous anaphylaxis (P.C.A.). The method employed was modified from that described by Ovary (1958).

(i) *Preparation of the sheep.* At least 2 days before the beginning of experiments, the flanks and abdomens of the sheep were clipped and the areas depilated using a preparation of barium sulphide 5, detergent washing powder 1, chalk 7, and corn starch 7 parts by weight. The constituents were mixed with water to form a thick cream, applied immediately to the clipped skin and left in contact for 3–4 min. The skin was scraped free of wool with a blunt spatula, washed with warm water and toilet soap and finally dusted with talc.

(ii) *Drug injections.* A range of concentrations of each of histamine, 5-HT, bradykinin, 40/80 and serial dilutions of anti-ovalbumin sheep serum contained in 0·2 ml isotonic saline were injected intradermally, for each experiment. Four h after the intradermal injection of serum, 15 min after injection of 48/80 and immediately after the injection of histamine, 5-HT or bradykinin, sheep received 20 ml 2% Coomassie Blue and 5 ml 10% ovalbumin in isotonic saline intravenously. A period of 30 min was allowed after intravenous challenge for the full development of blue lesions at the site of intradermal injection. The diameters of the blue spots were measured with calipers and the minimum concentration of a drug or minimum dilution of serum which gave a blue spot of 1·5 cm diameter was arbitrarily taken to be the threshold dose.

The sheep were subjected to eight different regimes incorporating four antagonist drugs: mepyramine, promethazine, methysergide and sodium meclofenamate injected intravenously 15 min before "challenge". In control experiments sheep received 5 ml of isotonic saline intravenously (see Table 1).

Table 1. *The scheme of treatment of eight sheep with four antagonists of anaphylaxis: mepyramine, promethazine, methysergide and sodium meclofenamate.* Serial dilutions of histamine, 5-HT, bradykinin, compound 48/80 and serum from an ovalbumin-sensitized sheep were injected intradermally on *each* of the thirty-two occasions (see Table 2). An interval of 4 days separates "treatments" in each animal.

	Treatment with antagonists (mg/kg)			
	1	2	3	4
Sheep 1	Nil (control)	Mepyramine 5	Promethazine 5	Methysergide 1
2	Mepyramine 10	Promethazine 10	Methysergide 2	Meclofenamate Na. 1
3	Mepyramine 5	Promethazine 5	Methysergide 1	Mepyramine 10
4	Promethazine 10	Methysergide 2	Meclofenamate Na. 1	Nil (control)
5	Promethazine 5	Methysergide 1	Mepyramine 10	Promethazine 10
6	Methysergide 2	Meclofenamate Na. 1	Nil (control)	Mepyramine 5
7	Methysergide 1	Mepyramine 10	Promethazine 10	Methysergide 2
8	Melcofenamate Na. 1	Nil (control)	Mepyramine 5	Promethazine 5

The inhibitory effect of antagonists is given as the multiple of the threshold dose of agonist which is required to re-establish the 15 mm blue lesion in the presence of the antagonist (Halpern & others, 1963). This is the same concept as dose-ratio (Gaddum, Hameed & others, 1955). The mean of four measurements was calculated for each treatment.

RESULTS

Histamine

The minimum threshold dose for histamine base producing a spot diameter of 15 mm, was 0·0025 $\pm$ 0·004 μg. Mepyramine was the most powerful antagonist. 5 mg/kg reduced the activity of histamine some 3000 times and 10 mg/kg reduced histamine-activity 5000 times. Promethazine at the same dose levels was about half as active as mepyramine. Methysergide and meclofenamate each had a small but significant antihistamine action. The effect of antagonists on the thresholds of the agonists is given in Table 2.

Table 2. *The influence of the antagonists of anaphylaxis (mepyramine, promethazine, methysergide and sodium meclofenamate) on the threshold doses of histamine, 5-HT, bradykinin, compound 48/80 and antibody necessary to produce cutaneous permeability changes in the conscious sheep.* Control threshold dose of each agonist = 1 (unity) in the absence of all antagonists. Each multiple is the mean of four separate measurements in different animals (see Table 1).

Antagonist	Dose mg/kg	Multiple of threshold dose				
		Histamine	5-HT	Bradykinin	Cpd. 48/80	P.C.A.
Mepyramine	5·0	3280	1·6	—	940	14
	10·0	5020	2·9	6·0	1710	20
Promethazine	5·0	1280	8·6	—	90	156
	10·0	3520	16	—	155	382
Methysergide	1·0	9·2	255	—	50	205
	2·0	20	600	10	120	529
Sodium meclofenamate ..	1·0	40	20	100	—	505

5-Hydroxytryptamine

The threshold dose of 5-HT was 0·18 ± 0·04 μg. The data in Table 2 show methysergide to be clearly the most efficient antagonist. 1 mg/kg diminished the 5-HT response by 250 times; 2 mg/kg inhibited 600 times. The high dose of mepyramine (10 mg/kg) inhibited slightly the 5-HT responses whereas smaller doses of these drugs (5 mg/kg) had no effect. Promethazine inhibited 5-HT more strongly than mepyramine. Meclofenamate (1 mg/kg) reduced the 5-HT response by a factor of 20.

Bradykinin

The minimum dose of bradykinin to produce a 15 mm blue lesion was 0·01 ± 0·005 μg of the synthetic compound (Sandoz). Sodium meclofenamate raised the threshold a hundredfold; methysergide caused a tenfold increase and mepyramine a sixfold increase in the bradykinin threshold.

Compound 48/80

The threshold dose of compound 48/80 was 0·05 ± 0·03 μg. The antihistamine drugs were powerful antagonists of the 48/80 response. Mepyramine (5 mg/kg) reduced the activity of 48/80 by a factor of 940 and promethazine (5 mg/kg) 90 times. Doubling the dose of antihistamine approximately doubled the inhibition of 48/80. Methysergide (1 to 2 mg/kg) increased the threshold to 48/80 by 50 to 120 times.

Passive cutaneous anaphylaxis (P.C.A.)

The activity of sera from four sensitized sheep varied in ability to produce P.C.A. Threshold dilutions of the sera were as follows: Sheep 1 = 10^{-2}; Sheep 2 = 10^{-2}; Sheep 3 = 6×10^{-4}; Sheep 4 = 3×10^{-3}. The "best" titre was in Sheep No. 3 and serum from this animal was used for all subsequent tests. Intradermal injections of this serum into four unprotected control sheep gave a consistent threshold around 6×10^{-4} serum dilution.

The antihistamine agents were the poorest antagonists of P.C.A. Mepyramine at 5 mg/kg and 10 mg/kg increased the threshold dose of antibody only slightly—namely 14 times and 20 times respectively. Promethazine at 5 mg/kg and 10 mg/kg was more potent, producing increases in threshold dilution from 156 to 382 times respectively. Methysergide was more potent than the antihistamine drugs. A dose of 2 mg/kg of methysergide increased the threshold of serum dilution some 500 times. Sodium meclofenamate similarly reduced the anaphylactic reaction of the skin vessels by a factor of 500.

DISCUSSION

A total of eight sheep were used and a scheme of treatments devised to allow four experiments per animal, each procedure separated by a 4-day interval. This method introduces potential problems associated with persistence of drugs namely the dye substance, the antigen and the antagonist.

Coomassie Brilliant Blue (George T. Gurr and Co. Ltd., London, N.W.9) was used throughout because it has been shown to be non-persistant (Feinberg & Dewdney, 1963). Coomassie Blue did not persist in the skin or subcutis of sheep for longer than 48 h.

There did not seem to be any disadvantage due to the possible persistence of antigen in circulation. Although some antibodies would undoubtedly be produced

during the 12-day period of exposure to antigen, there appeared to be no marked interference with the formation of distinct P.C.A. reactions. Three animals developed mild transient dyspnoea following protein injection on the 12th day.

Sheep do not readily show anaphylactic reactions within a 2-week period of "simple" protein injections as described here. This species seems to require a more extended and sophisticated regime for protein sensitization, including the use of adjuvants (e.g. Freund's) or "boosting" with alum-precipitated protein, or both, before being capable of showing a marked systemic anaphylaxis (Alexander & others, 1969). This is in contrast with cattle which readily become sensitized and show a severe reaction within 7 days of a single sensitizing dose of protein (Aitken & Sanford 1969).

It appears from the data that the sensitivity of the peripheral blood vessels of sheep to histamine is greater than in the laboratory species. The threshold dose in sheep was 0·0025 μg whereas in guinea-pigs it is 0·3 μg, in the rat 0·9 μg and in the mouse 0·15 μg (Halpern & others, 1963).

The sensitivity of sheep capillaries to 5-HT is about 70 times less than to histamine whereas the sensitivity to bradykinin only four times smaller.

The effectiveness of the antagonist drugs in sheep appears to be qualitatively similar to that in other species. Mepyramine and promethazine antagonized histamine strongly whereas methysergide and meclofenamate had much less antihistamine activity. Good correlation existed between the other active substances and their antagonists. 5-HT was inhibited by methysergide whereas the antihistamine drugs and meclofenamate had but a small effect on 5-HT.

Compound 48/80 was strongly inhibited by mepyramine and weakly by promethazine and methysergide. It is probable that compound 48/80 acts principally by releasing histamine but the inhibition by methysergide suggests that compound 48/80 may liberate some 5-HT in sheep, as has been shown in rats (Bhattacharya & Lewis, 1956). It would be of interest to clarify this point by direct determination.

Passive cutaneous anaphylaxis is well inhibited by meclofenamate and methysergide but relatively poorly by the antihistamine drugs. Thus it is unlikely that peripheral vascular permeability changes as a result of local anaphylaxis are due solely to the liberation of histamine in sheep. If histamine were the principle mediator of the anaphylactic response, one would expect powerful inhibition of mepyramine since mepyramine was shown simultaneously to inhibit strongly the actions of histamine itself on skin vessels in sheep.

On the other hand methysergide, 1 to 2 mg/kg, strongly inhibits 5-HT (threshold increased from 250–600 times) and the P.C.A. reaction is similarly reduced 530-fold. This contrasts with the weak inhibition of histamine and bradykinin by methysergide (9 to 20-fold reduction). This specificity of methysergide for 5-HT suggests that the amine is involved in cutaneous anaphylaxis in the sheep. It is further interesting that promethazine which exhibits some anti-5-HT activity, is intermediate between mepyramine and methysergide in inhibiting the P.C.A. reaction.

Sodium meclofenamate was the least specific antagonist showing some inhibition of all the active agents, although the inhibition by meclofenamate of bradykinin was at least double that of either histamine or 5-HT. Meclofenamate has been shown to be a powerful antagonist of bradykinin, SRS and antigen induced bronchoconstriction in the guinea-pig (Berry & Collier, 1964; Collier & James, 1967; Collier, James & Piper, 1968), but this antagonist is less effective in inhibiting the action of kinins on blood vessels than on bronchial muscle.

Meclofenamate and methysergide each inhibited the P.C.A. reaction to approximately the same extent. Alexander & others (1969) investigated the protection produced against experimental general anaphylaxis in sheep by mepyramine methysergide and meclofenamate. These authors described meclofenamate as the best antagonist whereas mepyramine and methysergide afforded little protection on the cardiovascular and respiratory systems. Meclofenamate antagonized the effects of exogenously administered bradykinin in sheep.

Evidence for the participation of various mediators has thus been obtained indirectly; there being no direct estimation of active agents. Nevertheless the establishment simultaneously of the specificity of each antagonist makes it possible to postulate the relative importance of each potential mediator and allows the conclusion that cutaneous anaphylaxis in sheep is mediated by the interaction of histamine, 5-HT and kinin; with the possible addition of SRS-A and other substances. Kinin and 5-HT appear to be more important than histamine in these circumstances, but it may not be valid to extend these conclusions to other sites or to generalized systemic anaphylaxis, where the relative importance of the mediators may well differ.

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