Short communications

Production of kinins in bovine anaphylactic shock

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Calves were sensitized by subcutaneous injection of horse serum in Freund's adjuvant. Subsequent challenge with intravenous horse serum induced increased blood kinin activity. This kinin activation correlated with the protracted fall in systemic blood pressure seen in the same animals.

The role of endogenous chemical mediators of anaphylaxis in ruminants (cattle and sheep in particular) was unknown until recently. It has now been shown that histamine, 5-hydroxytryptamine and dopamine are released by specific antigen from bovine lung (Eyre, 1971a, b, and unpublished observations). Other work, using the pharmacological inhibitor sodium meclofenamate, has implicated kinin in anaphylaxis in these species (Aitken & Sanford, 1969; Alexander, Eyre, Head & Sanford, 1970; Eyre, 1970, 1971c). Production of plasma kinins has already been described during anaphylaxis in the dog (Beraldo, 1950), guinea-pig, rat and rabbit (Brocklehurst & Lahiri, 1962), and mouse (Lima, 1967). We have investigated the whole blood kinin activity during experimental anaphylaxis *in vivo* in calves.

Methods.—Eight male Guernsey calves (35–50 kg, aged 4–8 weeks) were sensitized to horse serum and complete Freund's adjuvant (1 : 1 emulsion). The initial sensitizing dose (0·4 ml/kg) was given subcutaneously and was followed 7 days later by a similar injection. After a further 14 days anaesthesia was induced and maintained with pentobarbitone. Ventilation, femoral artery blood pressure (1 mm Hg≡1·333 mbar), and pulmonary artery blood pressure (monitored with a cannula inserted via the jugular vein) were measured on an E & M Physiograph by means of appropriate transducers. Calves were challenged by intravenous injection of 0·2 ml horse serum/kg body weight, infused over 2 minutes. Two similar unsensitized control animals were given identical intravenous injections of horse serum. Cardiorespiratory parameters were continuously recorded and blood was sampled from a carotid artery using siliconized needles and syringes. Kinin activity was measured over 25 min after the beginning of the antigen challenge. Kinins were extracted from 3 ml whole blood in 5 ml chilled absolute ethanol (Boreham, 1968), in a siliconized test tube, by vigorous shaking. After centrifugation the alcoholic extract was evaporated to dryness under reduced pressure at 35° C. The dry residue was resuspended in 2 ml de Jalon's solution and kinin concentration estimated by assay on isolated uterus of a rat treated with 0·1 mg/kg stilboestrol. The uterus was mounted in 10 ml de Jalon's solution containing atropine 0·1 μg/ml, mepyramine 0·5 μg/ml, and methysergide 5 μg/ml, gassed with 95% oxygen : 5% CO_2 mixture at 32° C. Synthetic bradykinin (Nutritional Biochemicals Co., Cleveland, Ohio) was used as the standard. The drug contact time was 30 s and interval between treatments 4 minutes.

Results.—Normal bradykinin equivalent values obtained from ten calves (±S.D.) were 1·2±0·3 ng/ml (range 0·4–2·1 ng/ml). A 3- to 8-fold increase in kinin activity was observed beginning 1–15 min after the initiation of antigen infusion in the eight sensitized calves, whereas in the two controls, bradykinin values did not alter significantly. Figure 1 shows the percentage increase in kinin activity obtained from eight sensitized and two unsensitized calves, together with a graph illustrating the mean percentage change in arterial blood pressure during anaphylaxis in eight sensitized calves.

The systemic blood pressure was lowered within 60 s of the beginning of infusion of the horse serum into sensitized calves and followed in some animals by a brief hypertension and a more prolonged hypotension in all cases before returning to normal. A biphasic rise in pulmonary artery pressure which slowly returned to normal and a brief period of apnoea were also major features of the response. Infusion of horse serum into two unsensitized controls caused a very slight fall in systemic blood pressure.

The increased kinin activity correlated with the lowering in systemic pressure

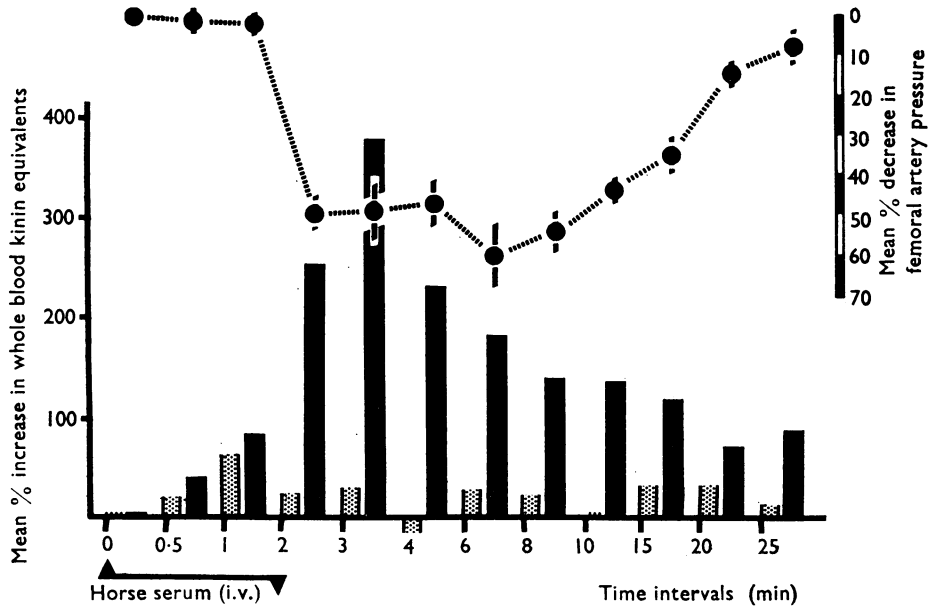

FIG. 1. Effects of intravenous infusion of horse serum (0·2 ml/kg between the arrows) for 2 min on blood pressure and blood kinin activity in eight calves sensitized to horse serum in Freund's complete adjuvant and in two unsensitized controls. All animals anaesthetized with pentobarbitone sodium. Upper graph ●- - - -● represents mean percentage decrease in femoral artery pressure (±S.D.) in eight sensitized calves after intravenously injected horse serum. Histograms represent mean percentage increases in whole blood kinin equivalents in the same eight sensitized calves (filled columns) and two unsensitized controls (dotted columns). Kinin activity was bio-assayed on oestrus rat uterus. Time intervals expressed in minutes.

observed during anaphylactic challenge. Bradykinin *per se* produced a transient hypotension with doses as low as 50 ng/kg when administered intravenously to anaesthetized calves. In addition, bradykinin caused a marked rise in pulmonary arterial pressure (2–37% increase with a dose of 0·5 μg/kg in 10 calves tested), and a reduced ventilation.

Discussion.—The results of these experiments indicate that considerable amounts of kinin are produced during active *in vivo* anaphylaxis in calves. The time of appearance of kinins in the peripheral blood correlates well with changes in cardiorespiratory parameters during anaphylaxis as measured in the same animals.

Sodium meclofenamate, a known kinin inhibitor (Collier, James & Piper, 1968), markedly antagonizes anaphylaxis both *in vitro* and *in vivo* in cattle (Aitken & Sanford, 1969; Alexander *et al.*, 1970; Eyre, 1970, 1971c; Wells & Eyre, unpublished). Furthermore, Aitken & Sanford (1969) also showed that antihistaminics and 5-hydroxytryptamine antagonists failed to protect cattle from the 'clinical' signs of anaphylactic shock. We have recently observed (unpublished results) that neither mepyramine nor methysergide had any significant inhibitory action on the lowering of systemic blood pressure, the rise in pulmonary artery pressure or the apnoea/dyspnoea associated with acute anaphylaxis in the calf.

We have suggested (Eyre, 1970, 1971a, b) that histamine and 5-hydroxytryptamine may play a smaller role in anaphylaxis of cattle than in some other species. The present results show that bradykinin is produced during bovine anaphylaxis and may contribute significantly to the anaphylactic syndrome in this species.

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