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Immunological release of histamine from bovine leucocytes

UNUSUAL ADRENERGIC MODULATION

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Summary. Sensitized bovine granulocytes release histamine when exposed to specific antigen. In comparison with *in vitro* systems in several other species, the modulation of this release by adrenergic agents is unique. β-Adrenoceptor stimulation potentiates (rather than inhibits), whereas α-adrenoceptor stimulation inhibits (rather than potentiates) histamine release. Adrenaline, which is generally considered to be a physiological antagonist of the anaphylactic reaction, potentiated histamine release in this study. Dopamine, which is present in high concentration in bovine mast cells, was without effect.

The results are discussed in terms of the possible role of granulocytes in bovine hypersensitivity.

INTRODUCTION

Histamine is released immunologically from mast cells and leucocytes of several species, following the interaction of specific antigen with antibody bound to the surface of the target cell. Modulation of this release by drugs has been studied principally in two systems: chopped lung of man, guinea-pig and monkey; and isolated human leucocytes (Assem and Schild, 1971; Ishizaka, Ishizaka, Orange and Austen, 1971; Bourne, Lichtenstein, Melmon, Henney, Weinstein and Shearer, 1974). In both systems, antigen-induced histamine release is inhibited by stimulation of β-adrenoceptors, apparently due to an increase in intracellular concentrations of cyclic AMP. This inhibitory response is greatly reduced by the β-adrenoceptor antagonist propranolol, but is unaffected by the α-adrenoceptor antagonist phentolamine (Lichtenstein and DeBarnardo, 1971; Assem and Schild, 1973). In chopped lung preparations, stimulation of α-adrenoceptors decreases the intracellular concentration of cyclic AMP and enhances antigen-induced histamine release (Kaliner, Orange and Austen, 1972).

In contrast, histamine release from human leucocytes does not appear to be modulated by α-adrenoceptor stimulation. Hence the α-adrenoceptor agonist phenylephrine produces little or no effect, and the α-receptor antagonist phentolamine does not alter the response to several catecholamines (Bourne, Lichtenstein and Melmon, 1972; Bourne *et al.*, 1974). Nevertheless, α-adrenoceptors have been demonstrated in a mixed population of human leucocytes, although the specific cell type carrying the receptor was not identified (Sokol and Beall, 1975).

The present report provides evidence of a system which does not fit into the presently accepted pattern of adrenergic modulation, namely the release

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of histamine from bovine granulocytes. This study was carried out as part of an attempt to clarify the role of granulocytes in bovine anaphylaxis, following the observation that large numbers of these cells occur in bovine pulmonary vessels at the height of the anaphylactic response (Wells, Eyre and Lumsden, 1973).

MATERIALS AND METHODS

Materials

L-Epinephrine bitartate, dopamine hydrochloride (Nutritional Biochemical Corporation, Cleveland, Ohio); D,L-isoproterenol hydrochloride (Sigma Chemical Company, St Louis, Missouri); propranolol hydrochloride (Ayerst, Montreal); phenylephrine hydrochloride; phentolamine base (Ciba, Dorval).

Reagents pertinent to the extraction and assay of histamine were of spectroscopic grade.

Preparation and incubation of leucocytes

The procedure for preparation of bovine granulocytes, incubation with antigen and assay of the released histamine has been described (Holroyde and Eyre, 1975b). Briefly, male Jersey and Guernsey calves 4–6 weeks old were sensitized to horse plasma (Holroyde and Eyre, 1975a). Blood was collected by jugular venepuncture, anticoagulated with EDTA and centrifuged to separate mononuclear and polymorphonuclear leucocytes. Following hypotonic lysis of erythrocytes, a granulocyte fraction of 94 per cent purity was obtained. This fraction was suspended in a Tris buffer containing bovine albumin, calcium and magnesium.

Aliquots of this suspension (approximately 18×10^6 leucocytes) were incubated for 30 min at 37° in the presence of sufficient horse plasma to cause the release of 40–60 per cent of the available histamine. Adrenergic agonists were added to the incubation medium in several concentrations, 2 min before antigen addition. When antagonists were studied, these were added 2 min before the agonists.

Histamine extraction and analysis

After incubation, the samples were centrifuged and the supernates assayed for histamine using a modification of the Shore fluorometric technique (Shore, Burkhalter and Cohn, 1959). Briefly, 2-ml aliquots of the supernate were shaken with 3 ml 0·4 N $HClO_4$ to precipitate protein, and centrifuged. Four-millilitre aliquots of the resultant supernate were shaken for 15 min with 10 ml butanol, 1·5 g NaCl and 0·5 ml 5 N NaOH. The aqueous phase was discarded and the organic phase shaken for 15 min with 4 ml NaCl-saturated 0·1 N NaOH. Eight-millilitre aliquots of the butanol phase were then shaken for 15 min with 4 ml 0·1 N HCl and 15 ml *n*-heptane. Two-millilitre aliquots of the aqueous phase were then assayed for histamine by adding 0·4 ml 1 N NaOH, followed by 0·1 ml 0·1 per cent orthophthalaldehyde, shaking well after each addition. After 4 min at room temperature the reaction was stopped by the addition of 0·2 ml 2 M citric acid.

Fluorescence was determined using an Aminco-Bowman Spectrophotofluorometer, at excitation/emission wavelengths of 358/446 nm respectively, and compared with a calibration curve constructed using known concentrations of histamine.

Total histamine available was determined by first boiling a 2 ml aliquot of an incubated sample with 3 ml 0·4 N $HClO_4$ for 10 min. The sample was then treated as described above. Suitable controls for reagent blanks, spontaneous histamine release, histamine recovery and drug fluorescence were included throughout.

RESULTS

Release of histamine is expressed as a percentage of the total amount available. Modulation of this release by drugs is expressed as a percentage change, compared to that released by antigen alone.

Isoprenaline (Fig. 1) caused potentiation of histamine release at low concentrations, and inhibition at high concentrations. Both these responses are statistically significant as determined by Student's *t*-test ($P < 0.05$). In the presence of 10^{-5} M propranolol (a concentration which produced no effect itself) the potentiatory phase was completely abolished, and the inhibitory phase was significantly enhanced. Phentolamine (10^{-5} M) tended to enhance the potentiatory phase (significant at 10^{-5} M isoprenaline), and to abolish the inhibitory phase. Higher concentrations of phentolamine could not be used as this drug itself substantially inhibited antigen-induced release (the results in Fig. 1 are corrected for this). Phenylephrine (Fig. 2) inhibited histamine release at the higher end of the concentration range tested (10^{-4}–10^{-3} M). Adrenaline (Fig. 2) potentiated

histamine release except at the highest concentration tested (10^{-3} M), where it tended to inhibit. Dopamine (10^{-6}–10^{-3} M) did not significantly affect histamine release.

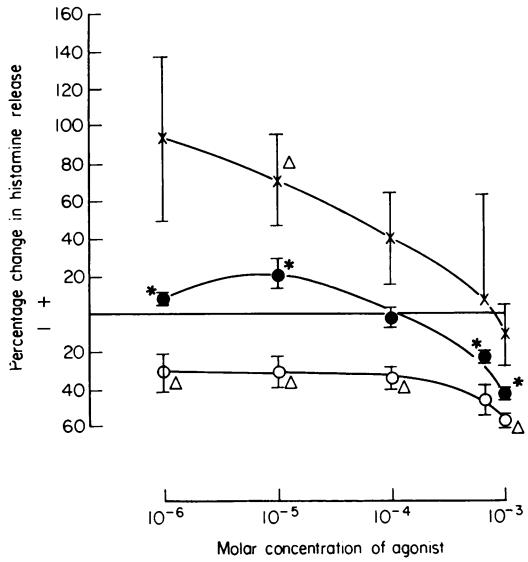

Figure 1. Modulation of antigen-induced histamine release from actively sensitized bovine granulocytes by: isoprenaline alone (●, n=6), isoprenaline+10^{-5} M propranolol (○, n=6) and isoprenaline+10^{-5} M phentolamine (×, n=4). Results are expressed as mean±s.e.m. (*) Significantly different from zero ($P<0.05$); (△) significantly different from isoprenaline alone ($P<0.05$).

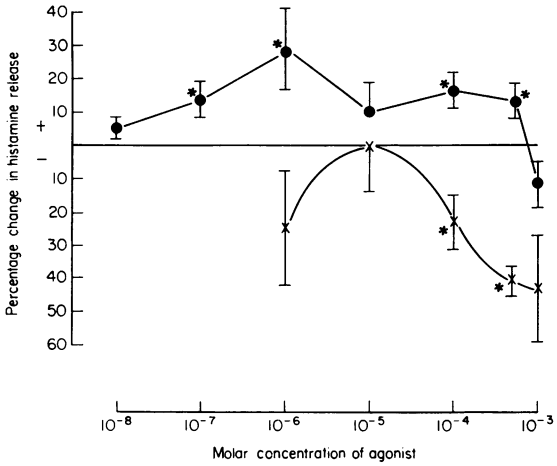

Figure 2. Modulation of antigen-induced histamine release by phenylephrine (×, n=3) and by adrenaline (●, n=5). Results are expressed as mean±s.e.m. (*) Significantly different from zero ($P<0.05$).

DISCUSSION

The present results clearly indicate a dual adrenergic modulation of histamine release from bovine granulocytes. Low concentrations of isoprenaline stimulate a β-receptor which enhances histamine release. High concentrations of isoprenaline stimulate an α-receptor which inhibits histamine release. The ability of isoprenaline to stimulate α-adrenoceptors has been reported in several tissues (Guimaraes and Osswald, 1969; Fernandez, Martinez and DeJalon, 1974; Holroyde and Eyre, 1975a), and is particularly evident in the present investigation: in the presence of propranolol, histamine release was substantially inhibited throughout the whole concentration range tested (10^{-6}–10^{-3} M). In the presence of phentolamine, the isoprenaline dose–response curve was displaced upwards, i.e. histamine release was further potentiated, and the inhibitory response to high concentrations of isoprenaline was abolished.

Phenylephrine, a preferential α-adrenoceptor stimulant, produced only inhibition of histamine release.

Dopamine is present in high concentrations in bovine mast cells and is released from sensitized bovine lung when exposed to specific antigen (Eyre, 1971). However, dopamine did not affect histamine release from bovine granulocytes, indicating its low affinity for adrenoceptors and the probable absence of a specific dopamine receptor.

It is particularly interesting that adrenaline (which inhibits the release of all mediators of anaphylaxis in all species so far studied) significantly potentiated the release of histamine in the present study. The resting plasma concentration of adrenaline in cattle is between 10^{-9} and 10^{-8} M (Callingham, 1975). The effect of systemic anaphylaxis on bovine plasma catecholamine concentrations has not been studied, but catecholamines are known to be released during anaphylaxis in the guinea-pig (Piper, Collier and Vane, 1967). In the latter species, the serum adrenaline concentration rapidly rises from approximately 10^{-8} M to 10^{-7} M, and in extreme cases approaches 6×10^{-7} M (Bernauer, Hagedorn and Filipowski, 1971). Assuming that similar concentrations could be achieved during anaphylaxis in cattle, it does not seem unreasonable to suggest that the release of histamine from circulating leucocytes could be enhanced.

The physiological significance of these results, particularly with regard to the progress of the

anaphylactic reaction *in vivo*, is at present open to speculation. However, it may be advantageous to the animal for histamine release from leucocytes to be potentiated under certain circumstances. This would be particularly true if the leucocyte-derived histamine were viewed not as a primary mediator of anaphylaxis, but as a modulator of the release of other 'more important' mediators. For instance, the release of slow-reacting substance of anaphylaxis (SRS-A) from bovine lung is inhibited by exogenous histamine (Burka and Eyre, 1975). This hypothesis is strengthened by the observation that release of histamine from bovine lung (a major source of the histamine released during bovine anaphylaxis) is inhibited by both α- and β-adrenoceptor stimulation and possibly by exogenous histamine (unpublished results). The leucocyte, being an obviously mobile cell, is in a unique position to function as a local modulator of the anaphylactic response.

Whatever the role of the leucocyte in bovine hypersensitivity, it is clear that this cell type is regulated by adrenoceptors in a manner completely different from any other comparable immunological system so far described.

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REFERENCES

Assem E.S.K. & Schild H.O. (1971) Inhibition of the anaphylactic mechanism by sympathomimetic amines. *Int. Arch. Allergy*, **40**, 576.

Assem E.S.K. & Schild H.O. (1973) β-Adrenergic receptors concerned with the anaphylactic mechanism. *Int. Arch. Allergy*, **45**, 62.

Bernauer W., Hagedorn M. & Filipowski P. (1971) Catecholamine release during anaphylactic shock in guinea pigs. *Naun.-Schmied. Arch. Pharmak.* **270**, 326.

Bourne H.R., Lichtenstein L.M. & Melmon K.L. (1972) Pharmacologic control of allergic histamine release *in vitro*: evidence for an inhibitory role of 3',5'-adenosine mono phosphate in human leukocytes. *J. Immunol.* **108**, 695.

Bourne H.R., Lichtenstein L.M., Melmon K.L., Henney C.S., Weinstein Y. & Shearer G.M. (1974) Modulation of inflammation and immunity by cyclic AMP. *Science*, **184**, 19.

Burka J.F. & Eyre P. (1975) Modulation of release of SRS-A from bovine lung by autonomic and autacoid agents. *Int. Arch. Allergy*. (In press.)

Callingham B.A. (1975) Catecholamines in blood. *Handbook of Physiology*, volume 7, p. 430. American Physiological Society, Washington.

Eyre P. (1971) Release of dopamine from bovine lung by specific antigens and by compound 48/80. *Brit. J. Pharmac.* **42**, 423.

Fernandez P.L., Martinez A.M. & DeJalon P.G. (1974) Phentolamine and propranolol on isoprenaline induced responses on rabbit aortic strips. *Experientia (Basel)*, **30**, 72.

Guimaraes S. & Osswald, W. (1969) Adrenergic receptors in the veins of the dog. *Europ. J. Pharmacol.* **5**, 133.

Holroyde M.C. & Eyre P. (1975a) Reactivity of isolated bovine mesenteric and hepatic veins to vasoactive agents and specific antigen. *Europ. J. Pharmacol.* **30**, 36.

Holroyde M.C. & Eyre P. (1975b) Immunologic release of histamine from bovine polymorphonuclear leukocytes. *Amer. J. Vet. Res.* **36**, 1801.

Ishizaka T., Ishizaka K., Orange R.P. & Austen K.F. (1971) Pharmacologic inhibition of the antigen-induced release of histamine and slow-reacting substance of anaphylaxis (SRS-A) from monkey lung tissues mediated by human IgE. *J. Immunol.* **106**, 1267.

Kaliner M., Orange R.P. & Austen K.F. (1972) Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. IV. Enhancement by cholinergic and alpha adrenergic stimulation. *J. exp. Med.* **136**, 556.

Lichtenstein L.M. & DeBarnardo R. (1971) The immediate allergic response: *in vitro* action of cyclic AMP-active and other drugs on the two stages of histamine release. *J. Immunol.* **107**, 1131.

Piper P.J., Collier H.O.J. & Vane J.R. (1967) Release of catecholamines in the guinea pig by substances involved in anaphylaxis. *Nature (Lond.)*, **213**, 838.

Shore P.A., Burkhalter A. & Cohn V.H. (1959) A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. exp. Ther.* **127**, 182.

Sokol W.N. & Beall G.N. (1975) Leukocytic epinephrine receptors of normal and asthmatic individuals. *J. Allergy clin. Immunol.*, **55**, 310.

Wells P.W., Eyre P. & Lumsden J.H. (1973) Hematological and pathological changes in acute systemic anaphylaxis in calves: effects of pharmacological agents. *Canad. J. comp. Med.* **37**, 119.