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Release of dopamine from bovine lung by specific antigen and by compound 48/80

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Summary

1. Dopamine was shown to be released from the isolated lung of calves sensitized with horse serum by compound 48/80 and by specific antigen.

2. Dopamine contracts the bovine pulmonary artery and pulmonary vein. Phentolamine, the α-adrenoceptor blocking agent, inhibits this activity of dopamine and also strongly reduces the Schultz-Dale response of the pulmonary vessels.

3. The results are consistent with the suggestion that dopamine (together with histamine and 5-hydroxytryptamine) may participate in the pulmonary vasoconstriction associated with anaphylaxis in cattle.

Introduction

Although there has recently been some interest in hypersensitivity reactions in domesticated ruminants, the significance of biogenic amines and other anaphylactic mediators in these species is largely unknown. The lung is a principal anaphylactic shock organ in cattle and sheep (Aitken & Sanford, 1969a: Alexander, Eyre, Head & Sanford, 1970 ; Eyre, 1970a). Although histamine and 5-hydroxytryptamine are involved, together with kinins and slow reacting substance in anaphylaxis in most other species, anaphylaxis in ruminants has not been investigated from this point of view.

It has recently been shown that histamine and 5-hydroxytryptamine (5-HT) are both released by antigen from bovine lung, Eyre (1971). The relative significance of these amines has not been determined, but it is well known that antihistamines are ineffective in treating naturally occurring allergic diseases of cattle. The antihistaminics and anti-5-HT agents do not efficiently protect cattle or sheep from experimentally induced systemic anaphylaxis (Alexander *et al.*, 1970 ; Aitken & Sanford, 1969b), but the anti-5-HT agent methysergide partially antagonizes bovine passive cutaneous anaphylactic reactions (Eyre, 1970b). A tentative suggestion has been made that histamine may be comparatively less important than other mediators in anaphylaxis in ruminants (Alexander *et al.*, 1970 ; Aitken & Sanford, 1969b). This report is part of an attempt to determine the nature of the mediators of anaphylaxis in the ungulate (hoofed) species.

The concentration of dopamine in the lung of ruminants is exceptionally high (Euler & Lishajko, 1957 ; Aviado & Sadavongvivad, 1970). Furthermore, this lung dopamine has been located in the mast cells (Coupland & Heath, 1961 ; Falck,

Nystedt, Rosengren & Stenflo, 1964). These facts suggested that dopamine might be liberated by antigen from bovine mast cells and that this amine could be a mediator which was unaffected by antihistaminics and other conventional antagonists of allergy and anaphylaxis.

Methods

Antigenic sensitization

Jersey or Guernsey bull calves between 3 and 8 weeks of age were sensitized to whole horse serum (0·2 ml/kg i.v.) followed by two injections at weekly intervals of 1·0 ml of horse serum emulsified in 1·0 ml of Freund's complete adjuvant divided into four or six parts and injected intradermally in the neck.

Lung preparation and challenge

Calves were killed with intravenous pentobarbitone 1–3 weeks after the last dose of antigen. One whole macroscopically normal lung was removed within approximately 5–10 min of death and transported immediately in ice-cold Tyrode to the laboratory. The edge of the apical lung lobe was excised and cut up with a McIlwain mechanical tissue chopper into rods of approximately $3 \times 1 \times 1$ mm. The chopped tissue was washed by suspending in Tyrode at 4° C for at least 1 h, then removed and lightly blotted on filter paper. Duplicate 100 mg $\pm$ 5 mg portions were then resuspended in conical flasks in 2 ml volumes (1) Tyrode alone, (2) 1:4 dilution horse plasma: Tyrode, (3) Tyrode containing compound 48/80 400 μg/ml. All flasks were continuously gassed with 95% oxygen, 5% CO_2 mixture and incubated for 15 min at 37° C in a Dubnoff shaking incubator.

Identical control experiments were conducted using lung samples from three adult Hereford steers slaughtered by shooting and one calf killed with pentobarbitone.

Dopamine assay

After incubation, the supernatants were decanted and set aside for amine assay. The tissue fragments were washed twice with 25 ml ice-cold Tyrode and homogenized in acid butanol with ground glass tissue grinders. The supernatants and homogenates were precipitated and extracted by the method described by Sadavongvivad (1970). The dopamine concentration in the final phase was estimated by the method of Carlsson & Waldeck (1958) using an Aminco-Bowman Spectrofluorimeter (excitation 345 nm and emission at 410 nm).

The Schultz–Dale reaction

It has been shown (Eyre, 1970a) that the pulmonary artery and vein of calves sensitized to horse serum contract *in vitro* to the specific antigen (Schultz-Dale effect). Spiral vein strips obtained from the calves described above, were set up in Krebs-Henseleit solution at 37° C and exposed to histamine, 5-HT, dopamine and horse plasma. A positive reaction to antigen indicated hypersensitivity in each calf. Also the vein strips permitted observations of the actions of dopamine *per se*.

Results

Lungs from six calves sensitized with horse serum and from four normal (unsensitized, control) animals have been studied and the results are shown in Table 1.

TABLE 1. *Release of dopamine from bovine lung* in vitro *by specific antigen and by compound* 48/80

Animal	Acid-extracted total lung dopamine (μg/g)	Percentage dopamine released challenged with		
		Tyrode only	Horse plasma 1:4 in Tyrode	Comp. 48/80 400 μg/ml Tyrode
Calves sensitized with horse serum				
4661	1·179	14·3	26·7	30·5
4662	0·713	17·7	24·7	15·5
4664	1·184	10·2	23·7	28·7
4671	2·922	11·4	28·8	25·3
4675	1·413	11·5	29·2	31·0
4670	1·569	9·3	21·0	20·2
mean±S.E.	1·497±0·69	12·4±2·8	25·7±2·9*	25·2±5·6*
Unsensitized controls				
Adult 1	1·738	9·9	9·0	32·2
Adult 2	1·957	10·0	10·5	27·2
Adult 3	1·973	8·5	11·8	29·1
Calf 4713	1·119	10·9	13·5	25·3
mean±S.E.	1·697±0·35	9·8±0·9	11·2±1·7	28·5±2·6*

*$P<0.001$ when compared with controls (Tyrode treated).

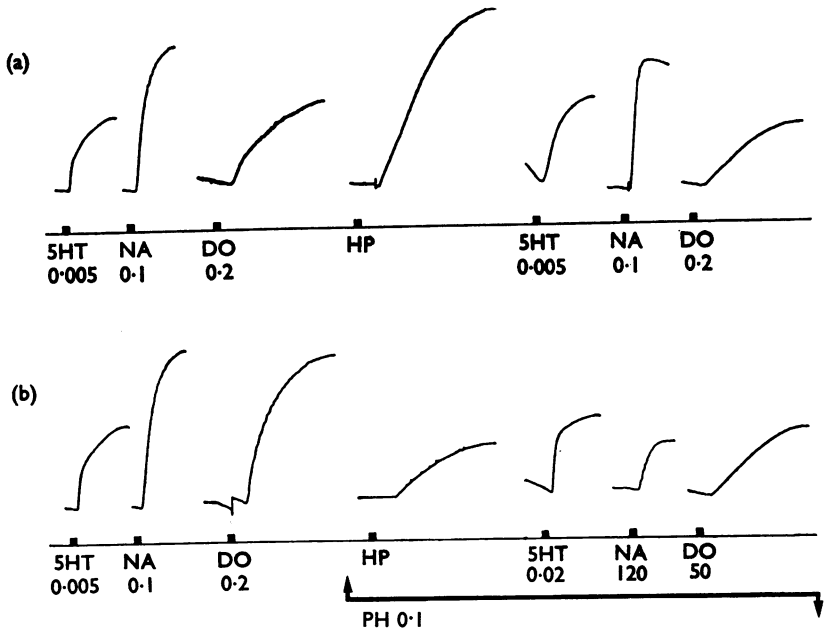

FIG. 1. Pair of isolated spiral strips (a & b) from a single pulmonary vein of a 6-week-old Jersey calf sensitized with horse serum in Freund's complete adjuvant. The tissues, which are in 20 ml Krebs-Henseleit solution at 35° C, are contracting to 5-hydroxytryptamine (5-HT), noradrenaline (NA), dopamine (DO), and horse plasma (HP: 0·5%). (b), Between the arrows phentolamine (PH) was present. Phentolamine inhibited noradrenaline and dopamine (dose-ratios 1,000 and 100 respectively: four observations each) and reduced the Schultz-Dale response to horse plasma 60% (four observations). Drug concentrations are expressed as μg base/ml bath fluid.

There appeared to be no difference between the concentration of dopamine in calf lung and in lung of steers. The spontaneous release of dopamine in both sensitized and unsensitized lung was high and varied from 10 to 17% (mean 12·4). Horse plasma liberated significantly greater amounts of dopamine ($P=0{\cdot}001$) from sensitized lung (25·7% $\pm$2·9). In the case of unsensitized lung, horse plasma released a similar amount of dopamine to that liberated spontaneously. Compound 48/80, however, consistently liberated dopamine in quantities significantly greater than spontaneous release in both sensitized and unsensitized lung.

In all six sensitized calves, strong positive Schultz-Dale responses were produced in isolated pulmonary veins (Fig. 1). It can also be seen in Fig. 1 that dopamine contracts the pulmonary vein and that the α-adrenoceptor blocking agent phentolamine antagonizes dopamine and reduces the response of the vessels to antigenic challenge by 60%. (Pharmacology of isolated pulmonary smooth muscles to be published in full elsewhere.)

Discussion

In so far as can be ascertained, this is the first report of the liberation of dopamine by specific antigen and by compound 48/80.

Experimentally induced systemic anaphylaxis in cattle and sheep is characterized by a fall in arterial blood pressure, increased resistance to pulmonary inflation and a marked pulmonary vasoconstriction (Aitken & Sanford, 1969b ; Alexander *et al.*, 1970). Isolated pulmonary artery vein strips taken from protein sensitized cattle react to specific antigen with a Schultz-Dale response (Eyre, 1970a).

Although we have recently shown that histamine and 5-HT are liberated by antigen from bovine lung (unpublished results) the manifestations of anaphylaxis are not inhibited significantly by antihistaminic or antiserotonin agents. This suggested that other mediators might be responsible for the antihistamine resistant responses. Kinins, SRS-A and dopamine may be implicated.

Aviado & Sadavongvivad (1970) showed that intravenous infusion of dopamine in a goat caused increased aortic and pulmonary artery pressure, whereas our recent findings show that dopamine causes a fall in carotid pressure and a rise in pulmonary pressure in cattle.

The α-adrenoceptor blocking agent phentolamine inhibits the pulmonary vasoconstrictor activity of both dopamine and antigen *in vivo* and *in vitro,* but fails to inhibit the fall in carotid pressure or the bronchoconstriction associated with antigen injection. Further work is in progress to determine whether *in vivo* anaphylaxis in cattle is associated with increased blood dopamine concentrations, and to investigate the actions of dopamine blocking agents together with α- and β-adrenoceptor blocking agents on cardio-respiratory parameters in an attempt to suggest a functional role for the high concentrations of this catecholamine in the mast cells of ruminants.

In view of the abundant presence of dopamine in cattle mast cells and its release during antigen challenge, together with the inhibitor action of phentolamine on the pulmonary vasoconstriction caused by dopamine and by antigen, it is difficult to avoid the suggestion that dopamine may be involved in the mediation of the anaphylactic reaction in the pulmonary blood vessels of this species. However, the involvement of dopamine in anaphylactic responses of non-pulmonary blood vessels

(vasodilation) or of the bronchi (bronchoconstriction) is not suggested by these results.

It is feasible that dopamine may participate in pulmonary vasoconstriction in anaphylaxis in species other than ruminants, although the concentrations of lung dopamine are much lower—cat 0·11, rabbit 0·05, dog 0·06 μg/g lung compared with goat 6·4 (Aviado & Sadavongvivad, 1970) and sheep (Bertler & Rosengren, 1959) and calf 1·5 (this article).

Studies of dopamine liberation by antigen in a wide variety of species would be of great physiological interest.

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REFERENCES

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. and SANFORD, J. (1970). Effects of anaphylaxis and chemical histamine liberators in sheep. *J. comp. Path.*, **80**, 19–30.

AVIADO, D. M. & SADAVONGVIVAD, C. (1970). Pharmacological significance of biogenic amines in the lungs: noradrenaline and dopamine. *Br. J. Pharmac.*, **38**, 374–385.

BERTLER, A. & ROSENGREN, E. (1959). Occurrence and distribution of dopamine in brain and other tissues. *Experientia*, **15**, 10–11.

CARLSSON, A. & WALDECK, B. (1958). A fluorimetric method for determination of dopamine (3-hydroxytyramine). *Acta physiol. scand.*, **44**, 293–298.

COUPLAND, R. E. & HEATH, I. D. (1961). The chromaffin cells of the liver capsule and gut in ungulates. *J. Endocr.*, **22**, 71–76.

EULER, U. S. VON & LISHAJKO, F. (1957). Dopamine in mammalian lung and spleen. *Acta physiol. pharmac. neerl.*, **6**, 295–303.

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, 5HT, bradykinin) and passive cutaneous anaphylaxis in sheep. *J. Pharm. Pharmac.*, **22**, 104–109.

EYRE, P. (1971). Histamine release from calf lung *in vitro* by specific antigen and by compound 48/80. *Arch. int. Pharmacodyn.* (in the Press).

FALCK, B., NYSTEDT, T., ROSENGREN, E. & STENFLO, J. (1964). Dopamine and mast cells in ruminants. *Acta pharmac. tox.*, **21**, 51–58.

SADAVONGVIVAD, C. (1970). Pharmacological significance of biogenic amines in the lungs: 5 hydroxytryptamine. *Br. J. Pharmac.*, **38**, 353–365.