## Atypical adrenergic modulation of allergic histamine release from bovine leucocytes

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Histamine is released from sensitized mast cells and leucocytes of several species, following exposure to specific antigen. Modulation of this release by adrenoceptor stimulants has been studied principally in two systems: isolated human leucocytes (Bourne, Lichtenstein, Melmon, Henney, Weinstein & Shearer, 1974), and chopped lung of man, guinea pig and monkey (see Assem, 1974). In both systems, $\beta$-adrenoceptor stimulation elevates intracellular cyclic AMP concentrations and inhibits histamine release. Stimulation of $\alpha$-adrenoceptors enhances histamine release from chopped lung, but is without effect on isolated human leucocytes. A study of the adrenergic modulation of allergic histamine release from bovine leucocytes revealed radical differences from the systems described above.

As previously described (Holroyde & Eyre, 1975), granulocytes were isolated (94% purity) from venous blood of 6 week old Jersey and Guernsey calves which had been sensitized to horse plasma. Aliquots of the granulocyte fraction (approximately $18 \times 10^6$ leukocytes) were incubated at 37°C for 30 min in Tris buffer (Holroyde & Eyre, 1975) together with sufficient horse plasma to release 40–60% of the total available histamine. Adrenoceptor agonists ($10^{-6}$–$10^{-3}$M) were added to the incubate 2 min before the horse plasma; adrenoceptor antagonists were added 2 min before the agonists. Histamine release was determined fluorometrically.

Isoprenaline ($n=6$) potentiated histamine release at $10^{-6}$ M and $10^{-5}$ M ($P<0.05$), but inhibited at higher concentrations ($P<0.01$). In the presence of $10^{-5}$ M propranolol, the potentiation was reversed to significant inhibition. In the presence of $10^{-5}$ M phentolamine, the potentiatory phase was significantly enhanced and the inhibitory phase somewhat reduced. Phenylephrine ($10^{-6}$–$10^{-3}$ M) produced only inhibition of histamine release. These results indicate the presence of both $\alpha$- and $\beta$-adrenoceptors on bovine granulocytes. Stimulation of $\alpha$-adrenoceptors causes inhibition of histamine release, whereas $\beta$-stimulation causes potentiation. This is the exact opposite of the situation in all other species so far described.

Adrenaline, which inhibits the release of most mediators of allergy in all species examined, significantly potentiated the release of histamine in this study ($10^{-8}$–$10^{-3}$ M, $n=5$).

It is clear that the bovine granulocyte is regulated by adrenoceptors in a manner radically different from any other comparable immunological system so far described.

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## An investigation of histamine aerosol induced reflex bronchoconstriction in the anaesthetized dog

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The role of the vagus nerve in histamine-induced bronchoconstriction in anaesthetized dogs appears variable. Some workers claim that histamine induced bronchoconstriction is entirely reflex (Gold, Kessler & Yu, 1972; Nadel, 1974), while others have attributed a significant proportion of the bronchoconstriction to the direct action of histamine on bronchial smooth muscle (Krell, Chakrin & Wardell, in press). The purpose of this communication is to try to offer some explanation for these differing views.

Using anaesthetized beagle dogs we have measured changes in airways resistance ($R_L$) and dynamic lung compliance (Cdyn) after administration of histamine aerosol. The dogs were respired at constant pressure with a Bird Mk. VII ventilator. The reflex component of histamine induced bronchoconstriction was determined by bilateral vagal cooling.

A comparison was made between the effects on $R_L$ and Cdyn of 4 breaths of 2 aerosols of different particle sizes in 11 dogs anaesthetized with pentobarbitone sodium (30 mg/kg i.v. followed by 0.1 mg $kg^{-1}$ $min^{-1}$). Using a Bird micro-nebulizer, which produced particles of 0.5 μm mean diameter, relatively small increases in $R_L$ were produced (e.g. 4 inhalations of an aerosol generated from a 0.5% w/v solution histamine produced increases in $R_L$ of

$0.7 \pm 0.02$ $cmH_2O\ l^{-1}\ s^{-1}$ $n=6$). A 'Vaponefrin inhalajet' nebulizer modified to produce an aerosol containing particles of 10 μm mean diameter produced much greater changes in $R_L$ (e.g. 4 inhalations of an aerosol generated from a 0.5% w/v solution histamine produced increases in $R_L$ of $6.5 \pm 1.4$ $cmH_2O\ l^{-1}\ s^{-1}$ $n=6$). However, the bronchoconstriction resulting from using histamine aerosols generated from either nebulizer was not inhibited by bilateral vagal cooling.

A further series of experiments was performed to compare the effects of histamine aerosol of 10 μm mean particle size on $R_L$ and Cdyn in dogs anaesthetized with pentobarbitone sodium with those anaesthetized with chloralose (80 mg/kg i.v. followed by 10–15 mg every 15 minutes). Histamine aerosols of 10 μm now produced marked increases in $R_L$ even when comparatively low concentrations of histamine solution were used to generate the aerosol (e.g. 4 inhalations of a 10 μm aerosol generated from a 0.125% w/v solution of histamine produced increases in $R_L$ of $12.7 \pm 1.9$ $cmH_2O\ l^{-1}\ s^{-1}$ $n=6$, in dogs anaesthetized with chloralose compared with $1.6 \pm 0.8$ $cmH_2O\ l^{-1}\ s^{-1}$ in dogs anaesthetized with pentobarbitone sodium). In dogs anaesthetized with chloralose the increase in $R_L$ was significantly inhibited by vagal cooling, while the falls in Cdyn were largely unaffected.

The effects of direct electrical stimulation of the efferent vagal nerves were also noted in these experiments. Stimulation of the vagus nerves in dogs anaesthetized with pentobarbitone sodium produced a mean increase in $R_L$ of $1.2 \pm 0.4$ $cmH_2O\ l^{-1}\ s^{-1}$, while vagal stimulation in dogs anaesthetized with chloralose produced a much greater increase in $R_L$ of $12.4 \pm 1.8$ $cmH_2O\ l^{-1}\ s^{-1}$. (Stimulation parameters: 20 Hz 1.ms at supramaximal voltage.) It was further noted that i.v. administration of pentobarbitone sodium to the chloralose anaesthetized dog significantly reduced the response to direct-efferent vagus nerve stimulation (5 mg/kg by 49.4% and 10 mg/kg by 69.5%).

These experiments indicate the importance of selecting the right experimental conditions to obtain a reflex bronchoconstriction to histamine aerosol in the anaesthetized dog. Our results show that using chloralose anaesthesia and an aerosol of mean particle size 10 μm a large reflex bronchoconstriction is produced. If, however, pentobarbitone sodium is used as the anaesthetic and an aerosol of mean particle size of 0.5 μm is used, the response is almost entirely direct.

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## Studies on the mechanism of action by which 5H-[1]-benzopyrano-[2,3-b]-pyridin-5-ol (AH 6696) inhibits gastric acid secretion

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Peptic ulceration in man can be treated by inhibiting the secretion of acid into the stomach. Interest in 5H-[1]-benzopyrano-[2,3-b]-pyridin-5-ol (AH 6696) arose because it is a potent inhibitor of gastric acid secretion in the rat and dog. The mechanism by which AH 6696 inhibits gastric acid secretion has been investigated.

In the urethane anaesthetized rat (Ghosh & Schild, 1958) AH 6696 (1, 3 or 10 mg/kg i.v.) caused a dose-dependent reduction of submaximal gastric acid secretion. Five female rats (80–110 g) were used per dose level and the $ED_{50}$ values for AH 6696 to inhibit gastric acid secretion induced by histamine, pentagastrin or bethanechol were 1.6, 2.8 and 2.4 mg/kg respectively.

On the guinea-pig ileum preparation (Krebs-Henseleit solution at 32°C gassed with 95% $O_2$:5% $CO_2$) the $EC_{50}$ values (μg/ml) for AH 6696 to inhibit contractions induced by barium chloride, acetylcholine, histamine and 5-hydroxytryptamine were [mean ± s.e. mean, $n=10$] $354 \pm 48$, $338 \pm 40$, $86 \pm 25$ and $28 \pm 6$ respectively. On the isolated right atrium preparation of the guinea-pig (Krebs-Henseleit solution at 37°C gassed with 95% $O_2$:5% $CO_2$) AH 6696 (10–100 μg/ml) failed to antagonize histamine-induced tachycardia ($H_2$-effect). Cardiovascular actions were investigated in 4 female