SMOOTH MUSCLE CONTRACTOR ACTIVITY OF COTTON DUST AQUEOUS EXTRACT *IN VITRO*. II - THE RESPONSE OF THE GUINEA PIG TRACHEA IN THE ABSENCE OF 5-HT INDUCED MECHANISMS IS ONLY PARTLY MEDIATED THROUGH HISTAMINE-SENSITIVE RECEPTORS

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Abstract

Using isolated guinea pig trachea that was relatively insensitive to 5-HT, it was shown that, in the absence of 5-HT induced mechanisms, the response to cotton dust aqueous extract (CDE) was only partially (75%) blocked by the antihistamine, chlorpheniramine at concentrations that completely blocked histamine. Methysergide also blocked the CDE-induced response (90%). Given that this preparation is insensitive to 5-HT, the mechanism of action of methysergide is unclear. Other studies have identified actions of methysergide unrelated to 5-HT, including interference with the action of eicosanoids. When the trachea was superfused, dose-dependent responses could be obtained with carbachol but not with CDE.

Introduction to Part II

The Response of the Guinea Pig Trachea in the Absence of 5-HT induced Mechanisms is only Partly Mediated Through Histamine-Sensitive Receptors.

Under isometric conditions, dose dependent contractions to 5-HT can be obtained in the guinea pig trachea. The ED_{50} for 5-HT in this preparation has been reported to be 13 times higher than in the rat fundus [1]. In the present investigation, however, it was found that, apart from a low, dose-independent response (which did not appear to be an artefact), the isotonic preparation was insensitive to 5-HT. The maximum dose given was 50µg/ml, approximately 1000 times its ED_{50} in the rat fundus. (This interesting phenomenon was not investigated further, but variations in responsivness arising from such differences in measurement technique have been reported. [2,3]) However, since it was found that the tissue was still responsive to CDE and that this response was blocked by methysergide, it called into question the mechanism of action of methysergide and suggested that, for this tissue, the "smooth muscle contractor agent" in CDE may not be producing its effects via endogenous 5-HT.

The preparation was found to be sensitive to histamine. Therefore, the possibility of the involvement of histaminergic mechanisms was investigated. The nature of the action of methysergide on the CDE-induced response was also probed.

The possible effects of CDE on the superfused trachea, under isotonic tension was also investigated.

Materials and Methods

A list of the materials used can be found in Table A of Part I of this series of reports.

Carbachol as Standard - As with the rat fundus experiments reported in Part I of this series, carbachol was used as an internal standard reference.

The Immersed Preparation

The trachea was isolated from Dunkin-Hartley guinea pigs.

This tissue is tapered, the cartillage rings being largest close to the nasopharynx and smallest at the tracheobronchial junction. The narrower portions of the trachea were the most responsive to the agonists studied. The tissue was spirally cut across seven segments of circular muscle and equilibrated under a tension of one gram in aerated ($95\%O_2+5\%CO_2$) Krebs solution (37°C).

Responses were measured isotonically, under a tension of 1 gram, using a Washington T-II isotonic lever transducer coupled to a Washington MD-1 recorder fitted with a CD 10 amplifier.

The Superfused Preparation (fig. 1)

The tissue was prepared as described in the previous section. However, rather than being placed in a bath of Krebs solution, it was suspended in air, with Krebs at 37°C being applied dropwise, as shown in figure 1.

Krebs solution was applied dropwise from nozzle A. When applying the test solution with pipette B (1-5 drops), the Krebs solution was stopped and the test solution left in contact with the tissue for one minute before resuming dropwise application of Krebs. In this manner, dose-dependent responses were obtained with carbachol (1-30µg/ml).

Results

The Immersed Preparation

Carbachol (fig. 2)

The ED_{50} of carbachol was 44(± 7)ng/ml. In all subsequent studies, therefore, a dose of 50ng/ml was used as reference.

CDE (fig. 3)

The interpolated ED_{50} of CDE was 0.31(±0.045)mg/ml. The response to this dose of CDE, however, was relatively small. 1.5mg/ml (ED_{82}) was selected as a control dose,

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producing an absolute response nearer to the carbachol ED_{50}.

Histamine (fig. 4)
Histamine caused a dose-dependent contraction whose maximum varied between 15 and 50µg/ml. Having reached its maximum, even a half-log increase (3-fold) in dose resulted in a profound relaxation. The maximum contraction obtained was of the same order of magnitude as CDE (1.5mg/ml,ED_{82}) and carbachol (5ng/ml,ED_{50}).

Methysergide (fig. 5)
Preliminary studies carried out with methysergide (10 & 30ng/ml) resulted in only partial blockade of CDE (1.5 & 5mg/ml). A higher dose of methysergide (100ng/ml) resulted in a greater blockade of CDE at a 1.5mg/ml dose level (-91(±10)%,n=5) and at 5mg/ml (-88(±17)%,n=5). Comparatively, methysergide (30 & 100ng/ml) did not significantly affect histamine (15µg/ml).

Chlorpheniramine (fig. 6)
At a concentration of 0.1 µg/ml, chlorpheniramine blocked histamine responses completely (blocked to -4(±2)% of control,n=4). Its effect on CDE, however, was less reproducible as borne out by the large errors.

At a higher dose of chlorpheniramine (1.0µg/ml), there is evidence that CDE spasmogenic activity is partially, but significantly, blocked (1.5mg/ml blocked to 26(±6)%, 5mg/ml blocked to 25(±6)% of control).

The Superfused Preparation
In this preparation, dose-dependent responses were obtained with carbachol (1-30µg/ml). However, CDE, up to a concentration of 100mg/ml, only resulted in a very low response which was unrelated to concentration and probably an artefact.

Discussion

Histamine Dose-Response Curve
Histamine-induced isotonic relaxation of methacholine contracted (50-70%) sheep bronchus has been reported to be selectively antagonised by the H_2-antagonist, burimamide [4]. The histamine-induced isotonic relaxation of similarly treated cat trachea, on the other hand, requires both mepyramine (H_1-antagonist) and burimamide for complete blockade [4]. It is, therefore, not surprising that both contractile and relaxant effects of histamine are also observed in the guinea pig trachea (GPT). The H_1-antagonist, chlorpheniramine, abolished the contractile effect of histamine. There was also evidence of a probable slight histamine-induced relaxation ($P < 0.07$ (2-tailed t)). The latter was abolished with an increased dose of the antagonist. (see fig. 6)

It is proposed that there are two opposing histamine-induced responses in GPT. An attempt has been made to represent these diagrammatically, here. (fig. 7)

Blockade by Chlorpheniramine, of the contractile response suggests that the latter is H_1 mediated. The probable slight residual relaxation, observed in the presence of Chlorpheniramine, suggests that the relaxation response may be via receptors which are less susceptible to Chlorpheniramine. However, a higher concentration of Chlorpheniramine does abolish all of the histamine-induced responses. It is likely that, at the higher concentrations, Chlorpheniramine's action becomes less specific.

Methysergide
Methysergide was capable of selectively blocking CDE-induced contractility, albeit partially, without affecting histamine responses.

Methysergide has been reported to cause the contraction of guinea pig ileum (isometric preparation) via histamine H_1 receptors, although it has not been established whether this is a direct or indirect action [5]. At the doses employed here, methysergide did not elicit a response. There was, however, a trend for the mean histamine response to increase with increasing doses of methysergide.

The results indicate that, in this preparation, the majority of the response to CDE was due to mechanisms, unrelated to 5-HT but, which were antagonised by methysergide.

Later (see Part III of this series of reports, also presented at these proceedings) it was shown that the CDE components responsible for contraction in this preparation were not the same as those active on the rat fundus. Therefore, whilst the contraction in the rat fundus may be due to 5-HT, it appears that methysergide is not sufficiently specific for confirmation of this hypothesis. Indeed, other studies [6], investigating the mechanism of action of methysergide in the rat fundus showed that methysergide significantly blocked responses to $PGF_{2\alpha}$ and arachidonic acid.

Chlorpheniramine
Concentrations of chlorpheniramine causing complete blockade of the histamine response appeared to partially block CDE responses. Therefore, part of the CDE-induced contraction of the isotonic spirally cut trachea seems to be via histamine-related mechanisms.

The Superfused Preparation
Carbachol resulted in dose-dependent contractions in both the superfused and the immersed preparations. However, with CDE, a dose-response relationship was only observed in the immersed preparation. A mediator release mechanism for the CDE induced responses would account for this difference, since in the immersed preparation,

mediators are able to reach their target cells through the medium of the physiological buffer.

Conclusions

In the isotonic guinea pig trachea, methysergide partially blocked CDE, but not histamine-induced contractions. Therefore, one or more components of CDE-induced contraction in GPT are not via mechanisms involving histamine. However, CDE is partially affected by the H_1-antagonist, Chlorpheniramine. Hence, the CDE-induced contraction in GPT involve more than one mechanism; one of the mechanisms involving histamine receptors and another being methysergide sensitive.

CDE-induced *in vivo* and *in vitro* histamine release from a number of cells has been extensively reported [e.g. 7,8,9,10,11]. Histamine antagonists have been only partially effective in reducing bronchoconstriction after cotton dust inhalation [11,12]. This suggests that other mechanisms are also involved.

The results obtained with the superfused trachea preparation provides evidence for the action of CDE on GPT being via a mediator release mechanism. As well as histamine [8] CDE-induced arachidonic acid metabolite release has been demonstrated in the guinea pig perfused lung [13]. Hence it is plausible that the histamine-independent component of the isotonic GPT response may be due to eicosanoid release. 5-HT related mechanisms may also be involved *in vivo,* where there is an isometric component to the response (i.e. when the response is auxotonic).

References

1. Cohen ML, Schenk KW, Colbert W, Wittenauer L. 1985. Role of 5-HT_2 receptors in serotonin-induced contractions of non-vascular smooth muscle. J. Pharmacol. Exp. Ther. 232:770-774.

2. Li W, Stephens NL. 1994. Auxotonic loading and airway smooth muscle shortening. Canadian J. Physiol.Pharmacol. 72:1458-63.

3. Sadeghi Hashjin G, Henricks PAJ, Folkerts G, Nijkamp FP. 1995. Preparation of bovine tracheal smooth muscle for in vitro pharmacological studies. J. Pharmacol. Toxicol. Methods. 34:103-108.

4. Eyre P. 1973. Histamine H_2 receptors in the sheep bronchus and cat trachea: the action of burimamide. Br. J. Pharmacol. 48:321-323.

5. Gunning SJ, Bunce KT, Humphrey PPA. 1988. Methysergide contracts guinea pig ilium via histamine H_1 receptors. Br. J. Pharmacol. 93:238P.

6. Riazi-Farzad B. 1995. Chemical and pharmacological studies of cotton dust. Ph.D. Thesis, UWCC, Cardiff.

7. Bouhuys A, Lindell SE, Lundin G. 1960. Experimental studies on byssinosis. Br. Med. J. 2:324-6.

8. Nicholls PJ, Nicholls GR, Bouhuys A. 1965. Histamine release by compound 48/80 and textile dust from lung tissue in vitro. In: Bouhuys A. Proc. of the second international symposium on inhaled particles and vapours. Pergamon Press, London. 69-74.

9. Bouhuys A, Lindell SE. 1961. 1961. Release of histamine by cotton dust extract from human lung tissue in vitro. Experimentia. 17:211-2.

10. Elisalde MH, Elisalde GS. 1982. The effect of aqueous extract of cotton bract on histamine release, calcium binding and cyclic nucleotide concentrations of the isolated mast cell. Proc. cotton dust res. conf. 6:24-6.

11. Bouhuys A. 1963. Prevention of monday dyspnea in byssinosis, a controlled trial with an antihistamine drug. Clin. Pharmacol. Ther. 43:311-4.

12. Fawcett IW, Merchant JA, Simmonds SP, Pepys J. 1978. The effect of sodium chromoglycate, beclomethasone dipropionate and salbutamol on the ventilatory response to cotton dust in mill workers. Br. J. Dis.Chest. 72:38-39

13. El-Mahdy N, Nicholls PJ. 1987. The effects of cotton dust and bract extract on guinea pig perfused lung. Proc. Cotton Dust Res. Conf. 11:57-61.

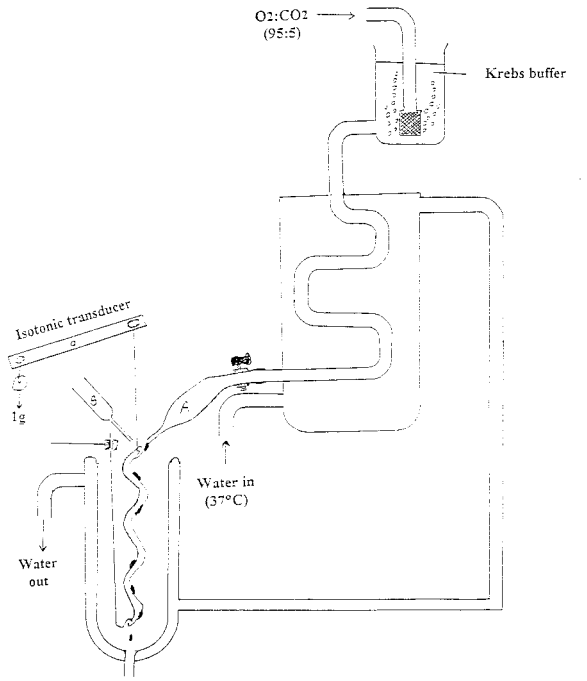

Figure 1. Diagrammatic Representation of the Superfusion Apparatus.

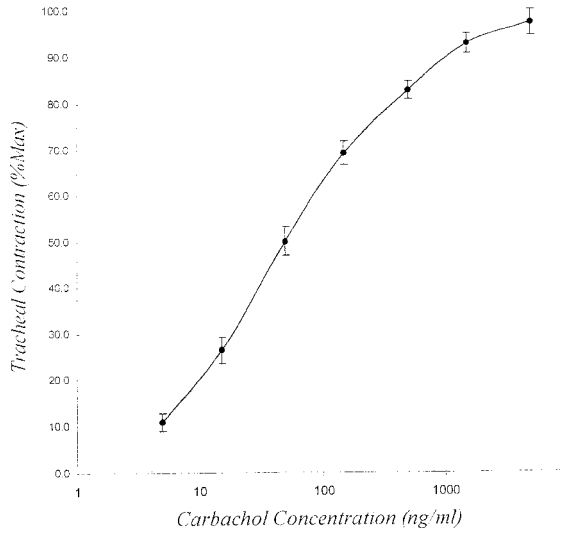

Figure 2. The Carbachol Concentration-Response Relationship in the Isotonic Guinea Pig Trachea as a Percentage of Its Maximum Response.

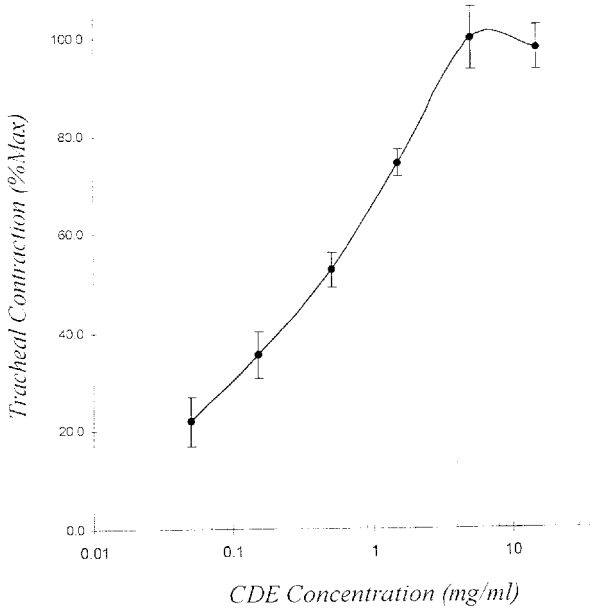

Figure 3. The CDE Concentration-Response Relationship in the Isotonic Guinea Pig Trachea as a Percentage of Its Maximum Response.

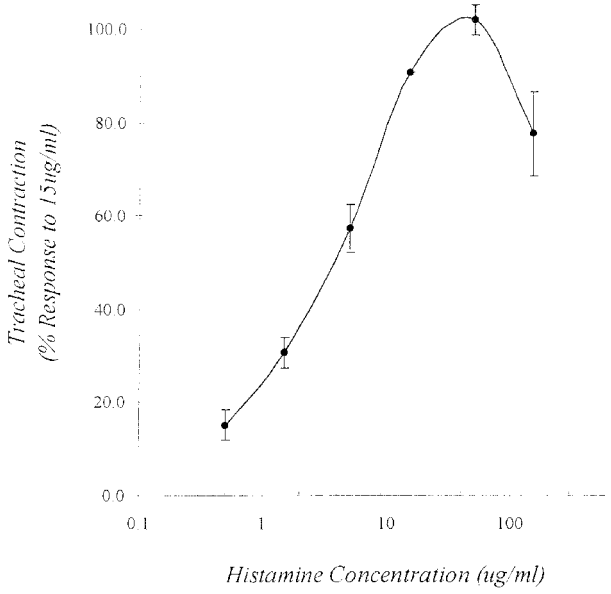

Figure 4. The Histamine Concentration-Response Relationship in the Isotonic Guinea Pig Trachea as a Percentage of Its Response to 15 μg/ml of Histamine.

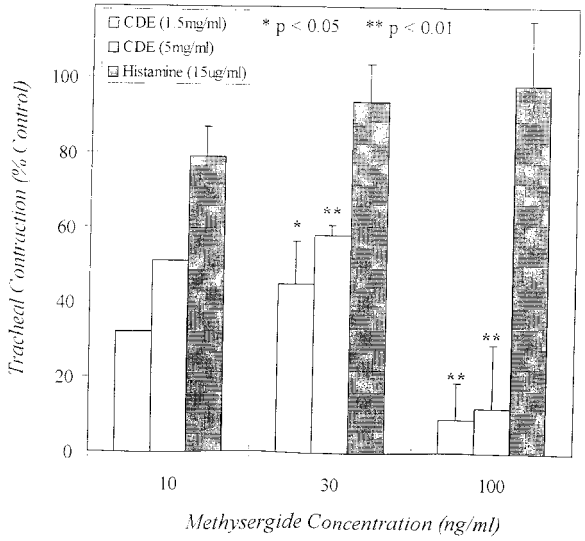

Figure 5. The Effect of Increasing Concentrations of Methysergide on Contractions Induced by Single Doses of CDE (1.5 and 5μg/ml) and Histamine (15 μg/ml) in the Isotonic Guinea Pig Trachea.

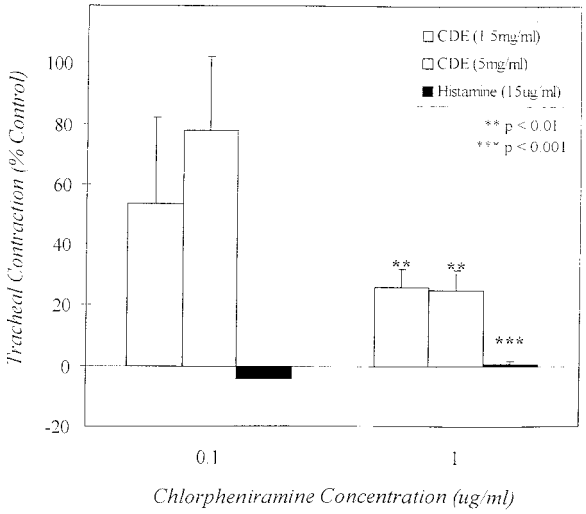

Figure 6. The Effects of Clorpheniramine (0.1 and 1.0μg/ml) on Contractions Induced by Single Doses of CDE (1.5 and 5μg/ml) and Histamine (15 μg/ml) in the Isotonic Guinea Pig Trachea.

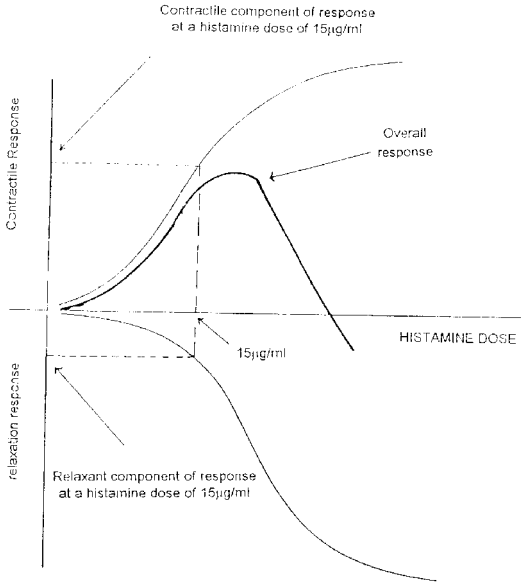

Figure 7. A Diagrammatic Representation of the Possible Components Responsible for the Total Histamine Concentration-Response Relationship in the Isotonic Guinea Pig Trachea.