Short communications

Histamine H_2-receptors in the sheep bronchus and cat trachea: the action of burimamide

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Histamine-induced relaxation of the sheep bronchus was antagonized by burimamide but not by mepyramine, indicating the presence of H_2-receptors. Mepyramine or burimamide alone produced partial antagonism of histamine-induced relaxation of the cat trachea. Both inhibitors added simultaneously effectively abolished the histamine response, suggesting that both H_1- and H_2-receptors are active in relaxing the cat trachea.

Introduction. — Histamine receptors which are blocked by 'classical' antihistaminics such as mepyramine have been defined as H_1-receptors (Ash & Schild, 1966). However, mepyramine and like-acting antihistaminics fail to antagonize the effects of histamine on gastric secretion (Ashford, Heller & Smart, 1949), heart (Trendelenburg, 1960) or rat uterus (Dutta, 1949). Mepyramine-resistant histamine receptors have been separately defined by Ash & Schild (1966).

Eyre (1969) reported that in sheep bronchus, histamine caused relaxation which was not inhibited by mepyramine nor by adrenoceptor blocking agents. It was postulated that the ovine bronchus possessed specific histamine receptors which were not of the H_1-type.

In the cat, histamine causes relaxation of tracheal muscle apparently mediated through H_1-receptors and adrenoceptors, since it was partially inhibited by mepyramine or by pronethalol (Maengwyn-Davies, 1968).

The recent development of burimamide, a specific histamine H_2-receptor antagonist (Black, Duncan, Durant, Ganellin & Parsons, 1972), suggested a further study of histamine-induced relaxation of the airway muscle of the sheep and cat.

Methods.—Segments of terminal bronchi from five sheep (Eyre, 1969) and of thoracic tracheae from two cats (Maengwyn-Davies, 1968) were removed from animals killed by pentobarbitone. Sheep bronchi were cut helically into strips, whereas cat tracheae were cut into rings and a chain of five or six rings prepared. Several such preparations from each individual animal were suspended in 20 ml Krebs solution at 35° C, aerated with 95% oxygen, 5% CO_2 mixture. Isotonic contractions were recorded with linear motion transducers and an 'E & M' Physiograph.

Concentrations of methacholine were chosen from the dose-response curves to provide submaximal contractions of each preparation, between 50 and 70%. Methacholine was left in contact with the tissues for 5 min or until maximum response, whichever was first. Histamine was then added and left in contact for 2 min or until maximum relaxant effect. The blocking drugs were always added 1–2 min before the methacholine. The activity of the antagonists was measured by determining the dose-ratio: the ratio of doses of agonist which give equal responses in the presence and absence of antagonist (Gaddum, Hameed, Hathway & Stephens, 1955). The drugs used were acetyl β-methyl choline chloride (methacholine), histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate (5-HT), isoprenaline hydrochloride, mepyramine maleate and burimamide.

Results.—*Sheep bronchus.* As previously reported Eyre, 1969), histamine (>0·5 μM) consistently caused dose-related relaxation of the tissue which was not antagonized by mepyramine (0·5–10 μM). On the contrary, in three out of five animals histamine relaxation was potentiated slightly by mepyramine. Figure 1a shows in contrast that burimamide (10 μM) inhibited histamine-induced bronchial relaxation (dose-ratio=20 ; n=4). Burimamide 50 μM more effectively antagonized the relaxant action of histamine (dose-ratio= 50 ; n=4). High concentrations of histamine (>100 μM) caused a small contraction of the bronchi in the presence of 50 μM burimamide. This concentration of burimamide slightly inhibited methacholine and 5-HT (dose-ratios=2·0 and 3·0 ; n=3 and 4 respectively), but did not displace the isoprenaline dose-response curve.

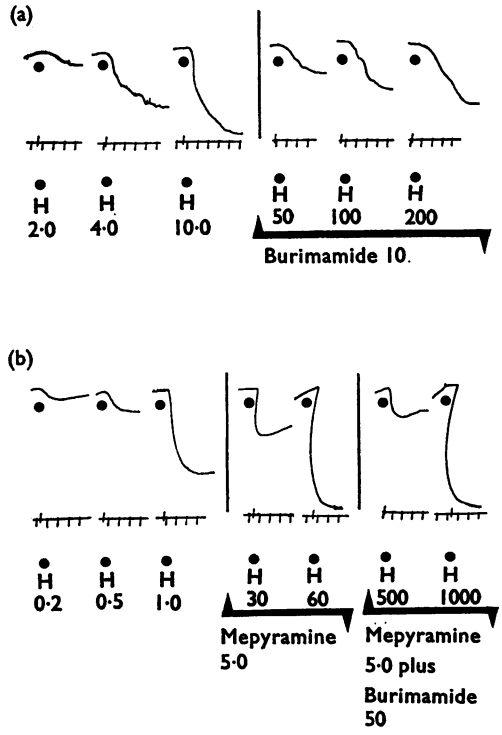

FIG. 1. (a) Isolated helical strip of sheep bronchus and (b) chain of six cat tracheal rings, each in 20 ml Krebs solution bubbled with 5% CO_2 in oxygen at 35° C. Each preparation is partially contracted (50–70%) by methacholine prior to the addition of various concentrations of histamine (H). The marker shows injection time and minutes. The antagonists mepyramine or burimamide are present between the arrows. All drug concentrations are μM.

Propranolol (1·0 μM) inhibited isoprenaline (dose-ratio>100 ; $n=3$) but did not diminish the inhibitory effect of histamine.

Cat trachea. Histamine and isoprenaline each caused dose-dependent inhibition of the partially-contracted preparation. Either mepyramine (5·0 μM) or burimamide (50 μM) administered separately produced partial inhibition of histamine (dose-ratios 100 and 20 respectively ; $n=2$). However, when both mepyramine (5·0 μM) and burimamide (50 μM) were added simultaneously (Fig. 1b), histamine relaxations were effectively abolished (dose-ratio=1,000 ; $n=2$). Neither mepyramine nor burimamide in the concentrations used, inhibited methacholine or isoprenaline. In the presence of propranolol (5·0 μM), relaxations due to histamine were reduced only slightly (dose-ratio <5) whereas propranolol markedly inhibited the effects of isoprenaline (dose-ratio >200 ; $n=2$).

Discussion.—The specificity of burimamide as an inhibitor of histamine H_2-receptors seems to be established (Black *et al.*, 1972 ; Wyllie, Hasselbo & Black, 1972). Therefore the fact that the relaxant action of histamine on sheep bronchi, which is totally mepyramine-resistant, is abolished by burimamide suggests that terminal airway smooth muscle of sheep possesses histamine H_2-receptors. That burimamide is a specific inhibitor was shown by the fact that this compound failed to inhibit methacholine, 5-HT or isoprenaline in this tissue.

Although the predominant action of histamine on sheep bronchi is relaxation, a minor secondary constrictor effect may be postulated since mepyramine potentiated slightly the histamine-induced relaxation, and in the presence of high concentrations of burimamide, histamine caused a small bronchial contraction.

An investigation of the actions of hist-

amine in other ungulate (hooved) species: calf (Eyre, 1970), goat, horse and pig (unpublished) has so far revealed no similar findings. In these other species, histamine caused mepyramine-sensitive bronchial contractions which indicates the presence exclusively of H_1-receptors.

With respect to the cat trachea, the suggestion of Maengwyn-Davies (1968) may require modification in the light of analysis using burimamide. That mepyramine and burimamide each partially counteracts histamine, suggests a dual histamine receptor mechanism involving H_2-receptors as well as H_1-receptors shown by Maengwyn-Davies. The β-adrenoceptor antagonist propranolol (5 μM) strongly inhibited isoprenaline but weakly inhibited histamine. Concentrations of propranolol greater than 10 μM blocked histamine relaxations more effectively but also inhibited unspecifically the responses to 5-HT and methacholine. It was further shown that burimamide in the concentrations tested did not displace the isoprenaline dose-effect curve of the tissue.

It seems reasonable to conclude that the cat trachea possesses H_1- and H_2-histamine receptors, both of which mediate relaxation of the tissue. In contrast to Maengwyn-Davies' conclusion, it appears from the present data that excitation of β-adrenoceptors (either by direct action or by virtue of catecholamine release) seems to be less important. It is however possible that catecholamine release by histamine could be mediated via H_2-receptors only, thus offering another explanation for the mepyramine-resistant action of histamine being blocked by burimamide, and fitting Maengwyn-Davies' observation that the effects of histamine were significantly reduced by pronethalol and also in tracheal preparations from which catecholamines had been depleted by pre-treatment with reserpine. It seems not unreasonable to postulate a triple mode of action of histamine on the cat trachea.

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REFERENCES

ASH, A. S. F. & SCHILD, H. O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmac. Chemother.*, **27**, 427–439.

ASHFORD, C. A., HELLER, H. & SMART, G. A. (1949). The effects of antihistamine substances on gastric secretion. *Br. J. Pharmac. Chemother.*, **4**, 153–161.

BLACK, J. W., DUNCAN, W. A. M., DURANT, C. J., GANELLIN, C. R. & PARSONS, E. M. (1972). Definition and antagonism of histamine H_2 receptors. *Nature*, **236**, 385–390.

DUTTA, N. K. (1949). Some pharmacological properties common to antihistamine compounds. *Br. J. Pharmac. Chemother.*, **4**, 281–289.

EYRE, P. (1969). The pharmacology of sheep tracheobronchial muscle—a relaxant effect of histamine on the isolated bronchi. *Br. J. Pharmac.*, **36**, 409–417.

EYRE, P. (1970). The Schultz-Dale reaction in bovine pulmonary smooth muscles. *Br. J. Pharmac.*, **40**, 166P.

GADDUM, J. H., HAMEED, K. A., HATHWAY, D. E. & STEPHENS, F. F. (1955). Quantitative studies of antagonists for 5-hydroxytryptamine. *Q. J. exp. Physiol.*, **40**, 49–74.

MAENGWYN-DAVIES, G. D. (1968). The dual mode of action of histamine in the cat isolated tracheal chain. *J. Pharm. Pharmac.*, **20**, 572–573.

TRENDELENBURG, U. (1960). The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. *J. Pharmac. exp. Ther.*, **130**, 450–460.

WYLLIE, J. H., HASSELBO, T. & BLACK, J. W. (1972). Effects in man of histamine H_2-receptor blockade by burimamide. *Lancet*, **2**, 1117–1120.

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