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Effect of drug-induced tone on the ability of histamine H_2 receptor agonists to relax guinea-pig tracheal chain

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Histamine causes bronchoconstriction in vivo and vitro by stimulation of H_1-receptors. Recently it has been suggested that histamine also stimulates H_2-receptors which mediate relaxation of the guinea-pig tracheobronchial smooth muscle, the evidence being that H_2-receptor antagonists increase the responsiveness of guinea-pig tracheobronchial smooth muscle to histamine (Okpako et al 1978; Drazen et al 1979; Duncan et al 1980). However, the use of a selective H_2-receptor agonist, dimaprit, has resulted in conflicting reports. Using tracheal spirals with drug-induced tone, Drazen et al (1979) were able to demonstrate bronchorelaxation to dimaprit, whereas Duncan et al (1980) were not able to do so.

This report compares the ability of isoprenaline, dimaprit and impromidine (the last two being selective H_2-receptor agonists) to relax the isolated tracheal chain preparation which exhibits spontaneous tone, and to demonstrate that the degree of tone induced in the tissue could account for the conflicting results stated above. As it is possible to take rings from either end of the trachea, a comparison of the effects of the above agents on the upper and lower portions of the trachea was also carried out.

Male Dunkin-Hartley guinea-pigs (250–450 g) were killed by cervical dislocation. The complete trachea was removed and cleaned of connective tissue. Four rings each consisting of two cartilage segments were cut from each end of the trachea. The rings were tied together so that the smooth muscle sections were on the same side of the chain (Chahl & O'Donnell 1967). The tracheal chain was mounted in a 10 ml isolated organ bath containing Krebs bicarbonate solution at 37 °C with ascorbic acid (1 mM), gassed with 95% O_2, 5% CO_2. Tissues were allowed to equilibrate for 1–1½ h, until a constant baseline was achieved, under a resting load of 150 mg. Cumulative dose-response curves to isoprenaline, dimaprit and impromidine were recorded at 90 min intervals using Ugo Basile 7006 Isotonic Transducers and a two-channel flat-bed recorder. All responses to relaxant drugs were expressed as a percentage of the maximum response to isoprenaline (10^{-5} M) under each of the three conditions. Results are a mean of four experiments for each drug under each condition. pD_2 values were derived from concentration-effect curves and are defined as the negative logarithm of the molar EC50 value. The EC50 value was obtained by using a least squares linear regression analysis. Comparison of the pD_2 values was made using Student's t-test for unpaired data.

Table 1 shows that there is no significant difference between the upper and lower portions of the trachea for either isoprenaline or dimaprit.

With the spontaneous tone preparation, dimaprit achieves nearly maximum relaxation of the tissue when compared to isoprenaline. However, it is 10^4 times less potent than isoprenaline. Impromidine, a more potent selective H_2 agonist (Parsons et al 1977; Durant et al 1978), does not achieve 50% of the maximum response to isoprenaline. It is surprising that impromidine is such a weak H_2-receptor agonist in the guinea-pig trachea when in other tissues involving H_2-receptor stimulation, e.g. rat atrium, rat uterus or rat gastric acid secretion (Parsons et al 1977; Durant et al 1978), it is 70–80 times more potent than dimaprit.

In comparison to the spontaneous tone preparation, increasing the tone of the tissue with carbachol (10^{-7} M) to between 8–15% of the maximum obtainable with carbachol does not significantly alter the pD_2 values to isoprenaline or dimaprit with the exception of dimaprit at the upper end of the trachea. However, when the concentration of carbachol

Table 1. The effect of increasing tone on the pD_2 values to isoprenaline, dimaprit and impromidine on tissues taken from the upper and lower portions of the guinea-pig trachea.

	Isoprenaline	Dimaprit	Impromidine
Spontaneous tone			
Upper trachea			
pD_2	8·68 ± 0·18 (4)	4·49 ± 0·03 (4)	Max. response at $9·6 \times 10^{-5}$ M (4)
Max. response	100%	85·8 ± 6·1	36·75 ± 7·3
Lower trachea			
pD_2	8·65 ± 0·06 (4)	4·7 ± 0·11 (4)	Max. response at $9·6 \times 10^{-5}$ M (4)
Max. response	100%	91·5 ± 3·6	29·5 ± 3·8
Carbachol (10^{-7}M)			
Upper trachea			
pD_2	8·33 ± 0·05 (4)	3·89 ± 0·1* (4)	
Max. response	100%	75·8 ± 9·5	
Lower trachea			
pD_2	8·45 ± 0·07 (4)	4·03 ± 0·16 (6)	
Max. response	100%	78·2 ± 12·5	
Carbachol (10^{-5} M)			
Upper trachea			
pD_2	7·1 ± 0·04** (4)	Max response at 5×10^{-4} M (4)	
Max. response	100%	4·5 ± 2·7	
Lower trachea			
pD_2	7·29 ± 0·05** (4)	Max. response at 5×10^{-4} M (4)	
Max. response	100%	7·5 ± 4·4	

pD_2 and maximum response values include ± s.e.m.
* $P < 0·05$
** $P < 0·001$ when compared with respective spontaneous tone pD_2 value.
( ) = Number of experiments.

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is increased (10^{-5} M) to give between 75–90% of the maximum tone, the sensitivity of the trachea to isoprenaline is significantly reduced and the ability of dimaprit to induce relaxation is virtually abolished.

Duncan et al (1980) induced maximal contraction of the tracheal spiral with acetylcholine, and were unable to relax it with dimaprit. They concluded that there were no H_2 relaxant receptors in the guinea-pig airway smooth muscle. Drazen et al (1979) induced contractions of the tracheal spiral which were 60–80% of the maximum obtainable with histamine, using either 2-(2-pyridyl)-ethylamine (2-PEA), a selective H_1 agonist, or carbachol. The tissue relaxed in response to dimaprit, suggesting a presence of H_2 receptors. These differing results can be explained in terms of the degree of tone induced in the tissue, i.e. functional antagonism. The greater the tone induced, the less likely will dimaprit cause relaxation of the tissue. The results from this work also indicate that the receptor reserve of H_2-receptors is significantly less than that of β-adrenoceptors.

In conclusion, in order to demonstrate that a compound has broncho-relaxant properties it is preferable to use a preparation which exhibits spontaneous tone rather than induced tone, since this may significantly alter the conclusions reached.

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α-Adrenoceptor activity of flutonidine (ST 600) in rat anococcygeus muscle and rabbit jejunum

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Imidazoline compounds are known to stimulate both α-adrenoceptors and histaminergic receptors (Sanders et al 1975; Kobinger & Pichler 1975; Schmitt 1977; Kobinger 1978). Mujic & van Rossum (1965) reported some imidazolines, e.g. naphazoline and tetrahydrozoline, caused relaxations in rabbit intestinal smooth muscles. They further reported that the antihypertensive effect of naphazoline and tetrahydrozoline in cats was mediated through direct stimulation of central α-adrenoceptors. Clonidine, another imidazoline compound is a centrally acting antihypertensive drug the actions of which are mediated through central α-adrenoceptors (Schmitt & Schmitt 1969; Schmitt et al 1973; Finch 1974).

Flutonidine, ST 600 [2-(5 fluoro-*o*-toluidine)-2 imidazoline HCl], a potent antihypertensive drug (Kho et al 1975), acts as a stimulant of central presynaptic and postsynaptic α-adrenoceptors, for its antihypertensive activity (Kho et al 1975; Marmo et al 1976, 1978). The cardiac effects of imidazolines like clonidine and tolazoline in guinea-pig hearts are reported to be mediated through histamine H_2 receptors (Csongrady & Kobinger 1974; Yellin et al 1975a, 1975b; Verma & McNeill 1977a, 1977b). In an earlier investigation, we reported the positive inotropic effects of ST 600 in guinea-pig isolated hearts were mediated via stimulation of histamine H_2 receptors (Veeranjaneyulu & Verma 1979). We have now examined ST 600 for its adrenoceptor activity in the rat anococcygeus muscle and rabbit jejunum. The anococcygeus muscle has a dense adrenergic innervation but apparently no cholinergic innervation (Gillespie 1972) and is a sensitive preparation for the study of the pre and post synaptic actions of α-adrenoceptor agonists and antagonists (Leighton et al 1979).

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Methods

Male rats (300–400 g) were killed by a sharp blow to the head and bled. The two anococcygeus muscles were prepared as described by Gillespie (1972). Each preparation was mounted in a 30 ml organ bath containing modified Krebs solution (composition mM: NaCl 116; KCl 5·4; $CaCl_2$ 2·5; NaH_2PO_4 1·2; $MgCl_2$ 1·2; $NaHCO_3$ 22·00 and glucose 11·00). The bathing solution was bubbled with 95% O_2 + 5% CO_2 and maintained at 37 ± 1 °C, at a pH 7·3. Cumulative dose responses were recorded on smoked drum using an isotonic frontal writing lever with 10 fold magnification. The muscles were maintained under a resting tension of 1 g. The preparation was stabilized for 30 min before the addition of any drug. Paired preparations were mounted at the same time, one preparation serving as control. The preparations were repeatedly washed at intervals of 10 min. Contact times for agonists and antagonists were 45 s and 15 min respectively. In another set of experiments rats were pretreated with reserpine (5 mg kg^{-1}