The inducement of tone and its inhibition in isolated tracheal muscle

We have recently described a preparation of the isolated intact trachea of the guinea-pig which can be used to assess the activity of bronchodilator drugs by determining their abilities to antagonize the temporary rise in intraluminal pressure induced by electrical stimulation (Farmer & Coleman, 1970). Subsequently we investigated the effect of various drugs on the resting intraluminal pressure in the non-electrically stimulated intact trachea preparation. The preparation set up, as described by Farmer & Coleman (1970), has an intraluminal pressure equal to or slightly above atmospheric pressure. Acetylcholine (0·1–30 μg/ml) and histamine (1–100 μg/ml) caused graded increases in intraluminal pressure of 1–25 mm Hg, and typical dose-response curves were obtained for these spasmogens. pA_2 values for atropine against acetylcholine and mepyramine against histamine were determined by the method of Arunlakshana & Schild (1959). Increasing doses of atropine caused successive shifts to the right of the acetylcholine dose-response curve and a pA_2 (30 min) of 8·46 $\pm$ 0·768 (n = 3) was obtained. A pA_2 value for mepyramine could not be obtained as the shifts in the histamine dose-response curves were not parallel. This is surprising as the antagonism of histamine by mepyramine on the guinea-pig ileum was shown by Arunlakshana & Schild (1959) to be competitive.

The small residual intraluminal pressure of the intact trachea preparation is reduced by β-adrenoceptor stimulants and doses producing maximal responses lowered the intraluminal pressure to just below atmospheric pressure. On washing, the intraluminal pressure rose to its previous level in about 10 min. The responses to β-stimulants were reproducible but pressure changes involved were too small to allow satisfactory quantitative evaluation. These results are similar to those previously described by Jamieson (1962), Wellens (1966) and Guirgis (1969). However, we have found that a suitably high intraluminal pressure could be developed if the tracheal lumen was momentarily exposed to atmospheric pressure immediately after the β-stimulant was washed from the bath. The tone then recovered quickly but to a level higher than before.

Repetition of this procedure resulted in successive increases in the level of the intraluminal pressure until equilibrium was reached usually after the fourth or fifth cycle. At this time the intraluminal pressure varied from 14–22 mm Hg and sometimes exceeded 30 mm Hg. Very occasionally the intraluminal pressure failed to

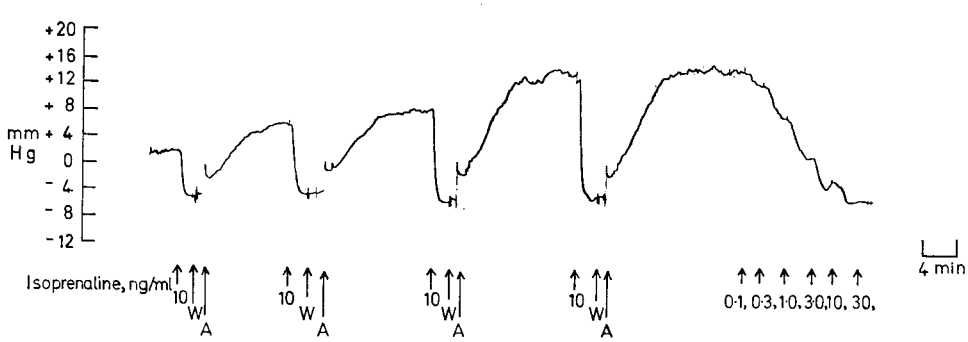

FIG. 1. The development of intraluminal pressure in the trachea by repeated dosing with isoprenaline and exposure to atmospheric pressure. The effects of graded doses of isoprenaline on the developed pressure are also illustrated. (W) wash, (A) exposure to atmospheric pressure.

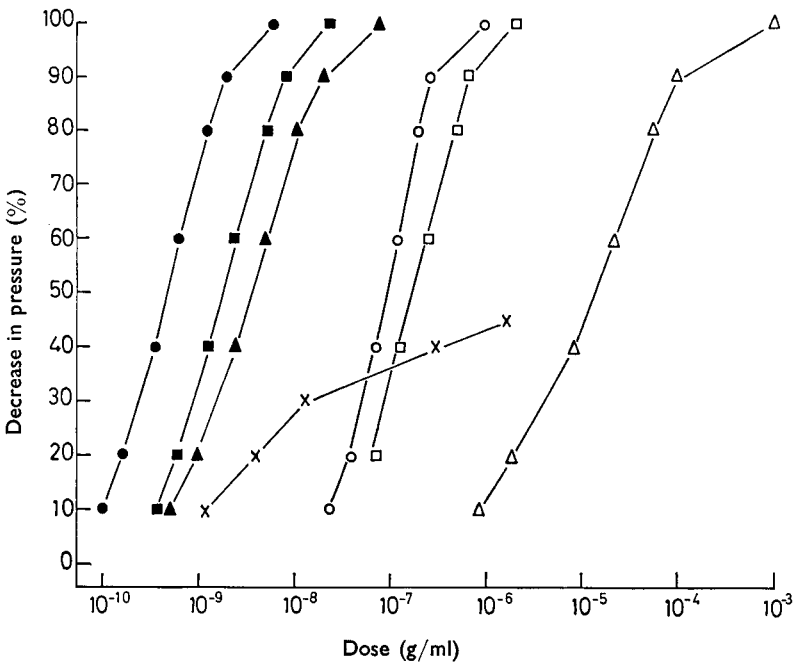

FIG. 2. Cumulative dose-response curves for a variety of agents with spasmolytic activity (●—●) isoprenaline, (■—■) salbutamol, (▲—▲) adrenaline, (○—○) noradrenaline, (□—□) papaverine, (△—△) choline theophyllinate and (×—×) atropine.

develop above 10 mm Hg and the tissue was discarded. The effects of various antagonists on the developed intraluminal pressure were studied in order to characterize this response. Atropine (1–3 μg/ml) partially reversed the developed intraluminal pressure (45–50%) while mepyramine (10 μg/ml) and BOL (10 μg/ml) had no significant effect. The developed pressure is thus due to both cholinergic and other unidentified components. Reduction of the developed intraluminal pressure now provided a suitable means for assessing the actions of bronchodilator drugs. The development of high intraluminal pressure following exposure to isoprenaline is illustrated in Fig. 1.

Cumulative dose-response curves for the spasmolytic actions of isoprenaline, adrenaline, noradrenaline, salbutamol, choline theophyllinate, papaverine and atropine are illustrated in Fig. 2. Order of potency was isoprenaline > salbutamol > adrenaline >> noradrenaline > papavarine >> choline theophyllinate; atropine caused only partial reversal of intraluminal pressure and could not be included.

The technique of inducing high intraluminal pressure in the intact trachea preparation and its subsequent reduction by spasmolytic agents offers a major advantage over all previously described intact trachea preparations. Furthermore, the procedure used did not alter the sensitivity of the preparation to spasmolytic agents when compared to those of the isolated tracheal chain and spiral preparations. The advantages of the intact trachea preparation over tracheal chain or spiral preparations are two fold. Firstly the intact trachea preparation is far easier and quicker to set up and the response to, and recovery from, the actions of spasmolytic agents is much shorter than those found using the chain or spiral preparations. It is concluded that the preparation described offers a simple, quick and sensitive means for detecting and evaluating the spasmolytic actions of bronchodilator drugs.

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Atropine-resistance of the urinary bladder innervation

The vertebrate urinary bladder is provided with a parasympathetic excitatory innervation. However, while the excitatory effects of acetylcholine on the bladder muscle are strongly antagonized by atropine or hyoscine, the nerve-mediated responses persist with only slight reduction in amplitude (Langley & Anderson, 1895). This evidence has led Henderson & Roepke (1934) and Ambache & Zar (1970) to argue that the excitatory innervation of the bladder is, at least in part, not cholinergic. Other workers have maintained that the innervation is solely cholinergic and have put forward apparently credible theories to explain the inability of muscarinic antagonists to prevent neuromuscular transmission. There are two primary conditions under which muscarinic antagonists such as atropine would not prevent cholinergic transmission to the bladder muscle. First, the receptors specifically occupied by acetylcholine released from nerves could be physically inaccessible to atropine. Second, atropine may reach the receptors but be unable to prevent acetylcholine from occupying the receptors.

There is no evidence to support the suggestion of Carpenter & Rand (1965) that the acetylcholine receptors in the neuromuscular junctions of the bladder are inaccessible to atropine.

Electron microscopic studies have not revealed the existence of any barriers isolating nerve-muscle complexes from the remaining extracellular space (Caesar, Edwards & Ruska, 1957; Thaemert, 1963; Nagasawa & Mito, 1967). In fact the relation between axons and smooth muscle cells in the bladder is similar to the arrangement found in the adrenergically-innervated vas deferens (Merrillees, 1968), yet neuromuscular transmission in the vas deferens is susceptible to blockade by competitive antagonists of α-adrenergic actions (e.g. Boyd, Chang & Rand, 1960). There is therefore no reason to believe that atropine cannot similarly reach all cholinergic receptors in the urinary bladder.

Since atropine is evidently able to penetrate into the neuromuscular junction, its inability to prevent acetylcholine from occupying the receptors indicates that either atropine is displaced from the receptors competitively by high local concentrations of acetylcholine (Huković, Rand & Vanov, 1965) or atropine cannot occupy the cholinergic receptors, i.e. they are not muscarinic. The suggestion that acetylcholine displaces atropine from muscarinic receptors competitively requires that either the amount of acetylcholine released is greater or the width of the synaptic cleft is smaller