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A new preparation of the isolated intact trachea of the guinea-pig

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A preparation is described in which the guinea-pig isolated intact trachea is subjected to repeated transmural electrical stimulation with alternating square wave pulses. The intraluminal pressure is continuously sensed by a pressure transducer. An increase in the intraluminal pressure is the predominant response to electrical stimulation. This response is prevented by low concentrations of atropine. After the initial rise a small fall in intraluminal pressure occurs; this is prevented by low concentrations of propranolol. The sensitivity of the preparation to various drugs is described.

Various preparations of guinea-pig isolated tracheal smooth muscle are used for the assessment of bronchodilator activity of drugs. The trachea may be cut into rings which are sewn together (Castillo & de Beer, 1947), or the rings severed through the cartilage and subsequently joined (Akcasu, 1959). Alternatively the trachea may be cut spirally to form a continuous preparation. Contractions or relaxations of these tissues may be measured isometrically or isotonically. Intact isolated tracheas have been used (Jamieson, 1962) and volume changes in response to transmural stimulation were studied by Foster (1964) and Carlyle (1964). Wellens (1966) also described an intact trachea preparation in which the effects of drugs on resting intraluminal pressure were measured by a pressure transducer.

β-Adrenoreceptor agonists cause relaxation of tracheal smooth muscle but quantitative determination of this activity is not easy because tracheal muscle preparations possess little intrinsic tone. This paper describes a preparation in which the isolated intact trachea is subjected to repeated transmural electrical stimulation with alternating square wave pulses to induce regular contraction of the tissue. The intraluminal pressure is continuously sensed by a pressure transducer.

EXPERIMENTAL

Methods and materials

Guinea-pigs were killed by a blow on the head and the trachea excised. By dividing the trachea halfway along its length, two preparations could be made from one animal. Each portion was mounted on the apparatus illustrated in Fig. 1 by tying the ends over blocks D and E. The physiological salt solution was in contact with the inner and outer surface of the trachea, although there was no direct contact between these fluids. It had the following composition in g/litre: NaCl, 8·0; $NaHCO_3$, 1·0; NaH_2PO_4, 0·32; glucose, 1·0; $MgCl_2$, 0·42; KCl, 0·2; $CaCl_2$, 0·4. The bath temperature was maintained at 37° and the solution gassed with air.

The apparatus consisted of a Perspex rod (A) 10 cm long, 6 mm diameter, on which was attached a foot (B) 1·5 cm long by 3 mm depth. Mounted on rod A was an adjustable collar (C) with clamping screw. Each end of the trachea was

tied to mounting blocks (D and E). The mounting block (D) was drilled to give two outlets, one for measurement of intraluminal pressure, the other for removal of air from the system. Platinum wire electrodes were mounted as shown and connected to an electronic stimulator by shielded copper wire let into the sides of rod A. Square wave alternating pulses of 1 ms duration and supramaximal voltage were applied to the tissue for varying durations (usually 7 s) at any given frequency.

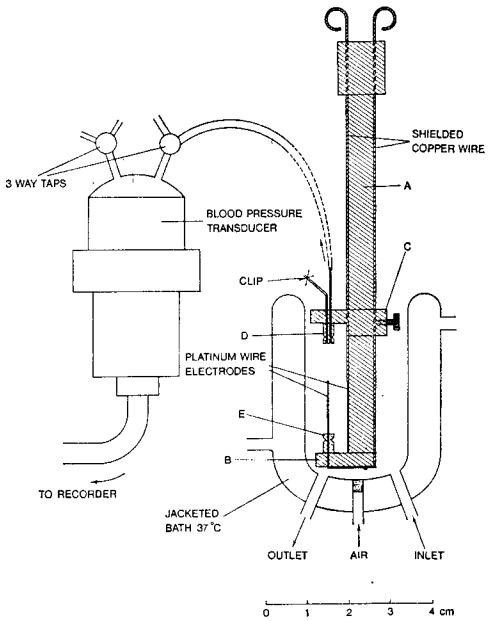

FIG. 1. Diagram of apparatus used to record pressure changes in the guinea-pig trachea induced by transmural electrical stimulation. A: Perspex rod. B: foot. C: adjustable collar. D and E: mounting blocks for trachea.

The following drugs were used: atropine methonitrate (BDH), isoprenaline sulphate (Burroughs Wellcome), adrenaline bitartrate (BDH), noradrenaline bitartrate (Bayer), papaverine hydrochloride (BDH), oxymetazoline hydrochloride (A & H), choline theophyllinate (A & H), phenylephrine hydrochloride (A & H), hemicholinium-3 (Aldrich), hexamethonium iodide (Koch-Light).

RESULTS

Nature of response. The application of the electrical stimulus to the tissue resulted in a rapid increase in intraluminal pressure followed by a decrease and a slow return to normal (Fig. 2a). The rise in pressure could be antagonized by atropine 10 ng/ml and the decrease in pressure by propranolol 50 ng/ml (Fig. 2a, b). A complete block in the rise but not the fall in intraluminal pressure was obtained with 10 μg/ml hemicholinium-3 which was not reversed by choline 400 μg/ml but was reversed by repeated washing of the preparation. Neither the rise nor the fall in intraluminal pressure was reduced by hexamethonium 10 μg/ml. With regular stimulation (20 Hz every 1 min) the resting intraluminal pressure became negative with respect to atmospheric pressure. If lower stimulation rates were applied in

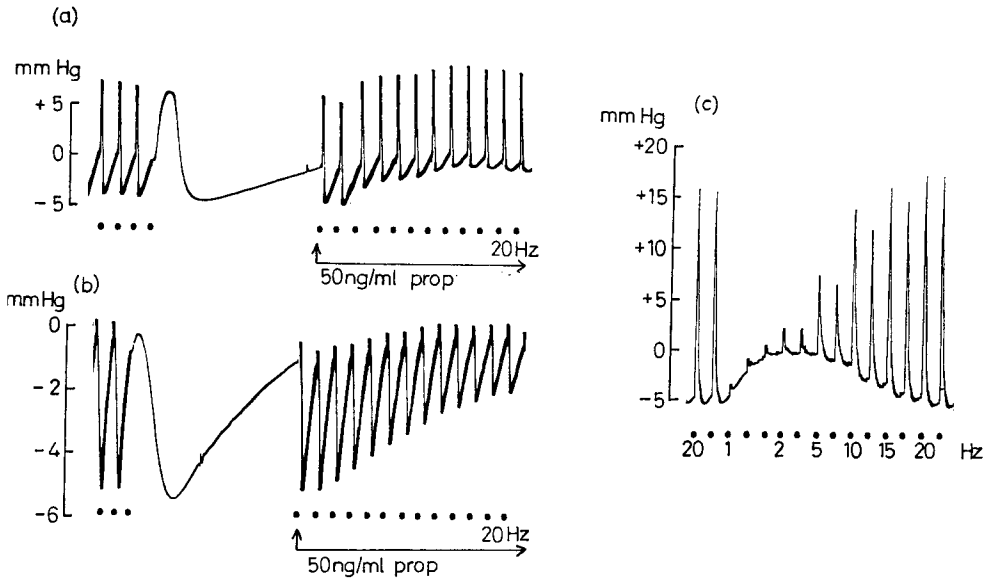

FIG. 2. Typical responses of the isolated intact trachea to transmural electrical stimulation. (a) Repeated stimulations at 20 Hz every 1 min, expanded part of record shows clearly the two components of the response. The decrease in intraluminal pressure is blocked by propranolol 50 ng/ml (prop). (b) Responses to stimulation at 20 Hz after the addition of 10 ng/ml atropine to the bath. Only a relaxation of the tissue is observed. This could be antagonized by propranolol 50 ng/ml. The sensitivity of the recording system was increased to demonstrate clearly the relaxation of the tissue. (c) Responses to stimulation at frequencies of 1, 2, 5, 10 and 20 Hz. Note the tendency of the resting intraluminal pressure to return to zero when lower rates of stimulation are employed. All drugs applied to outer surface.

order to show the relations between frequency and response (Fig. 2c), or stimulation was withheld, the resting intraluminal pressure returned to atmospheric pressure.

Effects of drugs on the rise in intraluminal pressure. The rise in intraluminal pressure could be prevented by drugs possessing anticholinergic, sympathomimetic or spasmolytic activity. Cumulative dose-effect curves for the inhibitory action of atropine, isoprenaline, adrenaline, noradrenaline, papaverine, oxymetazoline, choline theophyllinate and phenylephrine on the rise in intraluminal pressure are shown in Fig. 3. The inhibitory effect of isoprenaline was prevented by propranolol. Cumulative dose-effect curves were obtained for isoprenaline before and after the addition of 1, 5 and 25 ng/ml of propranolol. The dose-effect curve for isoprenaline took 50–60 min to prepare and was started 15 min after the addition of propranolol. The pA_2 value (contact time 45 min) was determined by the method of Arunlakshana & Schild (1959). The mean value $\pm$s.e. for three determinations was $8{\cdot}59 \pm 0{\cdot}78$.

DISCUSSION

A preparation of airway smooth muscle was required for the rapid evaluation of β-adrenoreceptor agonists. Most preparations to date have disadvantages due to the method of measurement or lack of intrinsic tone, or both. The whole trachea preparation described can be subjected to repeated periods of stimulation at selected frequencies by use of an automated gating device on the stimulator, and the intraluminal pressure continuously sensed and recorded. It was found necessary to use

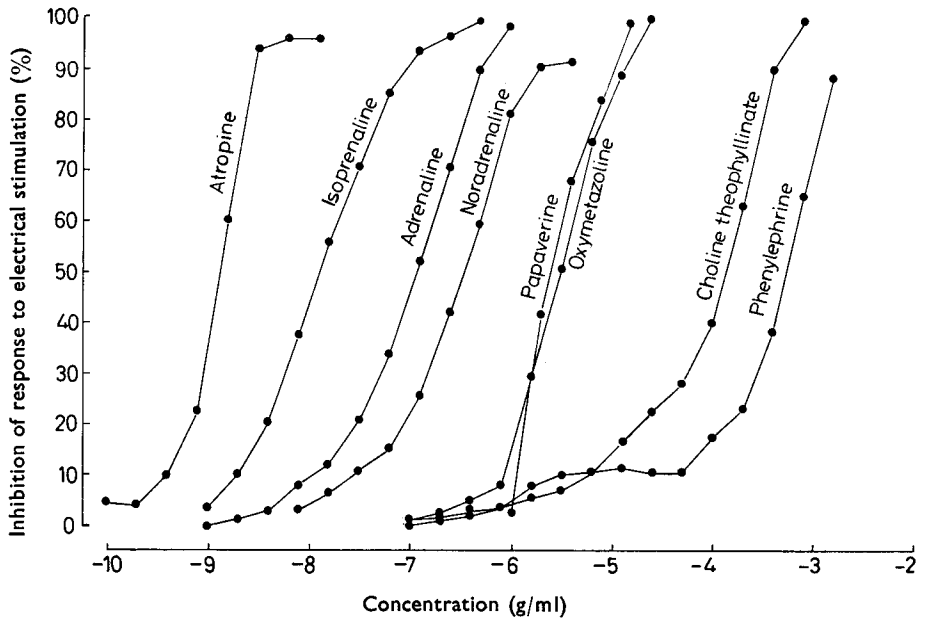

FIG. 3. Dose-effect curves for the inhibitory actions of drugs on the rise in intraluminal pressure induced by transmural stimulation of the isolated trachea. Frequency of stimulation 20 Hz. Drugs applied to the outer surface.

alternating square wave pulses since monophasic pulses resulted in gas production at the electrode surfaces. The gas production on the intraluminal electrode caused an increase in intraluminal pressure and deterioration of the preparation. The preparation gave a rise and then a fall in intraluminal pressure in response to stimulation. The rise in intraluminal pressure was usually very much greater than the subsequent fall. In some preparations no fall in intraluminal pressure was observed. The preparation of whole trachea of the guinea-pig as described by Foster (1964) and Carlyle (1964) gave a small contraction superceded by a rapid relaxation in response to electrical stimulation. These workers used a narrower pulse width for stimulation than was used here, which may have been a contributing factor. The pulse width of 1 ms used in the present preparation was found to be optimum for a contractile response. Lower pulse width resulted in a smaller contraction whilst greater pulse width did not increase the size of the contraction.

The contraction was due to excitation of postganglionic parasympathetic nerves since the response was blocked by atropine and hemicholinium-3 but not by hexamethonium. Similarly, the relaxation was due to stimulation of postganglionic sympathetic nerves since the response was reduced by propranolol and not by hexamethonium or hemicholinium-3. The preparation showed the expected sensitivity to a variety of drugs known to inhibit contractile responses of tracheobronchial smooth muscle. Of particular interest was the quantitative aspects of the interaction of propranolol with isoprenaline on the β-adrenoreceptors in this tissue. The trachea contains β-2 type receptors (Lands, Arnold & others, 1967) and propranolol gave a pA_2 value of 8·59 against isoprenaline on these receptors. Blinks (1967) gave a pA_2 value of 8·8 for propranolol against isoprenaline on the

β-1 type receptor the isolated driven left atria of the guinea-pig. Thus propranolol can be used to demonstrate the presence of β-adrenoreceptors in a tissue but it does not differentiate between β-1 type and β-2 type receptors.

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