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A note on the electrically transmurally stimulated isolated trachea of the guinea-pig

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The isolated trachea of the guinea-pig was stimulated electrically transmurally to give a biphasic response. The initial contraction was abolished by atropine and was rapidly succeeded by relaxation which was blocked by low concentrations of dichloroisoprenaline. The relaxation was also blocked by procaine, guanethidine, or by previous treatment of the animal with reserpine, but not with hexamethonium. It was concluded that the effects of transmural stimulation were mediated by post-ganglionic adrenergic nerves innervating beta receptors.

THE paired tracheal chain preparation from the guinea-pig (Foster, 1960) is useful in mode of action experiments with sympathomimetic drugs. Applied to the interaction between guanethidine or bretylium and catecholamines, it was seen that the potentiation slowly increased and reached a maximum only after about 3 hr. In an effort to see whether adrenergic neurone blockade by these drugs developed with the same time course in this tissue, the transmurally stimulated isolated tracheal preparation was developed. A description of this preparation was communicated to the joint meeting of the British and Scandinavian Pharmacological Societies, Copenhagen, 1960.

Methods

The apparatus in which the preparation was used is shown in Fig. 1.

The trachea was removed from a 600g guinea-pig which had been stunned and bled. A cannula was tied into each end of the trachea and a long platinum wire electrode was passed up through the lower cannula and tracheal lumen until its end lay in the upper cannula. The whole was fitted into a tissue bath containing Krebs solution at 38° bubbled with 95% oxygen and 5 % carbon dioxide. The lower cannula passed through a rubber sleeve, making a water-tight joint with the bottom of the bath, to a reservoir of Krebs solution. The upper cannula was connected to a graduated capillary tube through a three-way tap fitted with a short outlet tube. A second platinum electrode lay in the bath opposite the tracheal muscle.

The trachea was perfused with Krebs solution to wash out its lumen before being connected to the capillary tube of 1 mm internal diameter.

The fluid meniscus could be set at any starting position by opening the inlet from the reservoir and adjusting its height.

Any change in length of the tracheal muscle fibres produced a change in the volume of Krebs solution within the trachea and thus in the position of the meniscus in the graduated capillary tube.

Transmural stimulation was effected by a rectangular wave stimulator delivering pulses of 0·1 msec and 60 V for 30 sec; 3 pulses/sec was the

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frequency usually used. Stimuli were applied every 10 min and readings of the meniscus position were made at 2 min intervals or less. Three min after each stimulus the fluid in the bath was displaced by a larger volume of Krebs solution.

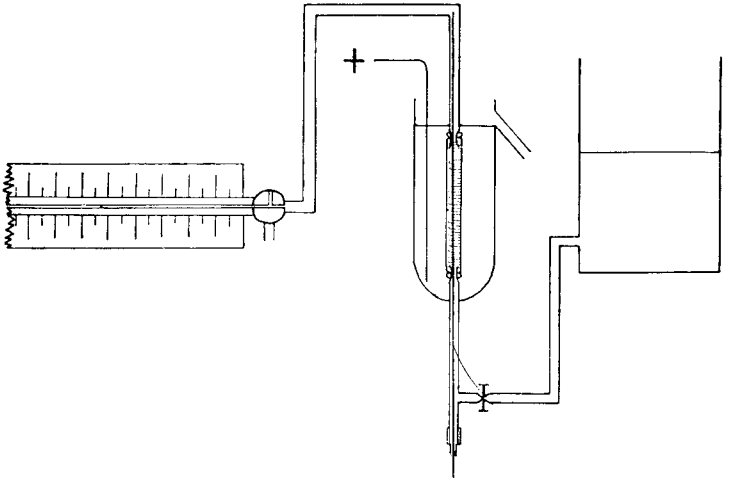

FIG. 1. The apparatus used to apply transmural stimulation to the tracheal muscle and record its effects. The apparatus for heating the tissue bath, displacing the Krebs solution in it, bubbling it with oxygen and carbon dioxide and applying stimulation across the electrodes is not shown.

The height of the reservoir, its inlet clip to the tissue bath and the three-way tap can be operated to perfuse the tracheal lumen or to set the starting position of the meniscus.

Results

Response of intact trachea to electrical transmural stimulation. Stimulation produced a biphasic response, initial contraction of the tracheal muscle being rapidly superceded by relaxation. Atropine, 0·4 μg/ml abolished the initial contraction and, since the relaxant response was the object of study, the alkaloid was included in all Krebs solution which came into contact with the trachea.

Analysis of mechanism of relaxation produced by electrical transmural stimulation. The relaxant response to transmural stimulation was not affected by hexamethonium, 10 μg/ml. It was blocked by procaine, 400 μg/ml, or by cocaine, 100 μg/ml, and was not seen using a trachea from a guinea-pig treated with reserpine, 5 mg/kg intraperitoneally, on each of the two preceding days. Concentrations of cocaine less than 25 μg/ml, increased the response. It was abolished by dichloroisoprenaline, 2 μg/ml, or by dichloronoradrenaline, 100 μg/ml.

Effect of frequency of stimulation on relaxant response. Frequency: response curves, over the range 1 to 12 pulses per sec, were linear or gently concave to the frequency axis.

The effect of bretylium on the relaxant response. The effect of bretylium is shown in Fig. 2. After the last two of a series of constant submaximal

responses, bretylium was kept in the bath continuously. Equilibrium was reached in about 45 min after the addition of bretylium when only 11% of the original response remained. Bretylium, 10 μg/ml produced a complete blockade in 20 min.

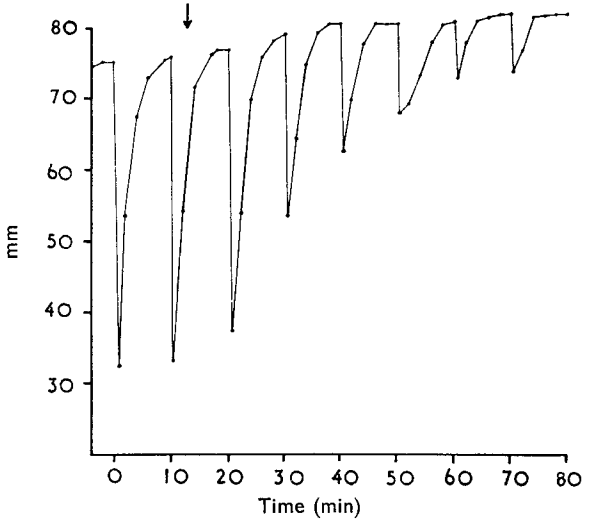

FIG. 2. The effect of bretylium, 2 μg/ml, kept in the bath after its addition at the arrow, on the response to transmural stimulation, applied at 10 min intervals, in the presence of atropine, 0·4 μg/ml. The scale position of the meniscus is plotted against time. Bretylium produces an increasing blockade of the relaxant response to stimulation.

The effect of guanethidine on the relaxant response and the interaction between cocaine and guanethidine. Fig. 3 shows the effects of concentration and time upon the blockade produced by guanethidine. The completeness of the blockade and also its rate of reaching equilibrium increased as the concentration of guanethidine was increased. Cocaine, 10 μg/ml in the presence of guanethidine 10 μg/ml altered the development of the blockade which was slower in onset but was no less complete than that achieved by guanethidine alone.

Discussion

The guinea-pig isolated trachea is a simple preparation which is rapidly set up and convenient to use.

Electrical transmural stimulation with the parameters used, in the presence of atropine produces relaxation. This effect is blocked by a low concentration of the β-blocking agent dichloroisoprenaline and by a far higher concentration of dichloronoradrenaline. The relaxation is therefore probably mediated mainly by β-adrenergic receptors. The blockade of the relaxant effect by local anaesthetic concentrations of cocaine and procaine suggests that the stimulation of nervous pathways is involved. This view

is confirmed by the blockade of the relaxant response by guanethidine, bretylium or by pretreatment of the guinea-pig with reserpine. The failure of hexamethonium to reduce the relaxant response suggests that the stimulation is effectively post ganglionic. The evidence strongly suggests that the effects of transmural stimulation were mediated by adrenergic nerves, apparently innervating β-adrenergic receptors.

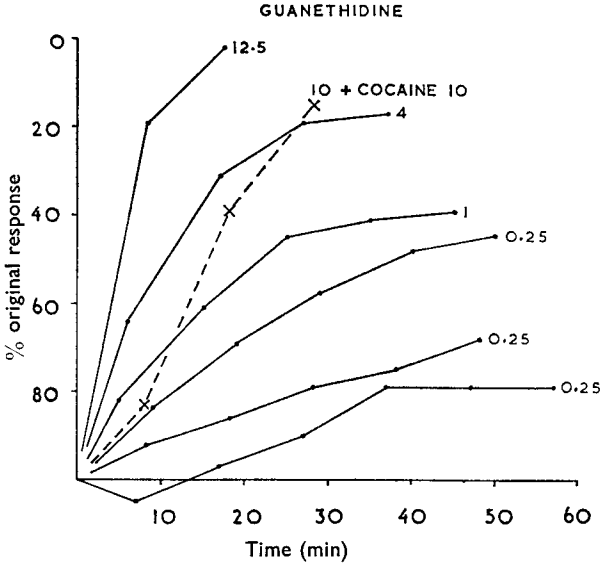

FIG. 3. The effect of guanethidine on the response to transmural stimulation in the presence of atropine, 0·4 μg/ml. The effect, as a percentage of original response to stimulation, is plotted against time of contact with guanethidine. Each line represents the result of a separate experiment and the figure opposite the end of each line denotes the concentration of guanethidine used in μg/ml. The broken line is the result obtained using guanethidine and cocaine in equal concentrations 10 μg/ml.

This preparation has proved a simple and convenient one for use when an isolated, adrenergic nerve, effector cell preparation is required.

Carlyle (1964) has adapted it to a study of cholinergic neuro-effector junctions of the tracheal muscle.

References

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