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An isolated parasympathetically-innervated oesophagus preparation from the chick

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An isolated parasympathetically-innervated preparation from the chick oesophagus is described. This preparation is essentially similar to mammalian parasympathetically-innervated smooth muscles in its responses to drugs, and because of its simplicity, robustness and cheapness it is recommended for use in student practical classes.

ISOLATED oesophagus preparations from the cat, the rabbit and the rat have been briefly described by Rabinovitch (1928), by Bain & McSwiney (1936) and by Hughes & McDowall (1954) respectively. Rand & Stafford (1964) have recently described in detail a study of the properties of isolated innervated oesophagus preparations from various species including that of the adult domestic fowl. While preparations from some species contain both striated and smooth muscle, those from the cat and the chicken contain only smooth muscle. This paper describes some pharmacological responses of the isolated innervated oesophagus of the chick which appears to possess several advantages as an experimental preparation over that taken from the adult bird.

Methods

Male chicks (Silver Link) aged between 1 and 7 days after hatching were starved overnight to empty the crop and then deeply anaesthetised or killed with ether. The upper oesophagus as far as the crop was removed together with as much as possible of one or of both parasympathetic nerve trunks. The oesophagus in the domestic fowl receives its parasympathetic innervation via branches from the vagus and glossopharyngeal nerves which run together along the course of the jugular veins on both sides (Rand & Stafford, 1964). Apart from about a 1 cm length at the free ends, the nerves were not separated from the jugular veins so that damage to the fine nerve branches passing to the oesophagus was avoided. The preparation was suspended in Krebs-Henseleit solution (NaCl 6·95, KCl 0·34, $CaCl_2$ 0·28, KH_2PO_4 0·162, $MgSO_4$ 0·294, $NaHCO_3$ 2·1, dextrose 2 g/litre) continuously gassed with 95% O_2 and 5% CO_2. The nerves were passed through stimulating electrodes of the type described by Burn & Rand (1960). Contractions of the oesophagus were recorded on smoked paper with an isotonic frontal writing lever loaded with 2 g and amplifying the contractions 6 times. The nerves were stimulated at various frequencies with rectangular pulses of 0·5 or 1 msec duration and of a strength such that the contraction for a given frequency was maximal. The body temperature of the chick is about 42° and in preliminary experiments the temperature of the bath

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solution was varied over the range 32–42°. Similar responses to nerve stimulation occurred throughout this range but at 32° spontaneous pendular movements were less pronounced and the preparation survived much longer. In all of the experiments reported, the bath temperature was maintained at 32°.

The drugs used were: acetylcholine chloride (Roche), physostigmine sulphate (BDH), nicotine hydrogen tartrate (BDH), tetramethylammonium bromide (BDH), dimethylphenyl piperazinium iodide (Light & Co.), hexamethonium bromide (May & Baker), mecamylamine (Merck, Sharpe & Dohme), pempidine tartrate (May & Baker), atropine sulphate (BDH),

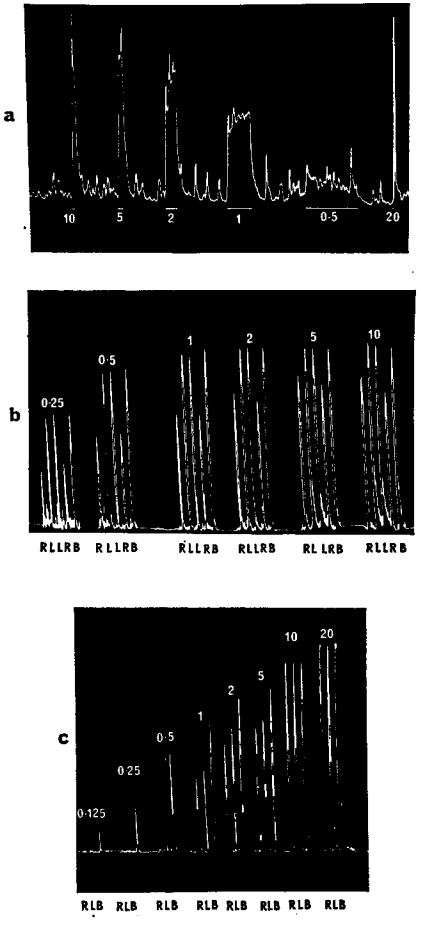

FIG. 1. a. Responses to a constant number of stimuli applied at different frequencies to the right nerve trunk. The numerals denote the frequency (stimuli/sec) applied for the periods marked by the horizontal lines. Each response is to 50 stimuli. b and c. Responses to stimulation of the right (R), the left (L) and both (B) nerve trunks for 10 sec at different frequencies denoted by the numerals above each group of responses. The responses marked B were produced by synchronous stimulation of both trunks in b and by asynchronous stimulation in c.

(−)-adrenaline (BDH), (−)-noradrenaline (Light & Co.), (−)-isopropylnoradrenaline bitartrate (Wyeth), phentolamine (Ciba), pronethalol (I.C.I.) and guanethidine sulphate (Ciba). The concentrations in the text refer to the bases.

Results

Stimulation of either nerve trunk caused contraction of the oesophagus. When the oesophagus was lying free in a petri dish, stimulation of the left nerve trunk caused it to curl to the left and stimulation of the right nerve trunk caused a similar movement in the opposite direction indicating that, as in the rabbit (Rand & Stafford, 1964), each nerve chiefly innervated the longitudinal muscle fibres on the corresponding side. The threshold frequency of stimulation in most preparations was 0·5 to 1/sec, but a few preparations responded to single shocks. The frequency required to produce maximal contractions varied in different preparations. In some it was as low as 2/sec but in most it was 10/sec. Fig. 1a illustrates responses, each to 50 stimuli applied to the right nerve trunk at different frequencies. Stimulation of the left nerve trunk produced slightly greater contractions than stimulation of the right (Fig. 1b and c). When both nerves were stimulated together and the stimuli to each nerve were synchronised (i.e. supplied by the same stimulator), the contractions produced were equal in size to those produced by stimulation of the left nerve alone (Fig. 1b). However, when both nerves were stimulated together but with the stimuli to each slightly out of phase, the contractions produced at low frequencies of stimulation were greater than those produced by stimulation of either nerve alone. For example, in the experiment illustrated in Fig. 1c, stimulation of both nerves at a frequency of 0·5/sec produced contractions similar in size to those produced by stimulation of the left nerve alone at a frequency of 2/sec. On the other hand, with a frequency of 20/sec, contractions of equal size were produced by stimulation of either nerve alone or of both together.

The right nerve trunk was easier to prepare than the left and in all the experiments in which the effects of drugs were studied, contractions of the oesophagus were elicited by stimulation of the right nerve at a frequency of 5 or 10/sec for 10 sec in every 2 min. With this pattern of stimulation comparable responses could be elicited for several hours. Regular stimulation of this type inhibited and usually completely abolished the spontaneous pendular movements of the oesophagus. On stopping the stimulation, the spontaneous activity usually slowly returned.

ACETYLCHOLINE, PHYSOSTIGMINE AND ATROPINE

Contraction of the oesophagus was produced by acetylcholine in concentrations of 0·025 μg/ml and above. The responses to acetylcholine and those to nerve stimulation were potentiated by physostigmine (0·05–0·1 μg/ml). Spasm of the oesophagus gradually developed about 10 min after the addition of physostigmine. Changing the bath fluid quickly restored the tone of the preparation to normal, but the potentiation of the responses to acetylcholine and to nerve stimulation persisted. Atropine

in a concentration of 0·01 μg/ml abolished the responses to acetylcholine and markedly depressed those to nerve stimulation. Larger concentrations of atropine (0·02 μg/ml and above) completely blocked the responses to nerve stimulation. Fig. 2 illustrates the effects of physostigmine and atropine on responses to acetylcholine and to nerve stimulation.

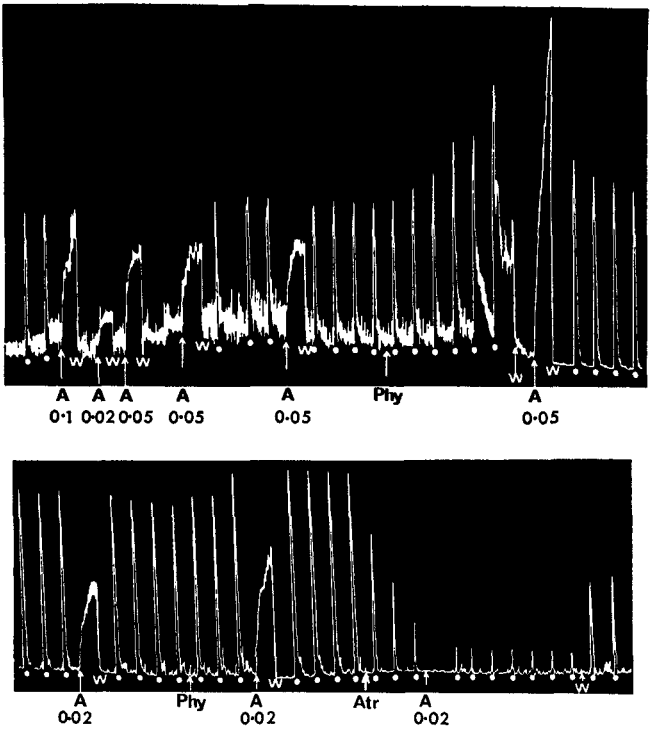

FIG. 2. Effects of acetylcholine, physostigmine and atropine. At the white dots, the right nerve trunk was stimulated at a frequency of 10/sec for 10 sec. Except when acetylcholine was added, the interval between stimulation periods was 2 min. At A, acetylcholine was added; the numerals denote the bath concentration in μg/ml. At Phy, physostigmine was added to give a bath concentration of 0·1 μg/ml in the upper record and 0·05 μg/ml in the lower. At Atr, atropine was added to give a bath concentration of 0·01 μg/ml. At W, the bath fluid was changed.

GANGLION STIMULANT AND BLOCKING DRUGS

The effects of nicotine, dimethylphenylpiperazinium (DMPP) and tetramethylammonium (TMA) were studied. In low concentrations (nicotine 2–5 μg/ml, DMPP, 0·5–1 μg/ml, TMA 2–4 μg/ml) all three enhanced or initiated rhythmic pendular movements and potentiated the responses to nerve stimulation. This effect of TMA is illustrated in Fig. 3a. Larger concentrations (nicotine 8–10 μg/ml, DMPP 2–4 μg/ml, TMA 4–6 μg/ml) produced an abrupt contraction of the oesophagus which often quickly waned before changing the bath fluid, especially with nicotine and DMPP (Fig. 3a).

The stimulant actions of nicotine, DMPP and TMA were abolished after the addition of hexamethonium (2–4 μg/ml), mecamylamine (0·5–1 μg/ml) or pempidine (0·5–1 μg/ml). The same doses of these ganglion blocking drugs produced a 50–80% depression of contractions evoked by nerve stimulation and in 6 out of 9 experiments additional amounts produced complete block. Fig. 3b illustrates the effect of pempidine on contractions

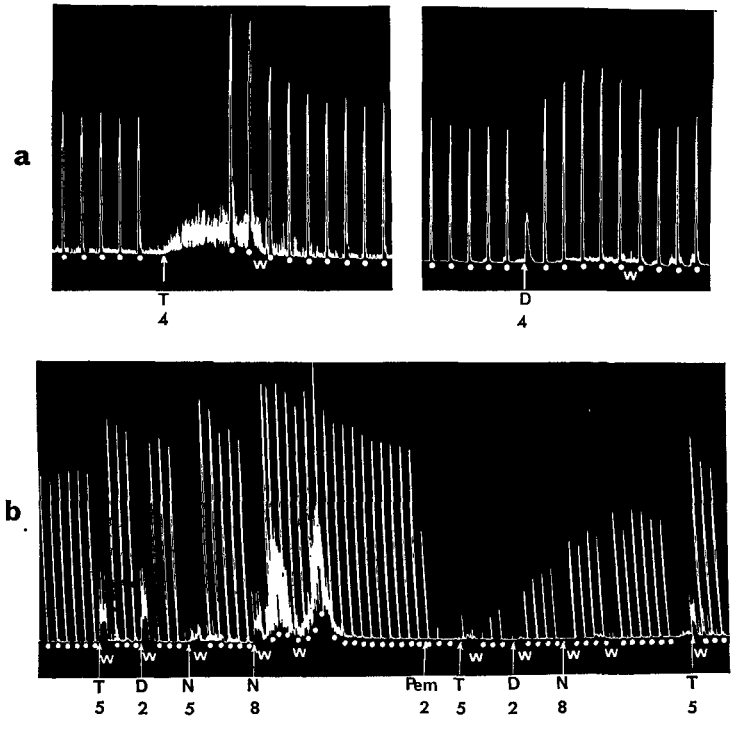

FIG. 3. Effect of ganglion stimulant and blocking drugs. At the white dots, the right nerve trunk was stimulated for 10 sec at a frequency of 5/sec in a and 10/sec in b. Except when ganglion stimulant drugs were added, the interval between stimulation periods was 2 min. At T, TMA, at D, DMPP, at N, nicotine and at Pem, pempidine was added to the bath. The numerals denote the bath concentrations in μg/ml. At W, the bath fluid was changed.

produced by nerve stimulation and by TMA, DMPP and nicotine. In two experiments with hexamethonium and in one with mecamylamine complete block of contractions evoked by nerve stimulation could not be obtained. In these 3 experiments the maximal degree of block was produced by 4 μg/ml hexamethonium and 1 μg/ml mecamylamine. Subsequent additions to a total concentration of 100 μg/ml of each drug did not produce a greater effect.

When added during partial block produced by a ganglion blocking agent, nicotine, TMA and DMPP restored the contractions evoked by nerve stimulation. The recovery from pempidine block illustrated in Fig. 3b was partly due to the addition of these ganglion stimulant drugs. In

other experiments, in which ganglion stimulant drugs were not added, recovery after pempidine was very slow despite frequent washings.

SYMPATHOMIMETIC AMINES AND ANTI-ADRENALINE DRUGS

(—)-Adrenaline (0·01–0·05 μg/ml), (—)-noradrenaline (0·02–0·1 μg/ml) and (—)-isopropylnoradrenaline (0·02–0·05 μg/ml) produced comparable inhibitions of the spontaneous pendular movements, when present, and of the contractions produced by nerve stimulation (Fig. 4, upper panels)

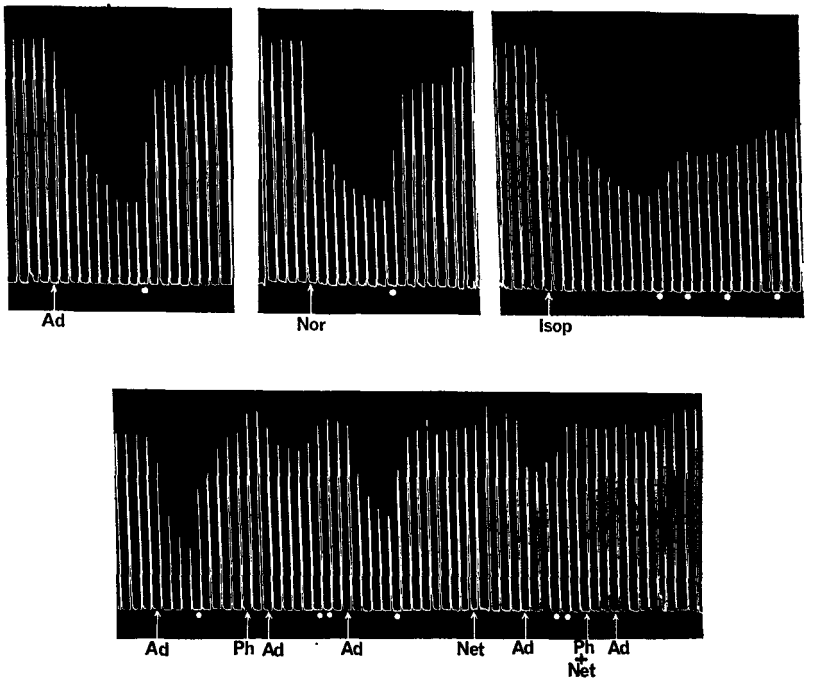

FIG. 4. Effects of sympathomimetic amines and anti-adrenaline drugs. Responses are to stimulation of the right nerve trunk (10/sec for 10 sec every 2 min). At Ad, (—)-adrenaline (0·05 μg/ml), at Nor, (—)-noradrenaline (0·1 μg/ml), at Isop, (—)-isopropylnoradrenaline (0·05 μg/ml), at Ph phentolamine (2·5 μg/ml) and at Net, pronethalol (1 μg/ml) were added to the bath. Phentolamine (2·5 μg/ml) and pronethalol (1 μg/ml) were added together before the last addition of adrenaline. At the white dots, the bath fluid was changed.

or by acetylcholine. In the same preparation, the depression of contractions produced by nerve stimulation was more marked the lower the frequency of stimulation. Contractions produced by acetylcholine and by nerve stimulation were reduced to a similar extent suggesting that the effect was due to inhibition of the smooth muscle rather than to a depression of ganglionic transmission of the type described by Marrazzi (1939). The relative potency of the three sympathomimetic amines varied in different experiments, but in most (—)-isopropylnoradrenaline and (—)-adrenaline were about equipotent and (—)-noradrenaline was about half

as active. The effects of adrenaline and noradrenaline were quickly removed by washing but that of isopropylnoradrenaline was persistent and full recovery was often not achieved despite repeated washing (Fig. 4, upper panels).

Phentolamine, in a concentration of 2·5 μg/ml. or pronethalol, in a concentration of 1 μg/ml, slightly potentiated the contractions produced by nerve stimulation and antagonised, but did not abolish the depressant effect of adrenaline added 4 to 10 min later (Fig. 4, lower panels). The addition of both anti-adrenaline drugs together in the above concentrations produced a greater antagonism of the adrenaline effect than either blocking drug added alone (Fig. 4, lower panels).

GUANETHIDINE

Guanethidine, in concentrations up to 2 μg/ml, was without effect on contractions of the oesophagus evoked by nerve stimulation. Larger amounts (up to 20 μg/ml) depressed the contractions.

Discussion

Rand & Stafford's (1964) results with the oesophagus of the adult fowl showed that it was relatively insensitive to drugs. In cases where the same drugs were used, we found concentrations 25–50 times smaller to be effective in preparations from the young chick. Rand & Stafford also found that preparations from the adult fowl responded slowly to drugs and their actions were reversed with difficulty. They attributed this to a tough connective tissue barrier which retarded the diffusion of drugs. The chick preparation, on the other hand, is relatively free of connective tissue and responds briskly to drugs which, in most instances, may be easily washed out.

The oesophagus receives its sympathetic innervation from the 1st thoracic segment. Had the point of stimulation of the nerve trunk been left *in situ*, it would have been craniad to the segment of oesophagus removed; it is unlikely therefore that the stimulated part of the nerve trunk contained any adrenergic inhibitory fibres. If such fibres had been present, however, blockade of their effects would have enhanced the responses to nerve stimulation; indeed a slight effect of this type was produced by the anti-adrenaline drugs. It is to be noted that the adrenergic neurone blocking drug, guanethidine, did not enhance the contractions, its only effect being to depress them in large doses. This result suggests that the only efferent fibres stimulated belonged to the parasympathetic division of the autonomic system. The slight enhancement of contractions by anti-adrenaline drugs may be attributed to their weak anticholinesterase action (Boyd, Chang & Rand, 1960), and the depression by large doses of guanethidine to its weak ganglion blocking action (Rand & Wilson, 1964).

The only unusual response from the preparation was the occasional inability of ganglion blocking drugs to block completely the contractions evoked by nerve stimulation. The resistant part of the contraction could

not be attributed to excitation of striated muscle fibres, since the time course of the responses, together with the fact that small concentrations of atropine always completely abolished them, demonstrated the absence of any contribution by such fibres. It is possible that in a few preparations, some fibres synapsed more centrally than the stimulating electrodes and that the stimulated nerve trunk therefore contained some post-ganglionic fibres. However, another possibility may also be considered. Martin & Pilar (1963a, b) have recently obtained evidence that at many of the synapses in the ciliary ganglion of the chick, transmission is mediated by electrical coupling between pre- and post-synaptic elements. Such ephaptic transmission is not susceptible to ganglion blocking agents and it may be that a similar transmission mechanism is occasionally present in some of the synapses of other parasympathetic ganglia in this species. Apart from this minor exception, the responses of the preparation to drugs were qualitatively similar to those expected from a parasympathetically-innervated mammalian smooth muscle preparation.

Several isolated sympathetically-innervated preparations are available for pharmacological investigations, for example, those of Finkleman 1930) and Huković (1961), but few tissues are easily isolated with their parasympathetic nerves. Because of its simplicity, robustness and cheapness, the isolated parasympathetically-innervated oesophagus of the chick provides a useful preparation, particularly for students' practical classes.

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