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STUDIES ON THE LONGITUDINAL MUSCLE OF THE ANTERIOR MESENTERIC ARTERY OF THE DOMESTIC FOWL

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SUMMARY

1. A simple, isolated preparation is described in which the activity of the longitudinal muscle of the anterior mesenteric artery of the domestic fowl is recorded isotonically.

2. The longitudinal muscle of the anterior mesenteric artery exhibited tone and marked spontaneous activity. Maximal shortening of this muscle was equal to about 60% of its resting length.

3. Acetylcholine in low concentrations produced powerful contractions of the muscle, whereas low concentrations of catecholamines produced relaxation by an action on β-receptors; much larger concentrations of adrenaline or noradrenaline contracted the muscle. A pharmacological analysis of the responses to electrical stimulation and to agonist drugs indicated that the muscle was supplied by excitatory, cholinergic nerves and by inhibitory, adrenergic nerves.

INTRODUCTION

Arterial smooth muscle in mammals is usually orientated circularly (Furchgott, 1955); hence, to study its properties in isolation it is necessary to use perfused segments (McGregor, 1965; de la Lande & Rand, 1965; Rogers, Atkinson & Long, 1966) or helical strips or rings (Furchgott & Bhadrakom, 1953; Furchgott, 1955). In the domestic fowl, however, Ball, Sautter & Katter (1963) have observed that the anterior mesenteric artery contains a well-developed longitudinal muscle layer located in its adventitia; a segment of the artery therefore may be used as a simple isolated preparation.

The experiments to be described in this paper were made to investigate

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the innervation of this longitudinal muscle. It will be shown that, unlike most mammalian arterial muscle which is innervated by excitatory, adrenergic nerves (e.g. see Keatinge, 1966), the longitudinal muscle of the anterior mesenteric artery (LMAMA) is supplied by excitatory, cholinergic nerves and by inhibitory, adrenergic nerves.

The work described in this paper formed part of a thesis approved for the degree of Doctor of Philosophy in the University of London. Some of the results have been briefly reported (Bolton, 1966*a*, *b*).

METHODS

Young domestic fowls were killed by dislocating their necks. The anterior mesenteric artery was severed at its origin from the aorta and at a point beyond the origins of several intestinal arteries. The mesentery at either side of the artery was cut and the segment so isolated removed. This segment was 3–7 cm long depending on the age of the fowl. The anterior mesenteric artery was anchored in an organ-bath by its aortic end and the other end was attached to an isotonic frontal writing lever magnifying 5 or 10 times and writing on smoked paper. In the illustrations the change in length of the artery is represented as a proportion of the resting length of the artery measured when under tension in the bath. Arteries were subjected to a load of 1 or 2 g depending on their diameter. Rectangular stimuli were applied to two annular electrodes which fitted loosely around the aortic end of the artery, close to its attachment in the bath. In all experiments, unless otherwise stated, 0·5 msec pulses of supramaximal strength were applied at supramaximal frequencies (10–20/sec) for periods of 5–20 sec with 3–8 min elapsing between periods of stimulation.

The solution in which the artery was immersed had the following composition (mM): NaCl, 118; KCl, 4·6; $CaCl_2$, 2·7; $MgCl_2$, 1·2; KH_2PO_4, 1·2; $NaHCO_3$, 25; glucose, 11. It was vigorously gassed with a mixture of 5 % carbon dioxide and 95 % oxygen and was maintained at between 41 and 42° C. The bath was washed by overflow.

The following drugs were used: (−)-adrenaline, acetylcholine chloride, bretylium tosylate, choline chloride, dihydroergotamine methanesulphonate, guanethidine sulphate, 4,4′biphenylene bis(2-oxoethylene)-bis(2-hydroxyethyl)-dimethyl ammonium bromide (hemicholinium), hexamethonium bromide, hyoscine hydrobromide, (−)-isoprenaline bitartrate, mecamylamine hydrochloride, nicotine hydrogen tartrate, (−)-noradrenaline bitartrate, pempidine tartrate, phentolamine hydrochloride, physostigmine salicylate, propranolol hydrochloride. The concentrations and doses of noradrenaline, adrenaline, and isoprenaline refer to the bases; those of other drugs refer to the above salts.

RESULTS

The LMAMA was studied in fowls of all ages, from chicks 2 days old to adult hens. Preparations from adult hens gave poor or sluggish responses to drugs and preparations from chicks less than about 2 weeks old gave poor responses to electrical stimulation and soon deteriorated. The best preparations came from chickens between 1 and 4 months old and these were used for most of the work.

There was considerable variation in the tone of different preparations, as judged by the size of the maximal relaxation produced by spasmolytic

agents, and in their spontaneous activity which consisted of rhythmic contractions. These took place at about 30 sec intervals in active preparations and each contraction consisted of several step-like components. Typical preparations, shortly after being set up, had moderate tone and marked spontaneous activity but both decreased with time, particularly in the first hour. During this time periods of spontaneous activity alternated with periods of relaxation (Fig. 1). Preparations with low (and some with moderate) tone were not spontaneously active. When high tone was induced with spasmogens, spontaneous contractions were small or absent.

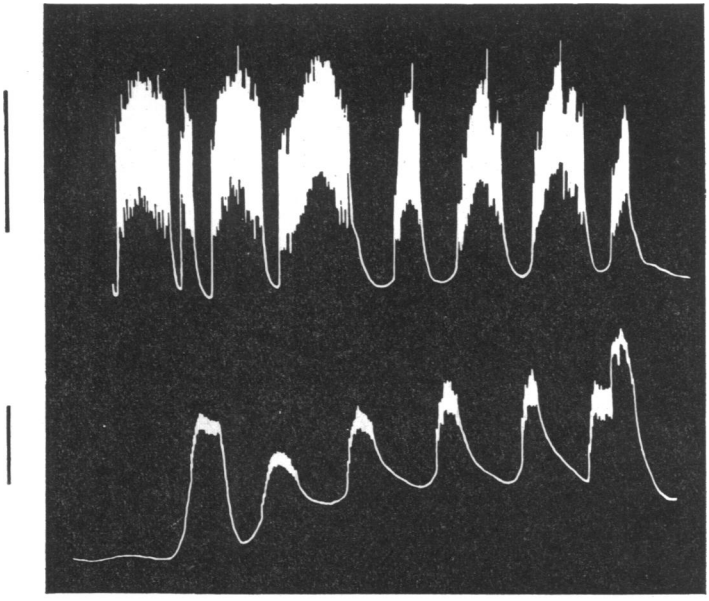

Fig. 1. Mechanical activity of the LMAMA recorded isotonically. Spontaneous activity alternating with periods of relaxation in an untreated preparation (upper tracing) and in a preparation to which physostigmine (50 ng/ml.) was added shortly after the beginning of the record (lower tracing).

In this and subsequent figures the vertical calibration represents a 10 % change in length of the muscle and the horizontal calibration a 10 min period unless otherwise stated.

Effects of electrical stimulation

As is shown in Fig. 2*a*, in low tone preparations electrical stimulation caused a contraction. When tone was raised by the addition of barium chloride (0·1–0·5 mg/ml.), electrical stimulation now caused a small contraction followed by a greater and longer-lasting relaxation. Similar responses were obtained when electrical stimulation was applied to untreated preparations with a moderate degree of tone. In general, the con-

tractions were less marked and the relaxations more marked, the greater the tone.

A likely explanation for these results is that the electrical stimuli were acting on both excitatory and inhibitory nerves. Further experiments were made therefore to test this possibility and to investigate the nature of the pathways and the transmitters which might be involved.

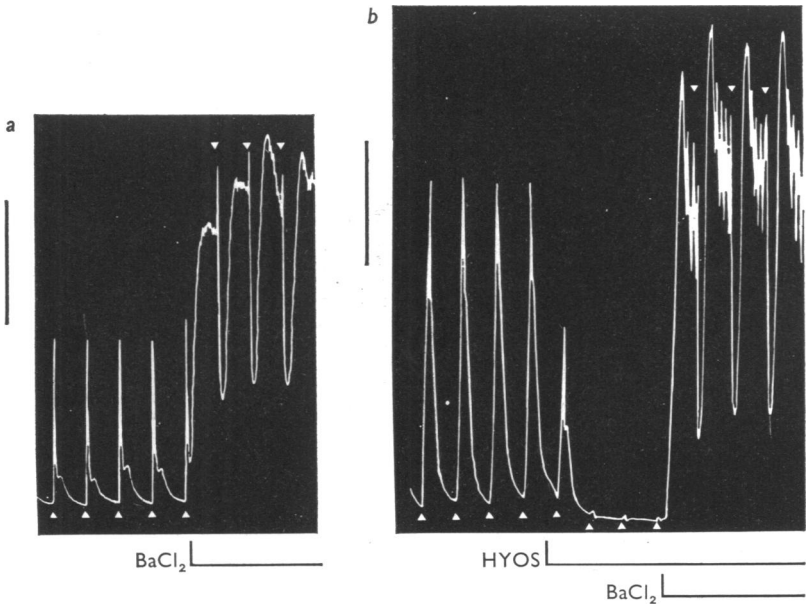

Fig. 2. The effects of electrical stimulation on the LMAMA (periods of stimulation indicated by the white triangles). (*a*) A low-tone preparation stimulated with 20 impulses/sec for 10 sec every 5 min 10 sec. At the bracket barium chloride (0·1 mg/ml.) was added. (*b*) A low-tone preparation stimulated with 10 impulses/sec for 20 sec every 5 min 20 sec. At the bracket hyoscine (HYOS, 10 ng/ml.) was added followed at the second bracket by barium chloride (0·1 mg/ml.) without washing out.

Excitatory pathway. It may be seen from Fig. 2*b* that after the addition of hyoscine (2 ng/ml. or more) the contractions in response to stimulation were virtually abolished but that relaxations were still obtained when the tone was raised with barium chloride. This suggests the existence of a cholinergic, excitatory pathway and a non-cholinergic, inhibitory pathway.

Further evidence in favour of a cholinergic excitatory pathway is the restoration of the hyoscine-blocked contractions by physostigmine in the continuing presence of hyoscine (Fig. 3) and the fact that acetylcholine itself (1 ng/ml. or more) contracted the muscle (Figs. 4, 5). Hemicholinium (0·5 μg/ml.), which can act prejunctionally to block cholinergic nerves (see

Schueler, 1960), virtually abolished the responses to electrical stimulation while the sensitivity to acetylcholine was scarcely altered. Choline (2 μg/ml.) reversed this blockade in the continuing presence of hemicholinium (Fig. 4).

The experiment illustrated in Fig. 5 indicates that the excitatory nerves activated by the electrical stimuli are preganglionic. Thus the contractions

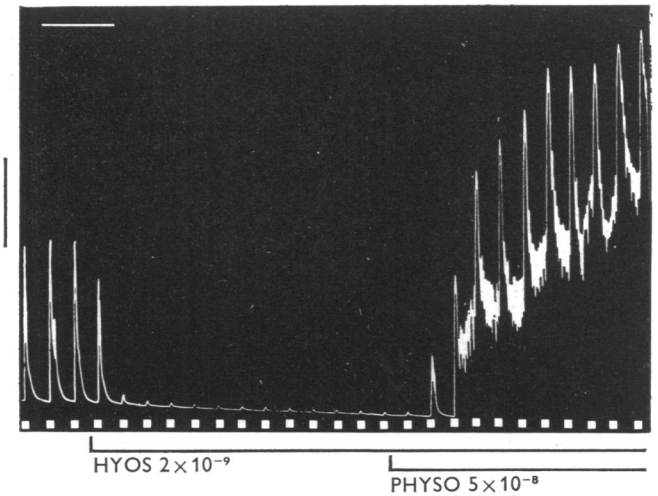

Fig. 3. The effects of 2 ng/ml. hyoscine (HYOS) on the responses of a low-tone preparation of the LMAMA to electrical stimulation (for 10 sec periods at the white squares). Physostigmine (PHYSO, 50 ng/ml.) was added without washing out the hyoscine. The preparation had been previously subjected to 0·5 μg/ml. propranolol for 1 hr. Electrical stimulation was of supramaximal strength and rate.

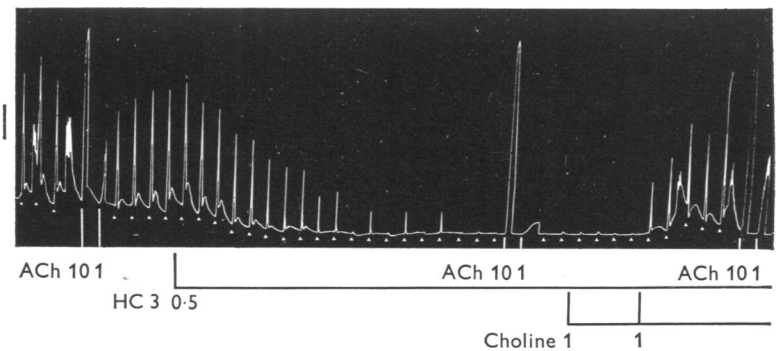

Fig. 4. The effect of hemicholinium on the responses of the LMAMA to electrical stimulation (at the white triangles) and acetylcholine (ACh, 10 and 1 ng/ml.). Hemicholinium (HC 3, 0·5 μg/ml.) was present for the period indicated by the bracket. Choline (initially 1 μg/ml., later increased to 2 μg/ml.) was added without washing out the hemicholinium. Electrical stimulation (for 10 sec every 5 min 10 sec) was of supramaximal strength and rate.

in response to electrical stimulation were abolished by hexamethonium in a concentration which did not affect the response to acetylcholine. Furthermore, contractions were obtained in response to nicotine and these too were abolished by ganglion blocking agents (Fig. 5).

Inhibitory pathway. As is shown in Fig. 6, relaxations could be obtained not only as a result of electrical stimulation but also in response to the catecholamines adrenaline, noradrenaline, and isoprenaline. The latter

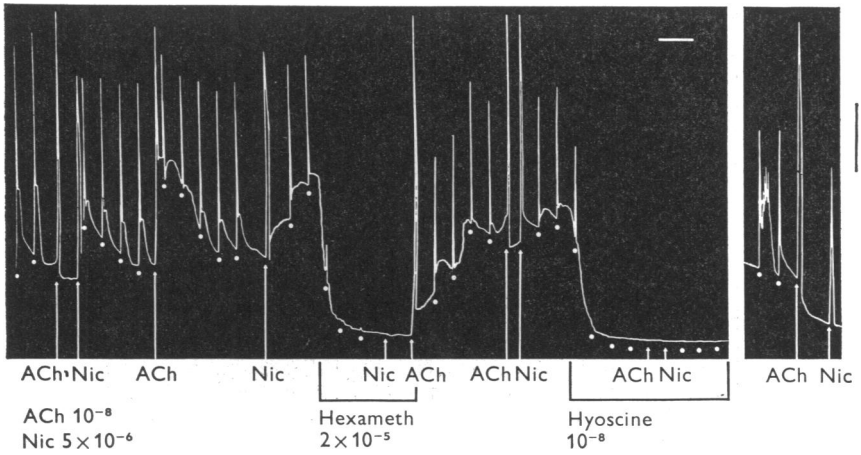

Fig. 5. The effects of hyoscine and hexamethonium upon the contractions of the LMAMA produced by electrical stimulation (for 10 sec periods at the dots), acetylcholine (ACh, 10 ng/ml.), and nicotine (Nic, 5 μg/ml.). Hexamethonium (Hexameth, 20 μg/ml.) and hyoscine (10 ng/ml.) were present for the periods indicated by the brackets. (The third dose of acetylcholine was added in the presence of hexamethonium.) Electrical stimulation was of supramaximal strength and rate. Stimulant drugs were left in the bath until the response reached a maximum and the drum was arrested while they were washed out. During the gap between the records the tissue was washed frequently over a period of about an hour.

drug was about 5 times more potent than adrenaline or noradrenaline, which were about equipotent. The relaxations in response to electrical stimulation or catecholamines were unaffected by phentolamine (1–5 μg/ml.) but abolished by propranolol (0·1–0·5 μg/ml.). This suggests that the inhibitory pathway is adrenergic and involves only β-receptors (Ahlquist, 1948). Larger concentrations (1 μg/ml. or more) of noradrenaline or adrenaline, but not isoprenaline, produced weak contractions of low tone muscle which were blocked by phentolamine (2 μg/ml.) or dihydroergotamine (2 μg/ml.).

As would be expected, the relaxations could also be abolished by the adrenergic neurone blocking agents bretylium or guanethidine in concentrations which did not affect the action of noradrenaline (Figs. 7, 8). The relaxations to electrical stimulation were scarcely affected by con-

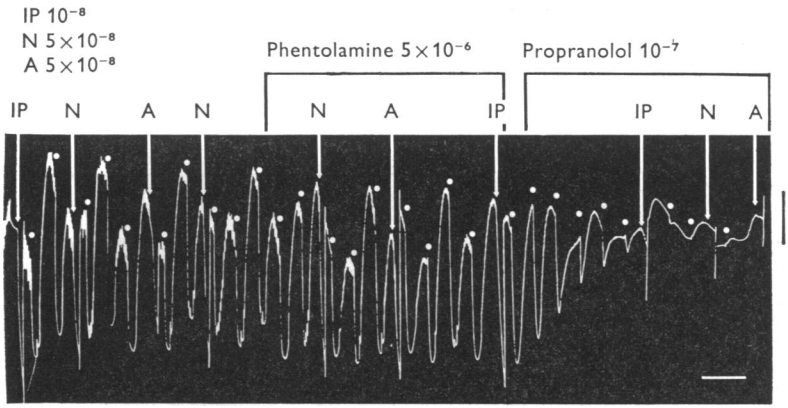

Fig. 6. A preparation of the LMAMA in which the tone has been raised by 0·2 mg/ml. barium chloride. Hyoscine (10 ng/ml.) is also present. Relaxations in response to electrical stimulation (for 10 sec periods at the dots), noradrenaline (N, 50 ng/ml.), adrenaline (A, 50 ng/ml.), isoprenaline (IP, 10 ng/ml.) are abolished by propranolol (0·1 μg/ml.) but not by phenotolamine (5 μg/ml.). Each dose of catecholamine was left in contact with the tissue for 90 sec and at the vertical line following the response the tissue was washed and the drum arrested until tone was regained. Electrical stimulation was of supramaximal strength and rate.

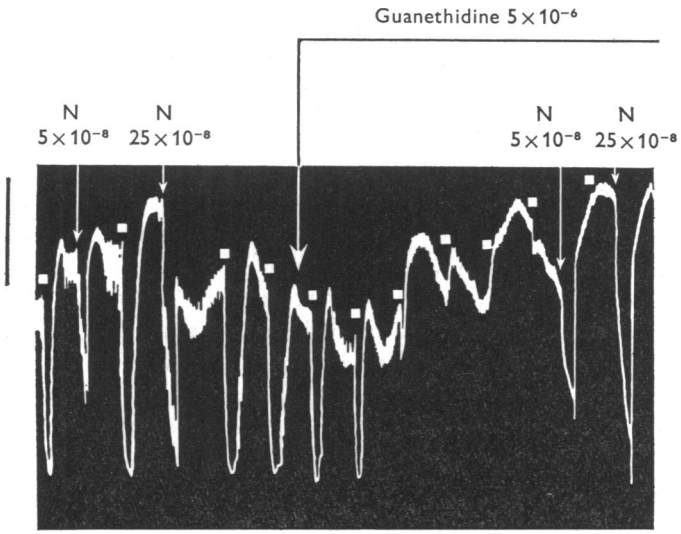

Fig. 7. A preparation of the LMAMA in which the tone has been raised with 0·2 mg/ml. barium chloride. Hyoscine (1 μg/ml.) is also present. Guanethidine (5 μg/ml.) blocks the relaxations in response to electrical stimulation (at the white squares) but does not affect the responses to noradrenaline (N, 50 and 250 ng/ml.). Noradrenaline was left in contact with the tissue for 90 sec and at the vertical line following the response the tissue was washed and the drum arrested until tone was regained. Electrical stimulation was applied for 20 sec every 5 min 20 sec and was of supramaximal strength and rate.

centrations of ganglion blocking agents (hexamethonium 200 μg/ml., pempidine 5 μg/ml., mecamylamine 2 μg/ml.) which abolished the contractions obtained with electrical stimulation (Fig. 8). There was thus no evidence that a ganglionic synapse was involved in the inhibitory pathway.

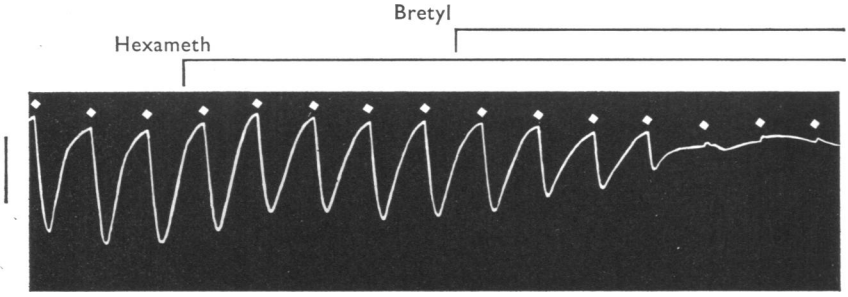

Fig. 8. A preparation of the LMAMA in which the tone has been raised with 0·1 mg/ml. barium chloride. Hyoscine (10 ng/ml.) is also present. Hexamethonium (Hexameth, 0·2 mg/ml.) only slightly reduces the relaxations in response to electrical stimulation (at the white diamonds) whereas bretylium (Bretyl, 10 μg/ml.) abolishes them. Electrical stimulation was applied for 20 sec periods every 8 min 20 sec and was of supramaximal strength and rate.

DISCUSSION

The responses of the LMAMA to electrical stimulation are best explained by assuming that the electrical stimuli excited preganglionically an excitatory, cholinergic nerve supply and simultaneously excited postganglionically an inhibitory, adrenergic nerve supply. Although this pattern of innervation is usual for non-vascular smooth muscle, it has hitherto not been observed for any vascular muscle other than the LMAMA of the domestic fowl.

The smooth muscle of *large* blood vessels is contracted by acetylcholine and by adrenaline or noradrenaline. Adrenaline and noradrenaline stimulate both α- and β-receptors but their action on the former type normally predominantes producing contraction (Furchgott, 1955). The LMAMA was interesting as it was at least 10–100 times more sensitive to acetylcholine than circular muscle of large blood vessels from either the mammal (Furchgott & Bhadrakom, 1953; Sutter, 1965) or the fowl (Bolton, 1967). Furthermore, the predominant effect of noradrenaline or adrenaline was on the β-receptors of the LMAMA (causing relaxation) and the effect of α-receptor stimulation (contraction) was only demonstrable by increasing the concentration about 100-fold. Unlike the LMAMA, circular smooth muscle from large blood vessels is usually not spontaneously active (Furchgott, 1955; Keatinge, 1966) and maximal shortening represents only about 20 % of its resting length (Furchgott & Bhadrakom, 1953); in

the LMAMA, maximal shortening probably exceeded 60% of the resting length (Fig. 4).

In view of its dual innervation and the marked changes in length it can undergo, it is possible that this longitudinal muscle has some specialized and unusual function. Longitudinal arterial muscle is a rarity, not being found in the other main arteries of the fowl (Ball *et al.* 1963; Bolton, 1967), nor is it generally encountered in mammalian arteries with the exceptions of the coronary and pulmonary arteries (Furchgott, 1955). Longitudinal muscle is however found in the anterior mesenteric artery of the turkey (Ball *et al.* 1963). It is possible that the LMAMA can change the blood pressure gradient along the artery and thus alter the blood flow through the intestines, its dual innervation enabling the rate of flow to be controlled by influences acting through the C.N.S.

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