J. Physiol. (1968), **196**, *pp.* 283–292
With 7 text-figures
Printed in Great Britain

ELECTRICAL AND MECHANICAL ACTIVITY OF THE LONGITUDINAL MUSCLE OF THE ANTERIOR MESENTERIC ARTERY OF THE DOMESTIC FOWL

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(*Received* 11 *July* 1967)

SUMMARY

1. The electrical activity and changes in tension of the longitudinal muscle of the anterior mesenteric artery (LMAMA) of the domestic fowl were recorded simultaneously using the sucrose-gap method.

2. Spontaneous activity consisted of recurring contractions each accompanied by a burst of action potentials.

3. In quiescent preparations, brief electrical stimuli, acetylcholine, or barium chloride produced contractions with the appearance of action potentials. Larger concentrations of barium chloride or acetylcholine produced depolarization and action potentials ceased although contraction was maintained. Whenever depolarization without action potentials occurred, it was associated with a smooth contraction, whereas action potentials were always accompanied by small rapid contractions superimposed upon the main contraction.

4. When the tone was raised with barium chloride (and in the presence of hyoscine) continuous action potentials occurred; under these circumstances brief electrical stimuli or noradrenaline produced relaxation, cessation of action potentials, and hyperpolarization.

INTRODUCTION

While the electrophysiology of visceral smooth muscle has been well studied (see Burnstock, Holman & Prosser, 1963, for a review) vascular smooth muscle has been relatively neglected. The discovery by Ball, Sautter & Katter (1963) that the anterior mesenteric artery of the domestic fowl possesses a well-developed longitudinal muscle layer located in the

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adventitia suggested the possibility of recording simultaneously electrical and mechanical activity in this muscle by using the sucrose-gap method.

In the preceding paper (Bolton, 1968) it was concluded that the responses to brief electrical stimuli resulted not from direct actions on the muscle, but from the stimulation of excitatory and inhibitory nerves. The aim of the present work is to record these responses to brief electrical stimuli and also those to neuromimetic drugs.

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).

METHODS

A 3–4 cm segment of the anterior mesenteric artery from 2- to 3-month-old chickens was set-up in the conventional sucrose-gap apparatus (Stämpfli, 1954; Burnstock & Straub, 1958). A thread was attached to a transducer for isometric tension recording. The transducer consisted of a RCA 5734 valve in a housing similar to that described by Talbot, Lilienthal, Beser & Renolds (1951). The output from this valve was displayed on one beam of a dual-beam oscilloscope. The electrical activity of the tissue was recorded using a pair of conventional silver–silver chloride electrodes via the usual cathode follower stage, and displayed on the other beam of the oscilloscope.

One side of the apparatus was perfused with potassium sulphate (17·8 g/l.) and the other with physiological saline of 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. This was gassed with 5% carbon dioxide and 95% oxygen. A temperature gradient existed in the vertical tube containing the active portion of the tissue; using a thermistor probe the temperature was found to rise from about 39° C at the top of the tube to 43° C at the bottom. The central compartment was perfused with 10% (w/v) de-ionized sucrose solution.

Annular platinum electrodes were set in the vertical tube containing the active portion of the preparation. An electronic stimulator (Model S4G, Grass Medical Instruments) was used to supply 0·5 msec pulses which were first passed through the stimulus isolation unit (available with this stimulator) set for monophasic stimuli. The stimuli used were of supramaximal strength. The part of the artery nearest the recording electrode in the sucrose-gap was more proximal *in situ* (with respect to the heart) than the end attached to the transducer. The doses of noradrenaline refer to the base and those of acetylcholine refer to the chloride salt. Drugs were given by injection in a small volume into the physiological saline or this was changed to one containing the drug.

RESULTS

Action potentials were usually simple spikes up to 8 mV in size. Each was usually followed by a hyperpolarization and as this decayed it merged into the slow-rising phase (prepotential) of the next spike (Fig. 1). If action potentials appeared abruptly, then the first one often did not show much evidence of a prepotential. Sometimes, however, small oscillations of the electrical record became bigger and hence action potentials were formed (Fig. 1*b*).

Spontaneous activity. This consisted of contractions which occurred at about 30 sec intervals. These large contractions consisted of several smaller contractions each of which was accompanied by an action potential. Between contractions there was electrical quiescence (Fig. 1*a*). In occa-

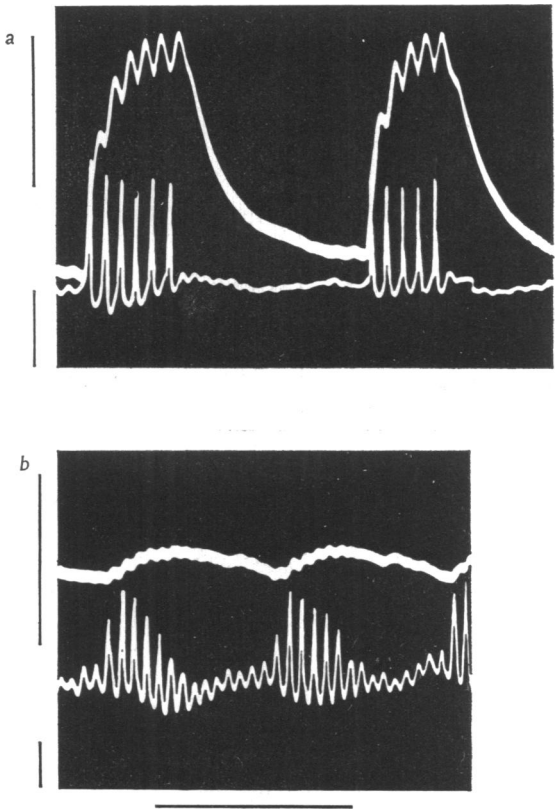

Fig. 1. Electrical activity and isometric tension changes recorded by the sucrose-gap method in two different preparations of the LMAMA. (*a*) Typical spontaneous activity. (*b*) Spontaneous activity of a type less frequently seen.

In this and subsequent figures the upper record is of the isometric tension and the lower record of the electrical activity, and the vertical calibrations by each panel are 1 g (upper) and 1 mV (lower) unless otherwise stated. The horizontal calibration is 20 sec, in this figure only. The action potentials have been retouched.

sional preparations these smaller contractions waxed and waned in size accompanied by parallel changes in the size of the electrical oscillations (Fig. 1*b*).

Excitation

Barium chloride. In quiescent preparations barium chloride (0·05–0·2 mg/ml.) produced a contraction. Either action potentials began immedi-

ately (Fig. 2*a*) or they were preceded by a depolarization. The latter was associated with a smooth rise in tension from which rapid small contractions were absent. Action potentials were always accompanied by rapid small contractions, and as depolarization occurred both became more frequent and diminished (Fig. 2*a*). Switching to physiological saline not containing barium chloride produced reverse changes and the initial contraction subsided, but several contractions of shorter duration followed.

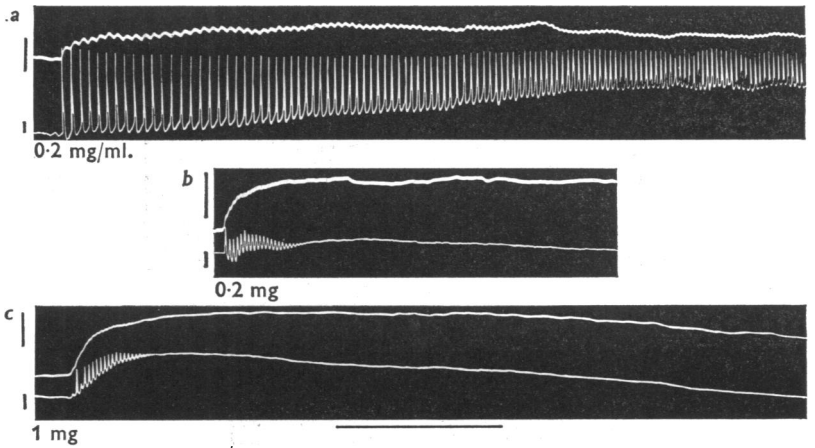

Fig. 2. The effects of barium chloride on the LMAMA. In *a* the physiological saline was changed to one containing 0·2 mg/ml. barium chloride and only the initial part of the response is shown. In *b* and *c* barium chloride in the doses shown was injected into the physiological saline. The horizontal line represents 60 sec in all records. The action potentials have been retouched.

Larger concentrations of barium chloride produced a burst of action potentials. These rapidly diminished and disappeared as depolarization proceeded but the contraction which occurred persisted for several minutes (Fig. 2*b*, *c*).

Responses to electrical stimulation. In quiescent preparations electrical stimulation produced a contraction very similar to those seen during spontaneous activity. As the rate of stimulation was increased, the contraction began progressively earlier after the beginning of stimulation and the frequency of action potentials and small rapid contractions increased. The maximal rate of rise of tension and maximal tension developed occurred at around 10 impulses/sec. At higher rates of stimulation, a relaxation followed the contraction and the tone fell below the level existing before stimulation. Thus the maximal duration of the contraction was obtained when stimulating at about 5 impulses/sec (Fig. 3).

During electrical stimulations at lower rates, oscillations of the electrical record preceded, and often followed, the appearance of action potentials

(Fig. 3). These oscillations were replaced in some experiments by a depolarization without action potentials which was associated with a slow rise in tension (Fig. 4).

In the present experiments, brief electrical pulses were applied through similar annular electrodes to those used previously (Bolton, 1968), and it is probable that the contractions observed here were again due to stimulation of excitatory, cholinergic nerves, especially as they were abolished

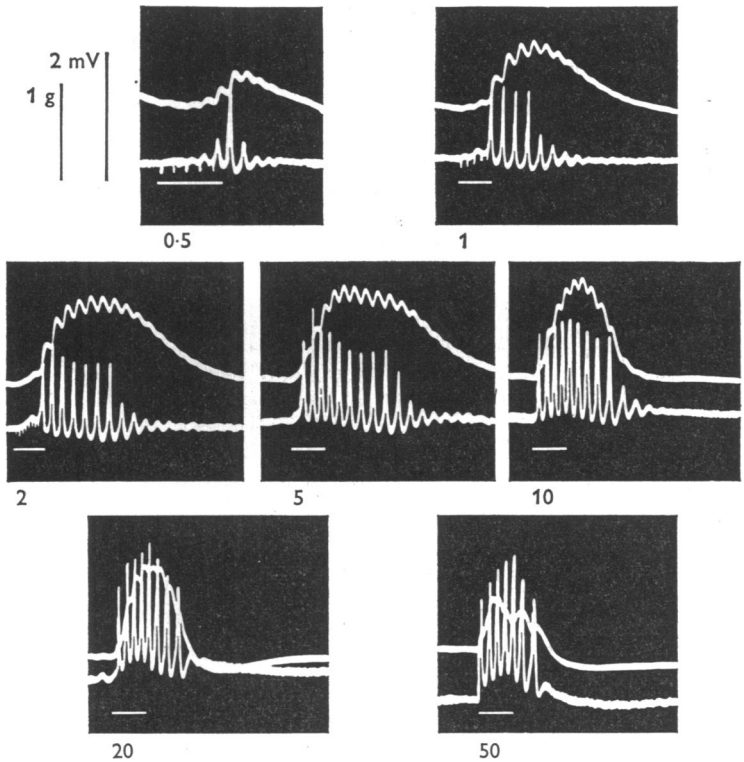

Fig. 3. The effects of brief electrical stimuli on a quiescent preparation of the LMAMA. Electrical stimulation at supramaximal strength was applied for 5 sec periods as indicated by the white lines except that, in the first panel, a 10 sec period of stimulation was used. The rate of stimulation per second is shown under each panel. The action potentials have been retouched.

by hyoscine (10 ng/ml.). The inhibitory nerves supplying the LMAMA have a higher optimal frequency (20–50/sec) than the excitatory nerves (optimal frequency 5–10/sec) (Bolton, 1967), and as both types of nerves are stimulated simultaneously (Bolton, 1968), this probably explains the biphasic nature of the response at higher rates of stimulation.

Acetylcholine. Low doses (10–20 ng) produced one or more contractions

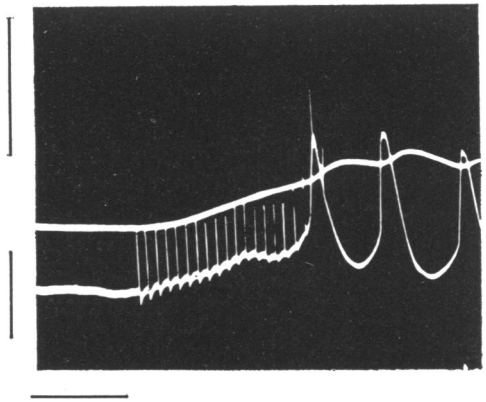

Fig. 4. The effects of brief electrical stimuli on the LMAMA. A depolarization without action potentials accompanies a smooth rise in tension; action potentials are associated with small rapid contractions. The horizontal calibration represents 2 sec. The action potentials and stimulus artifacts have been retouched.

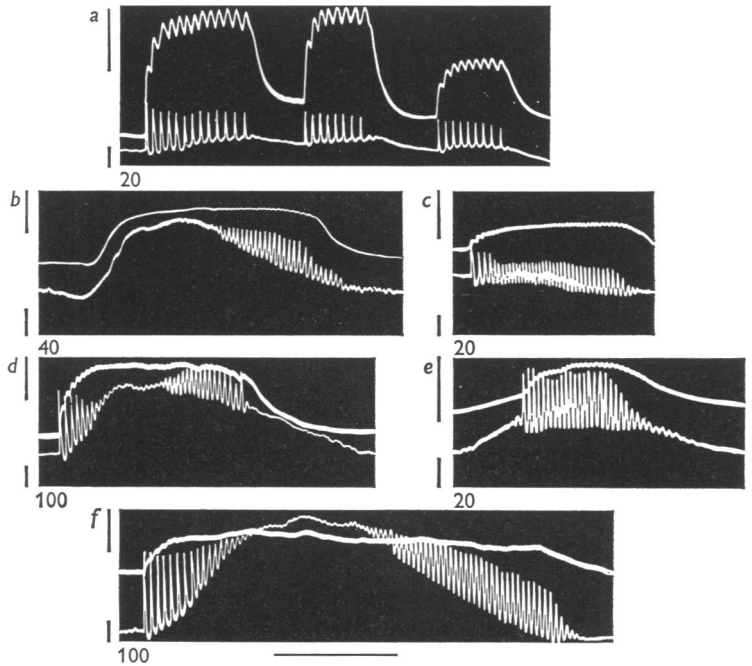

Fig. 5. Different types of response to acetylcholine in six different preparations of the LMAMA. The doses of acetylcholine shown below each panel (in ng) were injected into the physiological saline. With low doses (*a*, *c*) the response is one or more contractions. With larger doses depolarization occurs: this sometimes precedes action potentials (*e*) but generally it occurs simultaneously with them (*d*, *f*). In *b* action potentials are only observed during repolarization. The horizontal line represents 30 sec in all records. The action potentials have been retouched.

closely resembling those occurring during spontaneous activity (Fig. 5*a*, *c*). Larger doses of acetylcholine produced a combination of action potentials and depolarization; depolarization preceded (Fig. 5*e*) or followed (Fig. 5*d*, *f*) the appearance of action potentials. During depolarization tension was maintained. Action potentials also occurred during repolarization (Fig. 5*b*, *d*, *f*). These various types of response suggest that depolarization or hyperpolarization beyond certain limits precluded the generation or the conduction of action potentials.

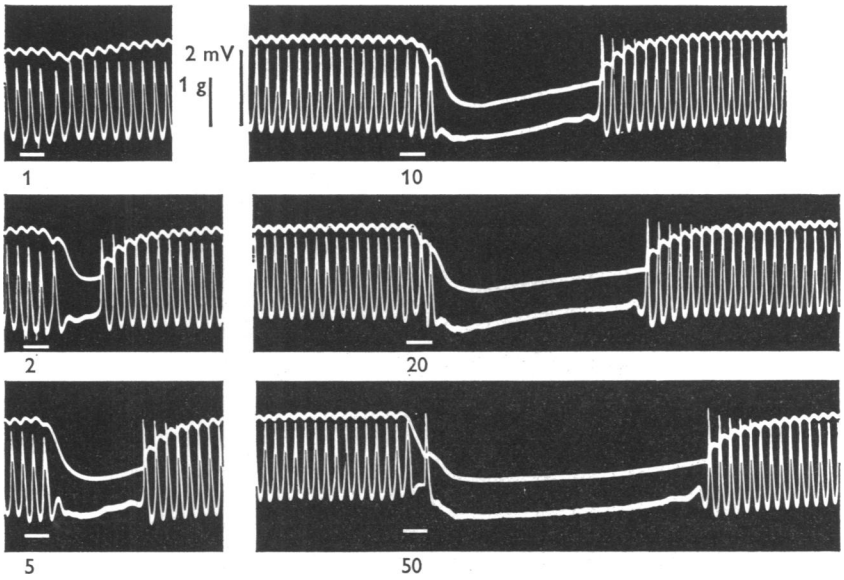

Fig. 6. The effects of brief electrical stimuli on a preparation of the LMAMA in which the tone had been raised by adding 0·2 mg/ml. barium chloride to the physiological saline. Hyoscine (10 ng/ml.) was also present. Electrical stimulation, at supramaximal strength, was applied for 5 sec periods as indicated by the white lines. The rate of stimulation per second is shown under each panel. The action potentials have been retouched.

It may be seen from the records shown in Figs. 1–5 that small rapid contractions were seen only in association with action potentials. Where depolarization occurred without action potentials, the tension rose smoothly and small contractions were not seen (Figs. 4 and 5*b*, *e*).

Inhibition

Electrical stimulation. When the tone of preparations was raised by adding barium chloride (0·1–0·2 mg/ml.) to the physiological saline, continuous small contractions and action potentials occurred. In the presence of hyoscine (10 ng/ml.), electrical stimulation produced relaxations.

At low rates of stimulation a small dip in the tension record followed stimulation; action potentials did not cease but became more widely spaced (Fig. 6). At higher rates of stimulation, relaxation began progressively earlier after the beginning of stimulation. Action potentials ceased and a hyperpolarization was observed. After the point of maximal hyperpolarization was reached, which approximated to the point of lowest tension, a slow depolarization and an accompanying smooth rise in tension occurred until a level of tension was reached where action potentials and small contractions began (Fig. 6). At 10–20 impulses/sec the size of the relaxation was maximal but the duration of the relaxation continued to increase up to 50 impulses/sec. It is likely, in view of the brief duration of the electrical stimuli and the results obtained previously (Bolton, 1968), that these responses resulted from the stimulation of inhibitory nerves and not to a direct effect on the muscle.

Noradrenaline. No response to noradrenaline was observed in quiescent preparations presumably because they were without tone. When the tone of preparations was raised by adding barium chloride (0·1–0·2 mg/ml.) to the physiological saline, the injection of noradrenaline (10 ng or more) produced a relaxation with the cessation of action potentials. Hyperpolarization was also sometimes observed. After a short period of quiescence, action potentials and small rapid contractions began again (Fig. 7). This response was unchanged by the presence of hyoscine and resembles the responses to electrical stimulation shown in Fig. 6.

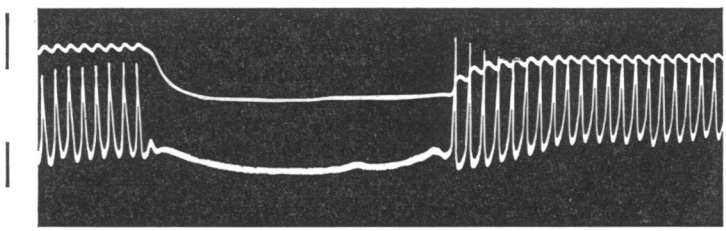

Fig. 7. The effects of noradrenaline injected into the physiological saline on a preparation of the LMAMA in which the tone had been raised by adding barium chloride (0·2 mg/ml.) to the perfusate. The horizontal calibration represents 30 sec. The action potentials have been retouched.

DISCUSSION

These observations on the LMAMA do not conform to the suggestion by Bozler (1941, 1948, 1962) that vascular smooth muscle behaves as a 'multi-unit' tissue. The well-developed 'single-unit' activity which occurs in the LMAMA is very similar to that observed in visceral smooth muscle (see Burnstock *et al.* 1963) and some vascular muscles (Roddie, 1962;

Cuthbert & Sutter, 1964; Funaki & Bohr, 1964; Johansson & Bohr, 1966). However, such activity is not usually found in the smooth muscle of large mammalian blood vessels such as the rabbit aorta (Furchgott, 1960), or sheep carotid (Keatinge, 1966).

The effects of noradrenaline and acetylcholine on the electrical and mechanical activity of the LMAMA resemble the actions of these drugs on visceral smooth muscle (see Burnstock *et al.* 1963). However, noradrenaline or adrenaline contract the smooth muscle of large blood vessels and increase the frequency of action potential discharge (Roddie, 1962; Funaki & Bohr, 1964; Cuthbert & Sutter, 1965), whereas noradrenaline produced the opposite effects on the LMAMA. Thus, in its responses to drugs, as in its activity and innervation (Bolton, 1968), the LMAMA resembles visceral smooth muscle more closely than it resembles smooth muscle from other large blood vessels.

I am most grateful to Professor W. C. Bowman and to Professor E. C. Amoroso for their advice and help, and to Professor G. A. H. Buttle for the privilege of working in his department. I am indebted to Mr D. Tullet for building the apparatus used in these experiments.

I thank the British Egg Marketing Board for a scholarship and for financial support for the work through grants to Professor W. C. Bowman and Professor E. C. Amoroso. This study was supported in part by grants from the United States Public Health Service, numbers GM 064089-05 and HD 01476-05.

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