Br. J. Pharmac. (1969), **35**, 112–120.

Spontaneous and evoked release of neurotransmitter substances in the longitudinal muscle of the anterior mesenteric artery of the domestic fowl

T. B. BOLTON

*Department of Pharmacology, School of Pharmacy, and *Department of Physiology, Royal Veterinary College, London*

1. Changes in length of the longitudinal muscle of the anterior mesenteric artery of the domestic fowl (LMAMA) were recorded isotonically.

2. The actions of physostigmine and hyoscine suggest that the tone of the LMAMA is dependent on a resting release of acetylcholine within the tissue.

3. Catecholamines inhibited the contractions obtained in response to stimulation of cholinergic nerves; this was a β-receptor effect. After β-receptor blockade, noradrenaline, but not isoprenaline, weakly facilitated the effects of stimulating cholinergic nerves.

4. In the presence of hyoscine, nicotine relaxed preparations of the LMAMA in which the tone had been raised with barium chloride. These relaxations were considered to be due to a release of catecholamine probably from sympathetic nerves.

The longitudinal muscle of the anterior mesenteric artery of the domestic fowl (LMAMA) differs from the smooth muscle of arteries such as the rabbit aorta (Furchgott, 1955) and the sheep carotid (Keatinge, 1966) in that it exhibits "single-unit" activity and is capable of much larger percentage changes in length. Moreover, it is unlike most other vascular muscle in that it is relaxed by noradrenaline and strongly contracted by low concentrations of acetylcholine. Furthermore, it is unique among vascular muscles so far described in that it is supplied both by excitatory cholinergic nerves and by inhibitory adrenergic nerves (Bolton, 1966, 1968a, b). Results presented in this paper suggest that neurotransmitter substances may also be released both spontaneously and in response to nicotine, thus providing further evidence for the existence of these two types of innervation. The physiological antagonism by catecholamines of the effects of acetylcholine released by cholinergic nerve stimulation is also examined.

* Present address.

Methods

The method has been described previously (Bolton, 1968a). The anterior mesenteric artery of domestic fowls 1–4 months old was severed at its origin from the aorta and at a point beyond the origins of several intestinal arteries; the segment so isolated was 3–7 cm long. This was immersed in physiological salt solution (pH 7.5) at 41°–42° C, and anchored by its aortic end around which two annular electrodes were placed. When it was desired to stimulate the nerves supplying the LMAMA, short bursts (5–20 sec) of rectangular 0.5 msec pulses, delivered at supramaximal rates (10–20/sec), were applied between these electrodes. The other end of the artery was connected to an isotonic frontal writing lever which magnified the contractions 5 or 10 times. The tension applied to the tissue was 1–2 g. In the illustrations the change in length of the preparation is represented as a percentage of the resting length of the preparation measured under tension in the bath.

The concentrations of catecholamines used are expressed as base, the concentrations of other drugs are expressed as the following salts: guanethidine sulphate, hexamethonium bromide, hyoscine hydrobromide, mecamylamine hydrochloride, nicotine hydrogen tartrate, pempidine tartrate, phentolamine hydrochloride, physostigmine salicylate, propranolol hydrochloride.

Results

Acetylcholine release

Initially the majority of preparations exhibited tone; such preparations were relaxed by catecholamines. When tone was present, rhythmical contractions usually occurred. These disappeared as the tone fell and were usually absent from preparations which showed low initial tone.

The addition of physostigmine (10 ng/ml. upwards) to preparations with low tone produced a rise in tone (Fig. 1a). Although it is possible that physostigmine may act on acetylcholine receptors itself or release acetylcholine from nerve endings (Mason, 1962; Carlyle, 1963; Ehrenpreis, Bigo-Gullino & Avery, 1965), it is unlikely that the low concentrations of physostigmine which were effective, were acting other than by preserving acetylcholine spontaneously released within the tissue. This is supported by the observation that the addition of hyoscine (1 ng/ml. upwards) produced relaxation of preparations with tone (Fig. 1b). It was possible alternately to raise and lower the tone by adding alternate, increasing concentrations of physostigmine and hyoscine (Fig. 1a, b).

The effects of stimulating cholinergic nerves supplying visceral smooth muscle are antagonized by catecholamines (Bowman & Everett, 1964; Kosterlitz & Watt, 1965), so it was of interest to discover whether catecholamines antagonized the effects of stimulating cholinergic nerves supplying the LMAMA. Bursts of 0.5 msec pulses applied to annular electrodes encircling the aortic end of the artery produced contraction of the LMAMA by stimulating cholinergic nerves; these contractions were blocked by ganglion-blocking agents showing that a ganglionic synapse is involved (Bolton, 1968a). Simultaneously, inhibitory adrenergic nerves are also excited using this method of stimulation and this results in a relaxation following the initial contraction (Bolton, 1968a, b). The effects of exciting inhibitory nerves were only occasionally seen in preparations with low tone (Fig. 2).

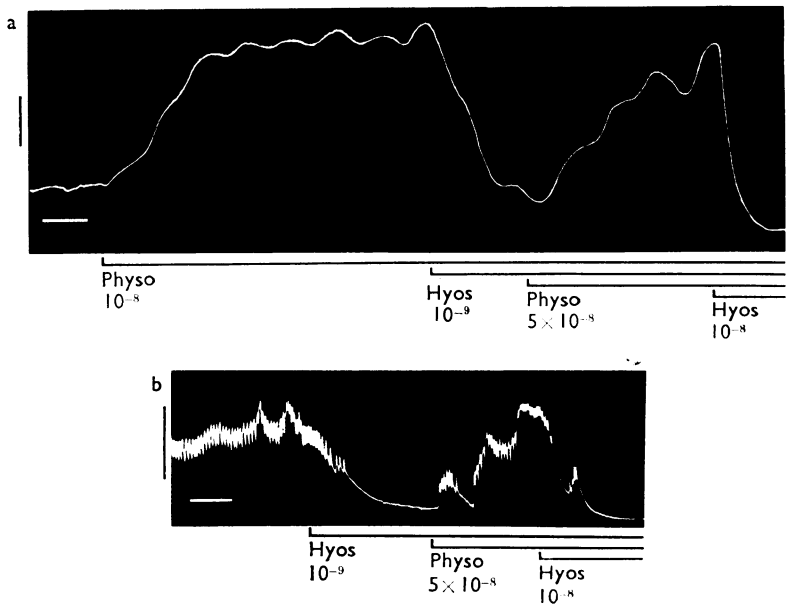

FIG. 1. Effects of physostigmine (Physo) and hyoscine (Hyos) on the tone of two preparations of the LMAMA. (a), A low-tone preparation which did not exhibit spontaneous activity when the tone was raised. Physostigmine caused an increase in tone which was antagonized by hyoscine. (b), A preparation with tone and spontaneous activity. Hyoscine reduced the tone and was reversed by physostigmine. In both preparations the tone could be alternately raised or lowered by adding alternate, increasing concentrations of physostigmine and hyoscine. All concentrations are in g/ml. and were added cumulatively without washing the tissue. In this and subsequent figures, the horizontal calibration is 10 min and the vertical calibration a 10% change in length of the LMAMA.

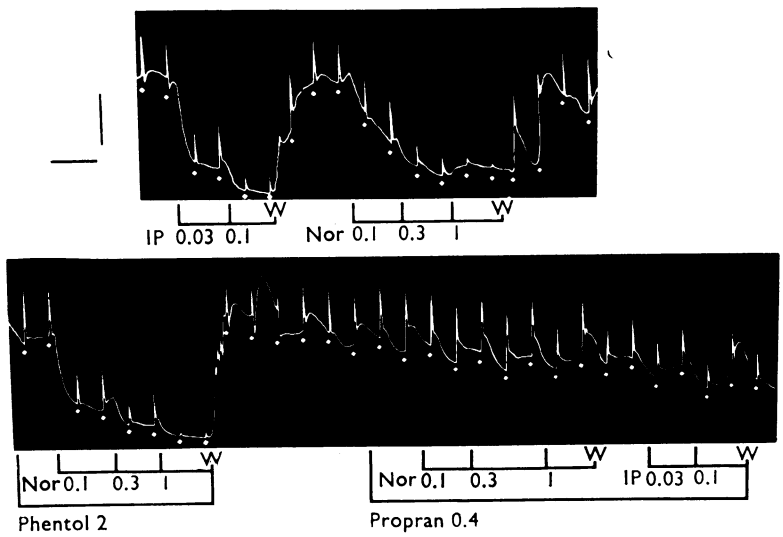

FIG. 2. Inhibition by catecholamines of the responses of the LMAMA to cholinergic nerve stimulation. The upper and lower records are continuous. Electrical stimulation (0.5 msec pulses of supramaximal strength and rate) was applied for 10 sec periods at the white diamonds. The stated concentrations (μg/ml.) of (−)-isoprenaline (IP) and (−)-noradrenaline (Nor) were added cumulatively, and the tissue was washed (W) as indicated. Control responses are shown in the upper panel and in the lower panel are shown the effects of phentolamine (Phentol, 2 μg/ml.) or propranolol (Propran, 0.4 μg/ml.) on the inhibition by catecholamines of the responses to cholinergic nerve stimulation.

The contractions in response to stimulating the cholinergic excitatory nerves in the manner described, were reduced or abolished by catecholamines. The characteristics of this inhibition showed that only β-receptors were involved. When the relative potencies of (−)-isoprenaline and (−)-noradrenaline in inhibiting cholinergic nerve effects were compared in five preparations, the mean concentrations of (−)-isoprenaline and (−)-noradrenaline producing 50% reduction of the responses were 17 and 276 ng/ml. respectively. In two experiments adrenaline was also a less potent inhibitor than (−)-isoprenaline. Propranolol (0.2–0.5 μg/ml.), but not

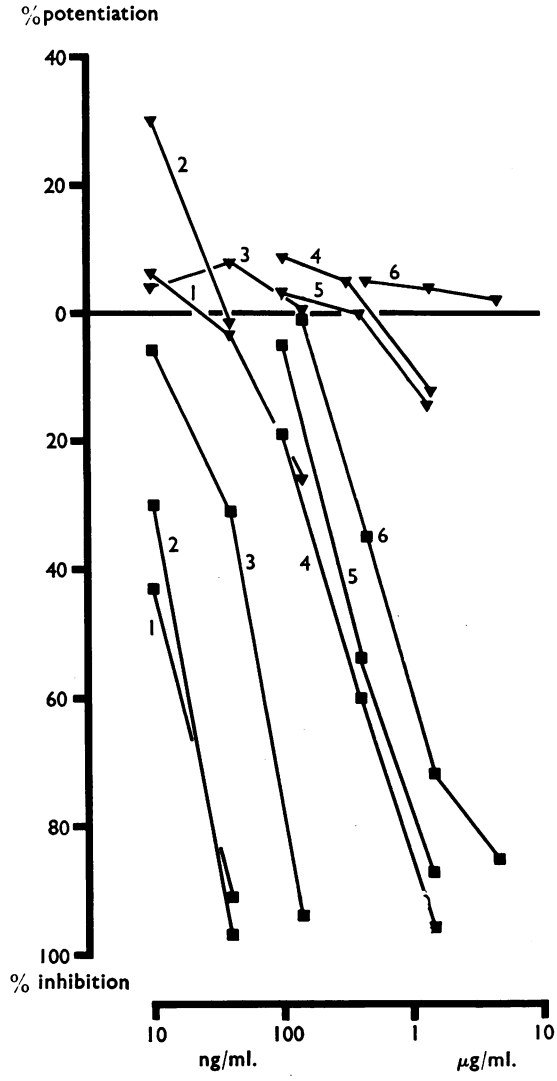

FIG. 3. Inhibition by noradrenaline of the responses of the LMAMA to cholinergic nerve stimulation. The % inhibition of the responses of six preparations (numbered 1 to 6) are plotted before (■) and after (▼) the addition of pronethalol (2 μg/ml., preparation 1) or propranolol (0.2–0.5 μg/ml., remaining preparations). All preparations show some potentiation of the responses after β-receptor blockade when catecholamine is added, although this potentiation is less pronounced or absent at higher concentrations. Isoprenaline never potentiated the responses after β-receptor blockade.

dihydroergotamine (2 μg/ml.) or phentolamine (2–5 μg/ml.), prevented this inhibition by catecholamines of the effects of cholinergic nerve activity (Figs. 2 and 3).

Although propranolol blocked the inhibition produced by (−)-isoprenaline of the contractions in response to cholinergic nerve stimulation, (−)-isoprenaline did not produce any potentiation of the responses to cholinergic nerve stimulation in the presence of propranolol. On the other hand, (−)-noradrenaline did potentiate the responses to cholinergic nerve stimulation in six experiments after β-receptor blockade, although the degree of potentiation was small, the greatest potentiation observed being 30% (Fig. 3). This potentiation is not apparent in the experiment illustrated in Fig. 2. It appears that (−)-noradrenaline inhibits the contractions elicited by stimulation of cholinergic nerves by an action on β-receptors, and this β-receptor inhibitory action normally masks a much weaker facilitatory action on the responses. As this postulated facilitatory action is presumably mediated by α-receptors, it would be expected that in the presence of an α-receptor blocking agent, the inhibition produced by (−)-noradrenaline would be potentiated. This was found to be the case ; in five experiments the mean concentration of noradrenaline producing 50% inhibition before the addition of phentolamine (2–5 μg/ml.) was 256 ng/ml. and in the same preparations in the presence of phentolamine this concentration was 210 ng/ml. In all the above comparisons the exact concentration of catecholamine producing 50% inhibition was obtained from the log dose-response graphs (compare Fig. 3).

Although propranolol antagonized the inhibitory action of (−)-isoprenaline on the contractions produced by cholinergic nerve stimulation, larger concentrations of (−)-isoprenaline (0.1–1 μg/ml.) still produced an appreciable inhibition of the contractions despite increasing the concentration of propranolol to 2–5 μg/ml. and also adding phentolamine (5 μg/ml.). This is possibly due to a papaverine-like action of isoprenaline which has been postulated (Ariëns, 1960).

Propranolol (0.2–0.5 μg/ml.) itself often potentiated the responses. This was probably due to the fact that the electrical stimuli applied between annular electrodes surrounding the artery stimulate both excitatory and inhibitory nerves (Bolton, 1968a, b) and in the absence of propranolol, the effects of stimulating the former are reduced to a small extent by excitation of the latter. Larger concentrations of propranolol (2–5 μg/ml.) produced a progressive reduction in the size of the responses, probably due to the local anaesthetic activity of propranolol (Morales-Aguilerá & Vaughan Williams, 1965).

Catecholamine release

As previously reported (Bolton, 1968a), relaxations produced by applying 0.5 msec pulses to annular electrodes encircling the artery were blocked by propranolol or by guanethidine. These relaxations were increased by raising the tone with barium chloride and were not appreciably affected by concentrations of ganglion-blocking agents which abolished the contractile responses obtained on excitatory nerve stimulation (Bolton, 1968a). These relaxations presumably result from the release of catecholamine, probably noradrenaline, from sympathetic nerve endings.

In preparations in which the tone had been raised with barium chloride (0.1–0.5 mg/ml.) and in the presence of hyoscine (10–100 ng/ml.), nicotine produced relaxations similar to those produced by sympathetic nerve stimulation (Fig. 4). These

responses to nicotine showed marked tachyphylaxis but no relaxations were obtained in the presence of the ganglion-blocking agents, hexamethonium (20 μg/ml.), pempidine (5 μg/ml.), or mecamylamine (1 μg/ml.). Relaxations could be obtained in the presence of phentolamine (2 μg/ml.) but in eight experiments no relaxations were obtained in the presence of propranolol (0.2–0.5 μg/ml.) as illustrated in Fig. 4a. After blockade by guanethidine (2–5 μg/ml.) of the relaxations obtained with electrical stimulation, no relaxations in response to nicotine were obtained in five preparations but in three other preparations relaxations occurred, as illustrated in Fig. 4b. In the presence of propranolol, nicotine sometimes produced contraction (Fig. 4a) and this contraction occurred despite increasing the concentration of hyoscine to 1 μg/ml. Hyoscine (10 ng/ml.) in the absence of barium chloride, has been

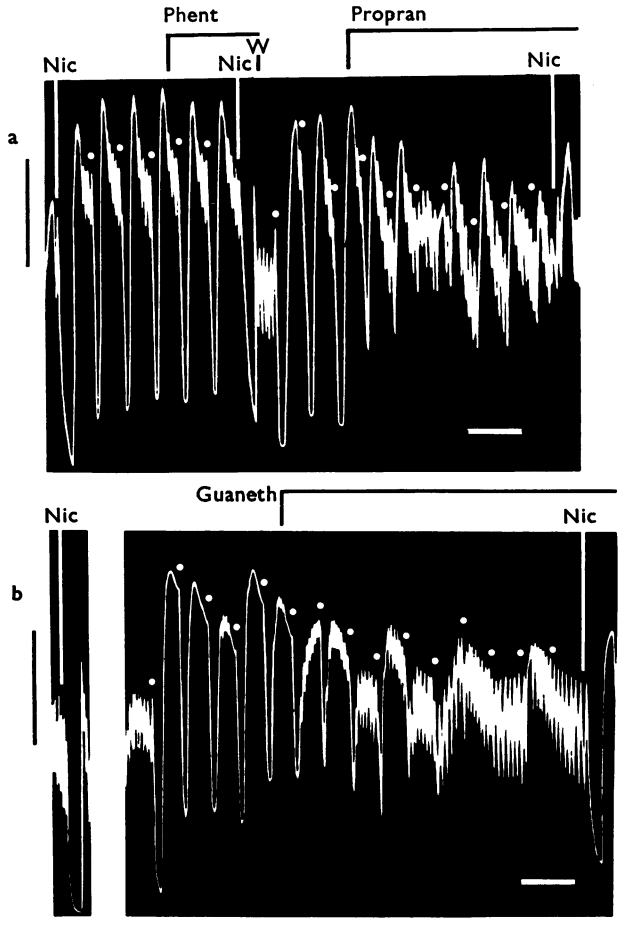

FIG. 4. Nicotine responses of two preparations of the LMAMA contracted with barium chloride (0.1 mg/ml.) and in the presence of hyoscine. (a), Responses to nicotine (5 μg/ml.) and to stimulation of the inhibitory nerves (for 20 sec periods at supramaximal strength and rate at the dots) were unaffected by phentolamine (Phent, 2 μg/ml.). After washing (W) the addition of propranolol (Propran, 0.2 μg/ml.) blocked the effects of inhibitory nerves and nicotine produced a contraction despite the presence of hyoscine (10 ng/ml.). (b), After blockade by guanethidine (Guaneth, 5 μg/ml.) of the responses to stimulation of the inhibitory nerves (for 10 sec periods at supramaximal strength and rate at the dots), nicotine (5 μg/ml.) still produced relaxation.

previously shown to block nicotine contractions of low-tone preparations of the LMAMA (Bolton, 1968a). Thus nicotine contractions of low-tone preparations seem to result from the stimulation of the ganglia of cholinergic nerves (Bolton, 1968a), while nicotine contractions of barium-sensitized preparations must result from a direct stimulant action of nicotine on the muscle, as in other smooth muscle (Evans & Schild, 1953).

Discussion

These experiments show that there are remarkable similarities between the LMAMA and mammalian visceral muscle. A spontaneous resting release of acetylcholine probably occurs in the smooth muscle of the intestine (Feldberg & Lin, 1949), the stomach (Paton & Vane, 1963), the chick amnion (Cuthbert, 1962, 1966), the trachea (Carlyle, 1964), and in cardiac (Day, 1956) and skeletal muscle (Brooks, 1954 ; Mitchell & Silver, 1963), but the results described in this paper are the first evidence that a similar resting release of acetylcholine may occur in vascular muscle. As the LMAMA is innervated by cholinergic nerves (Bolton, 1968a), in contrast to the smooth muscle of other large blood vessels, it is likely that the acetylcholine originates from these nerves.

In view of the fact that catecholamines inhibit the effects of stimulating the cholinergic nerves supplying visceral muscles (Bowman & Everett, 1964 ; Kosterlitz & Watt, 1965), it is not surprising that they have a similar effect on the responses to stimulating the cholinergic nerve supplying the LMAMA. This inhibition involves only β-receptors, in contrast to the situation in visceral muscle (Bowman & Everett, 1964 ; Kosterlitz & Watt, 1965), and as relaxation of the LMAMA by catecholamines also involves only β-receptors (Bolton, 1968a), it is probable that the underlying mechanism of these two effects is the same, that of hyperpolarization of the smooth muscle cell membrane resulting in a block of the generation and propagation of action potentials (Bolton, 1968b).

It does not follow, however, that the apparent facilitatory action of noradrenaline on cholinergic nerve activity in the LMAMA is due to an effect on the cholinergic nerves, as is the case of the cholinergic nerves supplying skeletal muscle (Bowman, Goldberg & Raper, 1962 ; Bowman & Raper, 1966) and some smooth muscle (Christensen & Daniel, 1966). First, in the LMAMA adrenergic nerves were being stimulated simultaneously with cholinergic nerves (Bolton, 1968a), and second, there is no doubt that α-receptors are present on the LMAMA (see responses to noradrenaline 1 μg/ml. in the absence and in the presence of phentolamine, Fig. 2). Hence, when β-receptors are blocked, exogenous noradrenaline might be expected to have an excitatory action on the smooth muscle of the preparation via α-receptors, and thus potentiate the responses to cholinergic nerve stimulation by a mechanism similar to that by which acetylcholine lowers the threshold to electrical stimulation in the guinea-pig taenia caecae (Burnstock, 1958).

Relaxations produced by nicotine were not obtained during β-receptor blockade, so it is likely that, like the relaxations in response to sympathetic nerve stimulation, they were due to a release of catecholamine. Unlike these latter responses, however, they were not always blocked by adrenergic neurone blocking agents. Others have made similar observations on the action of nicotine on visceral smooth muscle preparations (Gillespie & MacKenna, 1960 ; Jarrett, 1962 ; Bucknell, 1965 ; Burn-

stock, Campbell & Rand, 1966). This release of catecholamine by nicotine in the LMAMA is probably from sympathetic nerves as in other tissues (see Ferry, 1966).

I am grateful to Professor E. C. Amoroso and to Professor W. C. Bowman for their advice and help and to Professor G. A. H. Buttle for the privilege of working in his department. These results formed part of a thesis approved for the degree of Doctor of Philosophy in the University of London. The work was supported by the British Egg Marketing Board through a grant to Professor W. C. Bowman and by a scholarship to the author, and in part by grants from the U.S. Public Health Service, numbers GM 064089-05 and HD 01476-05.

REFERENCES

ARIËNS, E. J. (1960). Various types of receptors for sympathomimetic drugs. *Adrenergic Mechanisms*, pp. 264–270. London: Churchill.

BOLTON, T. B. (1966). A dually innervated longitudinal arterial muscle in the fowl. *J. Physiol., Lond.*, **186**, 129–130*P*.

BOLTON, T. B. (1968a). Studies on the longitudinal muscle of the anterior mesenteric artery of the domestic fowl. *J. Physiol., Lond.*, **196**, 273–281.

BOLTON, T. B. (1968b). Electrical and mechanical activity of the longitudinal muscle of the anterior mesenteric artery of the domestic fowl. *J. Physiol., Lond.*, **196**, 283–292.

BOWMAN, W. C. & EVERETT, S. D. (1964). An isolated parasympathetically-innervated oesophagus preparation from the chick. *J. Pharm. Pharmac.*, **16**, Suppl. 72–79*T*.

BOWMAN, W. C., GOLDBERG, A. A. J. & RAPER, C. (1962). A comparison between the effects of a tetanus and the effects of sympathomimetic amines on fast- and slow-contracting mammalian muscles. *Br. J. Pharmac. Chemother.*, **19**, 464–484.

BOWMAN, W. C. & RAPER, C. (1966). Effects of sympathomimetic amines on neuromuscular transmission. *Br. J. Pharmac. Chemother.*, **27**, 313–331.

BROOKS, V. B. (1954). The action of botulinum toxin on motor-nerve filaments. *J. Physiol., Lond.*, **123**, 501–515.

BUCKNELL, A. (1965). Effects of direct and indirect stimulation on isolated colon. *J. Physiol., Lond.*, **177**, 58–59*P*.

BURNSTOCK, G. (1958). The effects of acetylcholine on membrane potential, spike frequency, conduction velocity and excitability in the taenia coli of the guinea-pig. *J. Physiol., Lond.*, **143**, 165–182.

BURNSTOCK, G., CAMPBELL, G. & RAND, M. J. (1966). The inhibitory innervation of the taenia of the guinea-pig caecum. *J. Physiol., Lond.*, **182**, 504–526.

CARLYLE, R. F. (1963). The mode of action of neostigmine and physostigmine on the guinea-pig trachealis muscle. *Br. J. Pharmac. Chemother.*, **21**, 137–149.

CARLYLE, R. F. (1964). The responses of the guinea-pig isolated intact trachea to transmural stimulation and the release of an acetylcholine-like substance under conditions of rest and stimulation. *Br. J. Pharmac. Chemother.*, **22**, 126–136.

CHRISTENSEN, J. & DANIEL, E. E. (1966). Electric and motor effects of autonomic drugs on longitudinal oesophageal smooth muscle. *Am. J. Physiol.*, **211**, 387–394.

CUTHBERT, A. W. (1962). Actions of some anticholinesterases on the smooth muscle of the chick amnion. *Br. J. Pharmac. Chemother.*, **18**, 550–562.

CUTHBERT, A. W. (1966). Pharmacological responses of the smooth muscle of the chick amnion. *Physiology of the Domestic Fowl*, ed. Horton-Smith, C. & Amoroso, E. C., pp. 274–278. Edinburgh: Oliver & Boyd.

DAY, M. (1956). The release of substances like acetylcholine and adrenaline by the isolated rabbit heart. *J. Physiol., Lond.*, **134**, 558–568.

EHRENPREIS, S., BIGO-GULLINO, M. & AVERY, M. A. (1965). The effect of cholinesterase inhibitors and lipid soluble quaternary ammonium compounds on contractions of rabbit aortic strip. *Archs int. Pharmacodyn. Thér.*, **156**, 1–21.

EVANS, D. H. L. & SCHILD, H. O. (1953). The reactions of plexus-free circular muscle of cat jejunum to drugs. *J. Physiol., Lond.*, **119**, 376–399.

FELDBERG, W. & LIN, R. C. Y. (1949). The effect of cocaine on the acetylcholine output of the intestinal wall. *J. Physiol., Lond.*, **109**, 475–487.

FERRY, C. B. (1966). Cholinergic link hypothesis in adrenergic neuro-effector transmission. *Physiol. Rev.*, **46**, 420–456.

FURCHGOTT, R. F. (1955). Pharmacology of vascular smooth muscle. *Pharmac. Rev.*, **7**, 183–265.

GILLESPIE, J. S. & MACKENNA, B. R. (1960). The inhibitory action of nicotine on the rabbit colon. *J. Physiol., Lond.*, **152**, 191–205.

JARRETT, R. J. (1962). Action of nicotine on the rabbit muscular organ ileo-colic sphincter. *Br. J. Pharmac. Chemother.*, **18**, 397–404.

KEATINGE, W. R. (1966). Electrical and mechanical response of arteries to stimulation of sympathetic nerves. *J. Physiol., Lond.*, **185**, 701–715.

KOSTERLITZ, H. W. & WATT, A. J. (1965). Adrenergic receptors in guinea-pig ileum. *J. Physiol., Lond.*, **177**, 11–12*P*.

MASON, D. F. J. (1962). A ganglion stimulating action of neostigmine. *Br. J. Pharmac. Chemother.*, **18**, 76–86.

MITCHELL, J. F. & SILVER, A. (1963). The spontaneous release of acetylcholine from the denervated hemidiaphragm of the rat. *J. Physiol., Lond.*, **165**, 117–129.

MORALES-AGUILERÁ, A. & VAUGHAN WILLIAMS, E. M. (1965). The effects on cardiac muscle of β-receptor antagonists in relation to their activity as local anaesthetics. *Br. J. Pharmac. Chemother.*, **24**, 332–338.

PATON, W. D. M. & VANE, J. R. (1963). An analysis of the responses of the isolated stomach to electrical stimulation and to drugs. *J. Physiol., Lond.*, **165**, 10–46.

(*Received July* 12, 1968)