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THE RABBIT RECTOCOCCYGEUS: A GANGLION-FREE, PARASYMPATHETICALLY INNERVATED PREPARATION

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1 Isolated, desheathed preparations of the rabbit rectococcygeus muscle were relatively insensitive to spasmogens other than muscarinic drugs. Transmural stimulation with 1-50 pulses (0.2-0.4 ms at 10 Hz) elicited graded twitches which were abolished by tetrodotoxin and were therefore neurogenic; longer pulses sometimes triggered tetrodotoxin-resistant myogenic contractions.

2 Twitches elicited by 0.2-0.4 ms pulses were due to post-ganglionic excitation because they were not reduced by hexamethonium, pentolinium or dimethyltubocurarine, or by ganglion-paralyzing concentrations of nicotine.

3 The acetyl- and butyryl-cholinesterase activities of the rectococcygeus were determined manometrically and could be selectively inhibited by BW 284C51 (1:5-bis-(4-allyl-dimethyl-ammonium-phenyl)-pentan-3-one dibromide) and *iso*-OMPA (tetramono*iso*propylpyro-phosphortetramide), respectively. Single-pulse twitches were greatly potentiated in amplitude and duration only when both cholinesterases were inhibited.

4 The preparations could not be made to contract by nicotine (2.1-21 μM) even after cholinesterase inhibition, suggesting an absence of ganglion-cells; with nicotine (105-210 μM) small, atropine-susceptible responses were elicited, which were non-ganglionic because they were not reduced by tetrodotoxin.

5 Rectococcygeus preparations that had been treated with physostigmine released acetylcholine into the bath fluid on electrical stimulation.

6 The motor transmission was paralyzed by botulinum toxin (Type A) and abolished by atropine; the block of muscarinic receptors by atropine was effective against both endogenous and exogenous acetylcholine.

7 Inhibitory adrenoceptors and scanty motor α-adrenoceptors were detected in the smooth muscle.

8 Strong inhibitions of motor transmission and of rhythmic activity were produced by noradrenaline (but not by tyramine), by isoprenaline, and, after phentolamine, also by adrenaline and phenylephrine. These inhibitions were only slightly reduced by propranolol and rather more by pindolol.

9 Experiments on preparations retaining their extrinsic nerve supply suggest an absence of ganglionic relays in the last 1-2 cm of the motor nerve pathway to this muscle.

10 Some contrasting properties of the adjacent caudo-anal muscle, including the poor motor responses to transmural stimulation, are described.

Introduction

The rectococcygeus, a smooth muscle which anchors the rectum to the tail vertebrae and pulls the descending colon and rectum caudally during defecation, appears to be derived from the outer part of the longitudinal muscular coat of the rectum, with which it is continuous at its upper end. At its point of departure from the rectum it leaves behind the ganglionated, myenteric plexus of Auerbach. McKirdy's (1972) paper gives a brief account of a histological examination in which ganglion-cells could not be found within this muscle but nerve fibres were seen.

Although plexus-free, the rectococcygeus does receive an extrinsic parasympathetic motor

innervation from the sacral nerves. Langley & Anderson (1895; pp. 85 & 87) have reported visual observations of unilateral rectococcygeus contractions produced by faradic stimulation of the sacral or pelvic nerves on the same side, and absence of such contractions and of defecation after atropine. They state that in the rabbit the nerve fibres concerned 'run chiefly but not entirely in the posterior division of the pelvic nerve'. In further anatomical investigations they mention that this division of the pelvic nerve arises 'from a considerable ganglion situated on the posterior branch of the pelvic nerve soon after the anterior strand has emerged' (Langley & Anderson, 1896; p. 390). As can be seen from Figs 6 and 7 of their paper, this ganglion is located at some distance from the rectococcygeus muscle.

The pharmacological investigations described below show that the rabbit rectococcygeus provides robust, isolated preparations which appear to be ganglion-free and are therefore suitable for the study of cholinergic post-ganglionic transmission in a parasympathetic nerve.

A preliminary report of these results has appeared elsewhere (Ambache, Killick & Zar, 1973).

Methods

Male or female rabbits of several breeds were used, mostly New Zealand White, 0.6-4 kg; a few Polish or Netherland Dwarfs, 1.5-2.5 kg; and some Californians, 2.5-4 kg. The animals were stunned and bled out.

Procedure for exposure of the rectococcygeus muscle

A circular incision was made round the circumference of the abdomen at umbilical level and the skin was peeled off the lower half of the animal. The abdominal cavity was opened and, except for the last 12-15 cm of large intestine, the gut was removed between ligatures. The bladder was ligated at its neck and excised, together with the reproductive organs lying ventral to the rectum. The pubic bones were exposed by incisions through the muscles lying on their ventral surface; the front of the pelvic girdle was split with bone cutters approximately 1 cm on each side of the symphysis and removed. All soft structures lying ventral to the rectum were then carefully dissected away.

At this stage, if the rectum is lifted slightly, transillumination of the mesentery dorsal to the rectum reveals, enveloped in that mesentery, a fairly stout muscular band arising from the dorsolateral aspect of the rectum, about 5 cm above the anus, and running caudally and dorsally towards the coccyx (Fig. 1a); this is the rectococcygeus muscle. It should not be confused with the caudo-anal muscle, a much thinner muscular slip of very different pharmacological properties, which crosses it laterally.

For further exposure of the rectococcygeus, the rectum was transected 2-3 mm above the anus and was reflected cranially by cutting the mesentery as close as possible to the dorsal edge of the rectococcygeus. This exposes the distal end of the rectococcygeus as it disappears between the *flexor caudae* tendons towards its insertion into the third coccygeal vertebra; the precise insertion was determined in two animals by drilling a hole in the bone at the point of insertion or by transfixing the bone at that point with a steel needle and then macerating the soft tissues afterwards in hot 10% NaOH solution. At its rectal end the muscle consists of a broad, flattened band anatomically continuous with the dorsolateral part of the longitudinal muscle coat of the rectum. Figure 1b illustrates the boat-shaped appearance of the muscle at this end and the fact that in most, but not all, rabbits a distinct plane of separation is easily found by probing in the midline between the right and left halves of the muscle at its origin. The cleavage can be extended for about 1-1.5 cm along the muscle; beyond this point the two slips are firmly fused into a single round cord, which has to be bisected longitudinally when twin 'hemi-rectococcygeus' preparations are wanted.

Further experimental details

Each hemi-rectococcygeus was thoroughly de-sheathed *in situ* by dissecting off its mesenteric investments throughout its length. The preparations were then ligated and cut approximately 5 mm from origin and insertion, in order to avoid admixture of striated muscle fibres from skeletal muscles lying close to the coccygeal insertion of the muscle and of smooth muscle fibres from the longitudinal layer of the upper part of the rectum. Most of the experiments were performed on such bisected, hemi-rectococcygeus preparations but on a few occasions the whole of the rectococcygeus muscle was set up after careful desheathing.

For nerve-muscle preparations whole recto-coccygeus muscles were used, undivided. The mesentery containing the motor nerve supply was identified and detached from the wall of the pelvis; the muscle was excised into a dish with this triangular flap of mesentery and a ligature was placed at the apex of the flap.

Preparations were suspended at 35°C at an

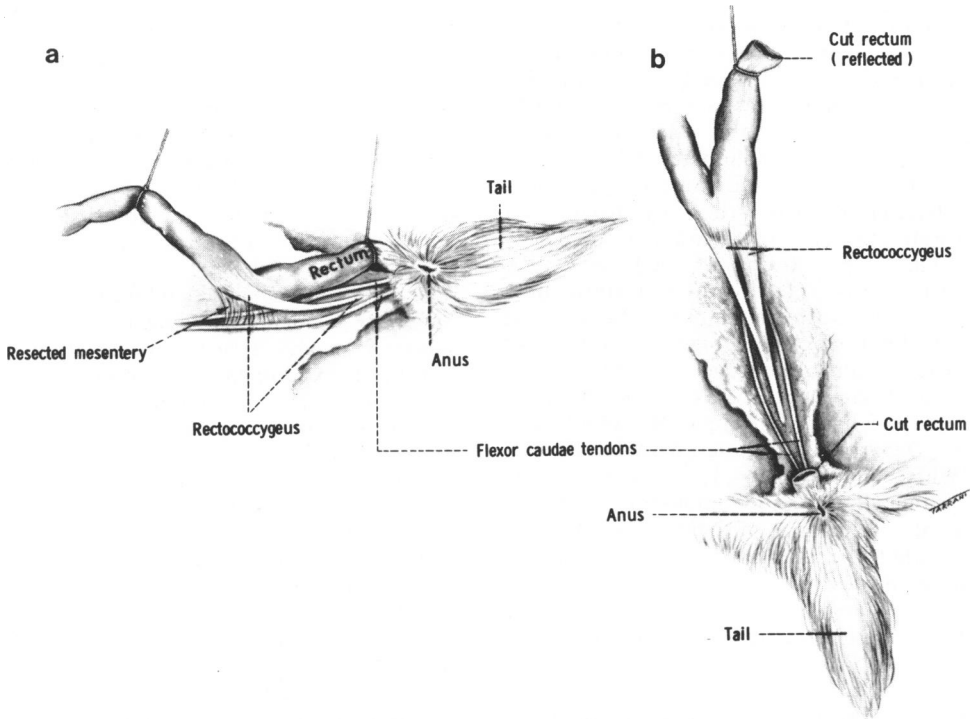

Fig. 1 Dissection of rabbit pelvis (semi-diagrammatic). (a) Right lateral view of the rectococcygeus muscle with resected mesentery containing the motor nerve supply. (b) Slightly oblique ventral view of rectococcygeus exposed by low transection and cranial reflection of the rectum.

initial tension of 1 g in Krebs-Henseleit solution of the following composition (mM): NaCl, 112.9; KCl, 4.69; $CaCl_2.2H_2O$, 2.52; KH_2PO_4, 1.18; $MgSO_4.7H_2O$, 1.5; $NaHCO_3$, 25.0 and glucose, 22.2. Organ baths with built-in vertical platinum-iridium electrodes were used, of volume usually 2 ml for desheathed preparations but 4.5 ml in the botulinum toxin experiments; nerve-muscle preparations were suspended in 4.5 or 5.5 ml baths. The Krebs-Henseleit solution in the organ baths and in the reservoirs was bubbled with a 95% O_2 and 5% CO_2 mixture. Muscular contractions were recorded isometrically by means of transducers of maximum loading capacity 25 g, coupled to potentiometric recorders (Servocorder model SR 652, Watanabe Instruments Corp., Tokyo); the time taken for a full scale deflection of the ink writers in these recorders is approximately 0.5 second. Intermittent field stimulation, usually at 1 min intervals, with single pulses or with trains of pulses delivered always at 10 Hz, was provided by a stimulator of low output impedance (Bell & Stein, 1971). Stimulation voltage was kept constant throughout each experiment. For nerve stimulation the ligated free end of the mesenteric flap was threaded through a moistened perspex-mounted electrode chamber placed above the level of the bath fluid. The two electrodes of platinum-iridium wire (SWG 30) each ended in a ring 3 mm in diameter; the separation of these two rings in the perspex mount was 6 mm. The distance between the electrodes and the muscle was 1-2 cm.

The following drugs were used: acetylcholine chloride (Hopkin & Williams), ATP (adenosine-5′-triphosphate disodium salt; Sigma), adrenaline hydrogen tartrate (B.D.H.), atropine sulphate (Hopkin & Williams), barium chloride (Hopkin & Williams), bradykinin triacetate (Sigma), BW 284C51 (1:5-bis-(4-allyl-dimethyl-ammonium-phenyl)-pentan-3-one dibromide; Burroughs Wellcome), carbachol (carbamyl choline chloride; Sigma), dimethyltubocurarine bromide (Burroughs Wellcome), guanethidine sulphate (Ciba), hexamethonium bromide (May & Baker), histamine dihydrochloride (Koch-Light), 5-hydroxytryptamine creatinine sulphate (Sigma), (−)-isoprenaline sulphate ($[isoprenaline]_2.H_2SO_4.2H_2O$; effective M.W. 278.3; Boots), pindolol (Sandoz), mepyramine hydrogen maleate (May & Baker), 5-methyl-furmethide (5-methyl-furfuryl-trimethyl-ammonium iodide; Dr H.R. Ing, Oxford), morphine

sulphate (Boots), pure muscarine chloride (Dr S. Wilkinson, Wellcome Research Laboratories, Beckenham), nicotine hydrogen tartrate (B.D.H.), (−)-noradrenaline bitartrate monohydrate (Koch-Light), pentolinium tartrate (May & Baker), phentolamine methanesulphonate (Ciba), (−)-phenylephrine hydrochloride (Sigma), physostigmine sulphate (Burroughs Wellcome), propranolol hydrochloride (I.C.I.), prostaglandins E_2 and $F_{2\alpha}$ (Dr J.E. Pike, Upjohn Co., U.S.A.), *iso*-OMPA (tetramono*iso*propylpyrophosphortetramide; Koch-Light), tetrodotoxin (Sankyo, Japan) and *p*-tyramine hydrochloride (Sigma). Non-molar concentrations refer to the respective salts.

Botulinum toxin, type A, was of a batch kindly supplied in 1965 by Dr J. Keppie, Microbiological Research Department, Experimental Station, Porton, Wilts., and kept at −15°C since then; its original potency was 41,667 mouse LD_{50}/mg. A 10-30 mg/ml solution of this powder in Krebs-Henseleit solution was prepared immediately before use.

Manometric determination of cholinesterase activities

Before excision the rectococcygeus was rendered visibly blood-free by perfusion with 2 litres of 0.9% w/v NaCl solution (saline) at 35°C through a cannula inserted into the abdominal aorta; a venous outlet was provided by an incision in the posterior vena cava. The excised, whole rectococcygeus muscle was desheathed, blotted between Whatman No. 1 filter papers and weighed; the weight of tissue obtained from adult rabbits was 70-160 mg. The tissue was stored frozen at −15°C for up to three days, and was then ground at room temperature with acid-washed sand in a mortar containing 25 mM $NaHCO_3$ solution in a volume chosen to give a final concentration of 25 mg of tissue per ml of homogenate. Aliquots of the whole, suspended homogenate were used immediately to determine enzyme activities by the Warburg manometric method at 37°C, with a gas phase of 95% N_2 and 5% CO_2. The rates of hydrolysis of 20 mM DL-acetyl-β-methylcholine and of 10 mM *n*-butyrylcholine were taken as measures of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities, respectively. The volumes of homogenate used per flask were adjusted according to the level of enzymatic activity, never exceeding ≡50 mg of tissue per flask; the final volume of fluid in each flask was made up to a total of 3 ml with 25 mM $NaHCO_3$. The CO_2 output from the 10th to the 40th min after the addition of substrate to the homogenate was measured and corrected for non-enzymatic CO_2 production and tissue blank. Values for the hydrolysis of the two substrates have been expressed as μl CO_2/100 mg wet tissue per hour.

The effects of pre-treatment with the following two anticholinesterases upon the cholinesterase activities were also investigated: (a) BW 284C51, a known selective inhibitor of AChE (Austin & Berry, 1953), which was added to homogenates 5 min before substrate, to give a final concentration of 1.8 μM of inhibitor; and (b) the selective, irreversible inhibitor of BuChE, *iso*-OMPA, added to homogenates 15 min before substrate to give a final concentration of 10 μM. For these experiments 2-3 muscles were pooled per homogenate; aliquots from the same homogenate were used to determine the AChE and BuChE activities in the absence and in the presence of the selective inhibitors.

In two other experiments (Table 1B) on the tissue from three animals, the rectococcygeus muscles, rendered blood-free by perfusion with Krebs-Henseleit solution at 35°C, were bisected and desheathed; each hemi-rectococcygeus was set up in an organ bath and subjected to field stimulation (1 pulse/min, 0.4 ms pulse width, 20 V) for 90 minutes. In each experiment one preparation was then exposed to *iso*-OMPA (50 μM) for 15 min and washed subsequently for 5 min, stimulation continuing throughout; the other, untreated half served as a control. The hemi-rectococcygeus preparations were then removed from the organ baths, blotted, weighed and frozen; their cholinesterase activities were determined subsequently as described above.

Estimation of acetylcholine content and release

Assays. For the determination of acetylcholine content or release bioassays were performed on plexus-free longitudinal muscle preparations from the guinea-pig ileum (Paton & Zar, 1968), sensitized by the addition of physostigmine (3.1 μM) to the Krebs-Henseleit solution in the reservoir. A threshold sensitivity to acetylcholine of the order of 1 ng/ml (5.5 nM) was achieved. The absence of nerve plexus was of considerable advantage for these assays: it permitted a tolerance of physostigmine, even in the high concentrations present in some of the test samples, and a relative steadiness of baseline not possible in plexus-containing preparations, which are thrown into spasm by physostigmine. In some cases twin, parallel preparations were suspended, each in a 0.7 ml organ bath, one with and the other without atropine, 0.72 mM. The fact that of the two preparations only the one without atropine was contracted by the test samples was taken to indicate that the activity of these samples was due

to acetylcholine; moreover, the contractions elicited by the test samples on the atropine-free preparation could later be abolished by subsequent addition of atropine to that preparation. In a few experiments mepyramine (0.25 μM) was also present in the reservoir, to exclude effects of histamine. The acetylcholine content of the test samples was estimated by matching assays in which the preparations were exposed to various doses of acetylcholine chloride alternating with the test samples at 2 min intervals.

Extraction of acetylcholine from rectococcygeus tissue. A modification of Feldberg's (1943) method was used. The muscle was removed from the animal, desheathed without bisection, blotted on Whatman No. 1 filter paper and weighed. The tissue was homogenized in a Griffith's tube with a mixture of 1 ml N/3 HCl and 2 ml of saline containing 30 μg of physostigmine sulphate. The homogenate was boiled gently for 60 s, cooled rapidly and stored at 2-4° C in a refrigerator for 30-60 minutes. Immediately before the assay, the cold homogenate was brought to pH 7.0 by electrometric titration with about 1 ml of *N*/3 NaOH; the pH was checked again after the extract had warmed to room temperature and any final adjustment to neutrality was then made. Phosphate buffer (0.2 ml of 0.02 M; pH 6.9) was added and the extract was allowed to settle before decanting the supernatant, aliquots of which were taken for assay.

Release of acetylcholine from rectococcygeus muscles during electrical stimulation. Desheathed 'donor' rectococcygeus muscle preparations, whole or bisected, were suspended in 1 ml organ baths and stimulated for 10-20 min with single pulses delivered at 1 min intervals; the contraction responses were recorded isometrically in the usual way, in order to check the effectiveness of the transmural excitation. Electrical stimulation was then discontinued and the preparations were exposed thereafter to physostigmine (3.1 μM). Following a >90 min period of equilibration, 'resting' test samples were collected: the tap was kept closed for periods of 3-10 min, after which the whole of the fluid bathing the preparation was drawn into a syringe and assayed for acetylcholine. After obtaining one or more such 'resting' samples, the rectococcygeus muscle was stimulated with 300-3000 pulses (0.4 ms; 10 Hz) and 3 or 5 min after the last pulse had been delivered the bath fluid was aspirated again for assay.

Plexus-containing preparations from guinea-pig ileum. Longitudinal muscle sheets were obtained by the method of Ambache (1954); their appearance is illustrated in Fig. 1 of the paper by Ambache, Freeman & Hobbiger (1971). Plexus-containing segments were selected.

Results

(A) Desheathed rectococcygeus preparations

Preparations were made from 64 rabbits. In seven of these experiments the whole rectococcygeus muscle was used without bisection; the remaining 57 were performed on desheathed, longitudinally bisected ('hemi-rectococcygeus') preparations. Forty-eight were entirely free from rhythmic activity throughout the course of experiments lasting several hours. The remaining 16 showed various degrees of spontaneous activity: some were quiescent for the first 2-4 h but developed small rhythmic activity thereafter; in the others the amplitude of the spontaneous rhythmic contractions was so great as to render difficult the assessment of some drug tests. When present, such rhythmic activity could not be abolished by tetrodotoxin or atropine. The impression was gained that carefully desheathed preparations were less prone to develop such spontaneous contractions.

Response to acetylcholine and to other spasmogens. Rectococcygeus preparations were relatively insensitive to most smooth muscle spasmogens, with the notable exception of muscarinic drugs. The threshold concentration for eliciting a contraction with acetylcholine could be as low as 55-110 nM (Figures 2 and 8). Graded responses elicited by increasing concentrations of acetylcholine in the range 0.055-275 μM are shown in the first panel of Fig. 2; it was not possible, however, to achieve a maximal contraction even with the highest of these concentrations (Figs 4 and 6), perhaps because of the cholinesterase content of this muscle (Table 1) and its relative thickness in large animals. Usually the response to acetylcholine consisted of a monophasic, sustained contraction, but occasionally this was preceded by a faster spike-like component, e.g. in Fig. 10, probably a manifestation of incipient rhythmic activity triggered off by the drug.

The effect of drugs which are not destroyed by cholinesterase, such as muscarine, 5-methylfurmethide (5-MF) or carbachol, resembled that of acetylcholine after physostigmine (see below) in that the contractions were always associated with the appearance of considerable rhythmic activity. Of these three drugs, pure muscarine was by far

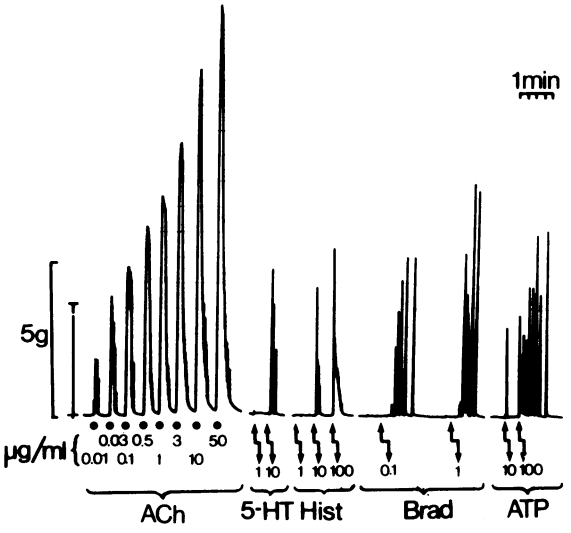

Fig. 2 Some pharmacological responses of the rabbit rectococcygeus muscle. Desheathed, longitudinally bisected ('hemi-rectococcygeus') preparation free from spontaneous rhythmic activity. Drug doses as indicated; contact times 30 s except for bradykinin, 1 minute. The responses to bradykinin (Brad) and to ATP occurred after these drugs were washed out. Hist, histamine; ACh, acetylcholine. For comparison, a twitch response (T) elicited by a single 0.2 ms pulse is shown at the beginning of the experiment.

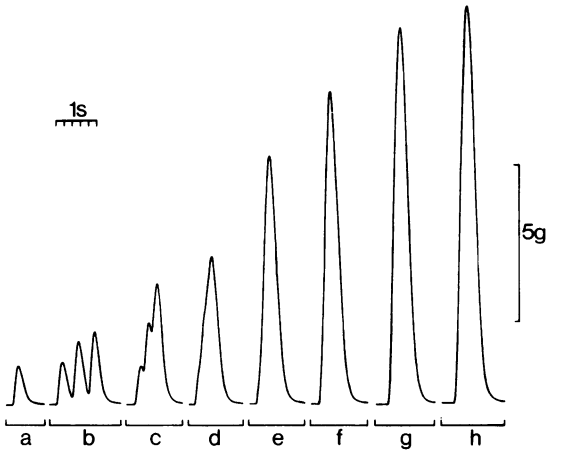

Fig. 3 Effect of stimulation frequency. Desheathed hemi-rectococcygeus; isometric recording at high paper speed. Pulse width, 0.2 ms; voltage supramaximal throughout. (a) Response to a single pulse, twitch duration: 2.5 s; (b-h) responses to trains of three pulses at various frequencies: (b) 0.5 Hz; (c) 1 Hz; (d) 2 Hz; (e) 5 Hz; (f) 10 Hz; (g) 20 Hz; (h) 50 Hz.

the most potent: in one comparison its activity was, on a molar basis, more than 10 times greater than that of 5-MF and 100-200 times greater than that of acetylcholine. Even after short exposures to low concentrations of muscarine, e.g. 23.8 nM (5 ng/ml), there was a marked and prolonged potentiation of single-pulse twitches.

The low activity of 5-hydroxytryptamine, histamine, bradykinin and ATP is seen in Figure 2. With bradykinin and ATP the responses were greatly delayed and consisted of spike-like contractions at irregular intervals. The fact, illustrated in Fig. 2, that these responses frequently occurred after the drugs were washed out would suggest that they were due to a triggering of latent rhythmic activity. Similar results were obtained with $BaCl_2$ (0.048-0.48 mM), which failed to induce contractions in the absence of electrical stimulation but considerably potentiated twitches evoked by transmural stimulation and triggered off atropine-resistant rhythmic activity. In some atropinized preparations histamine, too, triggered rhythmic activity which persisted after the drug was washed out.

Rectococcygeus preparations were also relatively insensitive to prostaglandins. In concentrations of 28.4-284 nM, prostaglandin E_2 caused a potentiation of twitches and of acetylcholine responses; and 0.284-1.42 μM produced a small increase in tone which was not maintained. In some experiments, prostaglandin E_2 generated much rhythmic activity. Twitch potentiation was recorded also with prostaglandin $F_{2\alpha}$ (14-28 nM).

Electrical stimulation. Twitches could be elicited by transmural stimulation with single pulses of 0.1-0.4 ms width. The duration of such a twitch, measured on fast-moving paper, was 2.5-3 s (Figure 3a). Figure 3b-h also shows the increase of the response to three pulses on varying the frequency between 0.5 and 50 Hz. Figures 4b and 6b illustrate the graded responses obtained at 10 Hz as the number of pulses was increased from 2 to 50, with the voltage constant.

The neurogenic origin of the twitch-response to these brief pulses of 0.1-0.4 ms duration was indicated by its extinction after the administration of tetrodotoxin (0.63-1.57 μM; 0.2-0.5 μg/ml). When the motor responses to 1-50 pulses were paralyzed by tetrodotoxin, the sensitivity of the smooth muscle to acetylcholine remained unaltered (Figure 4).

With wider pulses (0.4 ms) tetrodotoxin sometimes failed to eliminate completely the

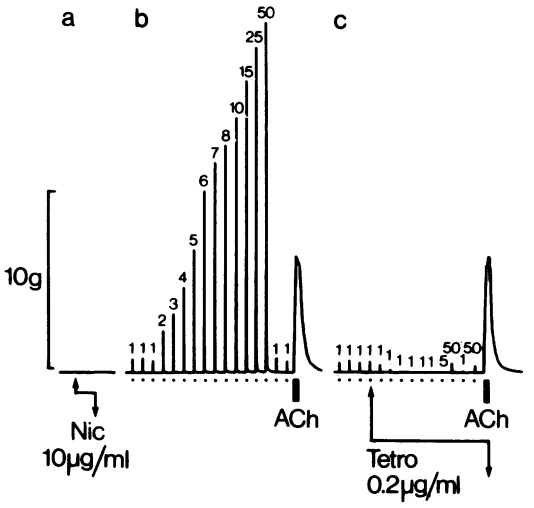

Fig. 4 Desheathed, rabbit hemi-rectococcygeus preparation free from spontaneous contractions. (a) Absence of response to nicotine (Nic, 10 µg/ml; 21 µM) administered for 2 min; (b) at the dots, twitches elicited by field stimulation (1-50 pulses); in this and in subsequent figures the voltage was kept constant throughout each experiment and, unless otherwise stated, twitch-intermittence was fixed at 1 min (serving as a time-scale), pulse width at 0.2 ms and frequency at 10 Hz. At the vertical bars, acetylcholine (ACh, 50 µg/ml; 275 µM) for 30 s; (c) between the arrows, tetrodotoxin (Tetro, 0.2 µg/ml; 0.63 µM).

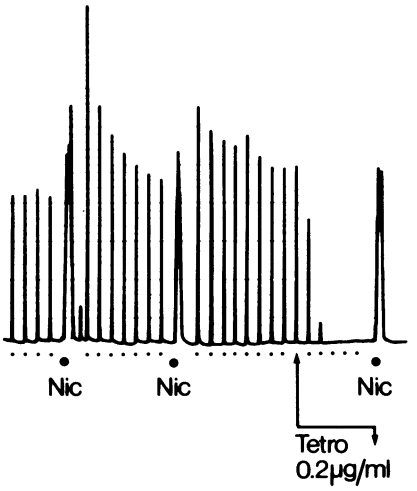

Fig. 5 Non-ganglionic action of nicotine in rabbit hemi-rectococcygeus preparation free from spontaneous rhythmic activity. The twitches were elicited by single pulses (small dots, 0.2 ms, one per minute). Large doses of nicotine (Nic, 50 µg/ml; 0.1 mM), administered for 30 s during interruptions of electrical stimulation. Between the arrows, tetrodotoxin (Tetro, 0.2 µg/ml; 0.63 µM) abolished the twitches without affecting the nicotine contraction. For explanation see text.

response to single pulses, suggesting the addition of a small myogenic component to the predominantly neurogenic contraction; in confirmation, this response was atropine-resistant. In subsequent experiments only neurogenic responses were investigated, by the use of pulses of 0.2-0.4 ms (usually 0.2 ms).

Nicotine. Motor responses could not be elicited with nicotine (2.1-21 µM; 1-10 µg/ml) (Fig. 4a), even after cholinesterase inhibition (Fig. 8d); high concentrations of nicotine (105-210 µM) induced small contractions followed by a potentiation of subsequent twitches. However, these nicotine contractions were resistant to tetrodotoxin (Fig. 5) and therefore could not be ascribed to ganglion stimulation; but they appeared to be mediated by a release of acetylcholine at the motor nerve endings because they were potentiated by physostigmine and abolished by atropine.

Ganglion-blocking drugs. Contractions induced by field stimulation were not reduced, but rather augmented, by hexamethonium (0.28 mM), pentolinium (0.19 mM), dimethyltubocurarine (up to 61.5 µM) or prolonged application of nicotine (1 mM). These drugs excluded any contribution from preganglionic nerve fibres; and the use of dimethyltubocurarine also excluded the presence of skeletal muscle fibres.

Evidence for cholinergic motor transmission

(a) Susceptibility to atropine. In preparations free from spontaneous activity, the twitches elicited by pulses of 0.2-0.4 ms width were always abolished by atropine (0.14-1.4 µM). Thus in Fig. 6, although after atropine (0.14 µM; 0.1 µg/ml) there was still a small response to the high dose of acetylcholine (275 µM), the originally much larger twitches induced electrically by 20-50 pulses were completely paralyzed. At a greater pulse width (0.5-1 ms) twitch extinction by atropine was incomplete, particularly with long trains of 10-50 pulses; these small, atropine-resistant contractions were not suppressed by tetrodotoxin and were therefore myogenic.

In a few preparations which were prone to spontaneous activity even before atropine, electrical stimulation (0.2-0.4 ms) after the

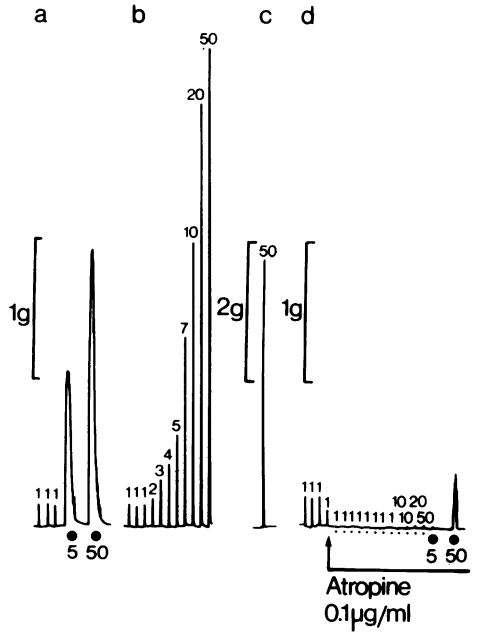

Fig. 6 Twitches elicited by single pulses or by trains of 2 to 50 pulses. At the dots (•), acetylcholine (ACh, 5 or 50 µg/ml; 27.5 or 275 µM) administered for 30 s during interruptions of electrical stimulation. The response to 50 pulses in (b) was off scale and was therefore recorded again in (c) at half the normal sensitivity (see 2 g calibration); (d) return to initial recording sensitivity. At the arrow, atropine (0.1 µg/ml; 0.14 µM) to the end of the experiment, abolishing all responses to 1-50 pulses and to acetylcholine, 5 µg/ml; the effect of acetylcholine, 50 µg/ml, was greatly reduced.

administration of atropine appeared to trigger off contractions. These responses were also unaffected by tetrodotoxin; in some experiments they were of constant height, whether induced by 2, 10 or 20 pulses.

(b) Paralysis by botulinum toxin. Exposure for 30 min to 1-3 mg of Type A botulinum toxin led to a subsequent gradual paralysis of motor transmission. Whereas in untreated preparations stimulated at 1 min intervals motor transmission remained unimpaired for several hours, in preparations treated with botulinum toxin virtual extinction of twitches, elicited by 1-50 pulses of 0.2-0.3 ms width, occurred within 1.5-2.5 h after removal of the toxin. The motor paralysis produced by the toxin was not associated with any reduction in the contractile responses to acetylcholine.

(c) Potentiation by physostigmine. In the absence of electrical stimulation, physostigmine (7.7 µM; 5 µg/ml) produced a delayed slow contraction with superimposed rhythmic activity, which took 10-15 min to subside after the drug was washed out (Figure 7a). When the baseline was regained, responses both to field stimulation and to acetylcholine showed considerable potentiation (Figure 7d and b). The spasmogenic and the potentiating actions of physostigmine were abolished by atropine (Fig. 7e and f) but were not affected by hexamethonium (0.28 mM).

(d) Presence of acetyl- and butyryl-cholinesterases (AChE and BuChE). The effects of physostigmine pointed to the presence of cholinesterase(s) in this tissue. AChE and BuChE activities were measured manometrically, with acetyl-β-methylcholine and *n*-butyrylcholine as selective substrates, and are given in Table 1. They were of the same order as found previously in plexus-free longitudinal muscle tissue from the guinea-pig colon (Ambache *et al.*, 1971) and are probably slightly higher than the actual activities present in the tissue, because AChE and BuChE are each capable of hydrolysing the substrate of the other, though very slowly (Augustinsson, 1963; Ambache *et al.*, 1971). This and perhaps also the fact that inhibitor-specificities are not absolute could account for the marginal inhibitions of each enzyme by the selective inhibitor of the other. Thus, BW 284C51 (1.8 µM; 1 µg/ml), a selective inhibitor of AChE, produced a 90% inhibition of the acetyl-β-methylcholine hydrolysis with only 12% inhibition of the *n*-butyrylcholine hydrolysis; this choice of BW 284C51 concentration was governed by analogous results illustrated in Fig. 2 of the paper by Ambache *et al.* (1971), which shows the degree of inhibition of intestinal AChE at eight concentration levels of BW 284C51. In contrast, the selective inhibitor of BuChE, *iso*-OMPA (10 µM; 3.5 µg/ml), produced an 87% inhibition of the *n*-butyrylcholine hydrolysis with only 8% inhibition of the acetyl-β-methylcholine hydrolysis.

(e) Effect of selective inhibitors of AChE and BuChE upon the motor transmission. In 10 transmurally stimulated preparations the administration of either selective anticholinesterase alone, in concentrations which, as shown in Table 1, produce 87-90% inhibition of the respective enzymes, caused little or no change in single-pulse twitches, but responses to trains of 2-5 pulses were slightly augmented. There was, for example, little or no change in the height of single pulse twitches even after soaking for 60-70 min in BW 284C51.

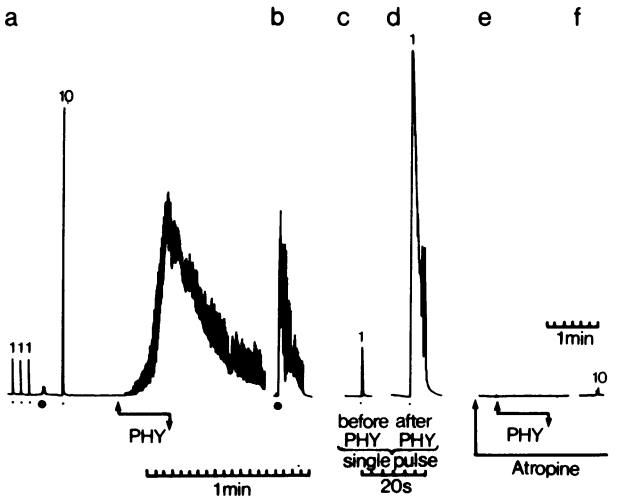

Fig. 7 Rabbit, desheathed, *whole* rectococcygeus preparation free from spontaneous contractions. The twitches were elicited by single pulses or trains of 10 pulses, as indicated by the numeral superscripts. At the large dots, acetylcholine (ACh, 50 ng/ml; 275 nM) for 30 seconds. Between the arrows, physostigmine (PHY, 5 μg/ml; 7.7 μM) for 6 minutes. (a) Slowly developing contractile effect of physostigmine in the absence of electrical stimulation, subsiding 11 min after the drug was washed out; (b) potentiated acetylcholine response 14 min after removal of physostigmine; (c and d) comparison of a single-pulse twitch recorded at higher paper speed before (c), and 12 min after the removal of physostigmine (d), note the increase in height and duration of the twitch-response at d and its multiphasic character; (e and f) extinction of physostigmine-induced contraction by atropine (0.5 μg/ml; 0.72 μM) administered 17 min earlier and virtual paralysis of the motor response to 10 pulses.

But the addition of the second cholinesterase-inhibitor to the first, and their presence together, produced an eight- to fifty-fold potentiation, in height and in duration, of single-pulse twitches. In five of these experiments BW 284C51 (1.8 μM), and in the remaining five *iso*-OMPA (10 μM), was given first; the order in which the cholinesterases were inhibited appeared to be immaterial (Figure 8). Unlike physostigmine, the administration of these anticholinesterases alone or together did not induce any contraction or rhythmic activity in the absence of electrical stimulation.

Table 1 Cholinesterase activities of rectococcygeus homogenates

	AChE		*BuChE*	
A: Unstimulated tissue				
Control rates: (μl CO_2 100 mg^{-1} wet wt. h^{-1}) (mean of six different tissue pools with s.e. mean)	101 ± 12		1025 ± 53	
Inhibition in the presence of BW 284C51 (1.8 μM)		90%		12%
Inhibition in the presence of *iso*-OMPA (10 μM)		8%		87%
B: Electrically stimulated preparations*				
Control rates: (μl CO_2 100 mg^{-1} wet wt. h^{-1})	90		757	
Inhibition after pre-treatment with *iso*-OMPA (50 μM)		8%		91%

Manometric determinations of the rates of hydrolysis of acetyl-β-methylcholine (as a measure of acetylcholinesterase (AChE) activity) and of *n*-butyrylcholine (as a measure of butyrylcholinesterase (BuChE) activity) by whole homogenates of blood-free rabbit rectococcygeus muscle. Except where stated otherwise, values represent the mean of two determinations on different tissue samples.
* See methods section.

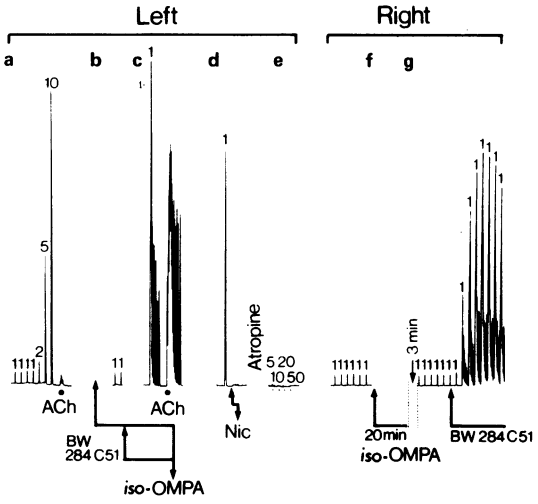

Fig. 8 Complementary role of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE): substantial potentiation of motor transmission occurs only when both cholinesterases are inhibited. Twin desheathed hemi-rectococcygeus preparations from the same rabbit; both free from spontaneous contractions. Twitches elicited by field stimulation at 1 min intervals with single pulses throughout, except for the responses to 2-10 pulses in (a); pulse width, 0.4 ms. On the left preparation, AChE-inhibition by BW 284C51 (1.8 μM; 1 μg/ml) preceded BuChE-inhibition by *iso*-OMPA (10 μM; 3.5 μg/ml); on the right, the order was reversed.

Left preparation. At the dots, acetylcholine (ACh, 20 ng/ml; 110 nM) for 30 seconds. At the large arrows, BW 284C51, alone for 10 min and then together with *iso*-OMPA. (a) Initial response to 1-10 pulses and to acetylcholine; (b) absence of twitch potentiation after 9 and 10 min in BW 284C51 alone; (c) great potentiation of twitch and of acetylcholine response after 13 min exposure to the two anticholinesterases together; (d) 10 min after the anticholinesterases were washed out, twitch potentiation still present but no response to nicotine (5 μg/ml; 10.5 μM) given for 1 minute; (e) complete abolition by atropine (1.4 μM) of all responses to 5-50 pulses.

Right preparation. (f) Before anticholinesterases; between f and g, treatment with *iso*-OMPA for 20 minutes; (g, beginning 3 min after the *iso*-OMPA was washed out) shows the absence of twitch potentiation until BW 284C51 was introduced.

In three experiments, muscles were exposed for 15 min to *iso*-OMPA (50 μM), which by itself produced a mean increase of 25% in the response to single pulses, and were then thoroughly washed in the organ-bath for the next 5 min, so as to remove the unbound *iso*-OMPA. Thereafter, the three treated preparations were pooled into two samples for manometric determination of the BuChE activity, which was compared with that of the three contralateral, untreated preparations, also pooled into two samples. Although, as shown in Table 1B, this treatment with *iso*-OMPA had resulted in an irreversible BuChE-inhibition of 91%, there was remarkably little potentiation of motor transmission.

(f) Presence of acetylcholine and its release upon electrical stimulation. The acetylcholine content of this tissue, expressed in terms of the chloride salt, was, in two experiments, 0.82 and 0.94 μg/g.

The release of acetylcholine from desheathed, whole rectococcygeus preparations treated with physostigmine was small or barely detectable in the absence of electrical stimulation ($<$0.5 ng per muscle per 8 or 10 min collection period). In two experiments, transmural stimulation with trains of 300-3000 pulses of 0.4 ms width repeatedly resulted in large increases of acetylcholine output, e.g. using 3000 pulses, from $<$0.5 ng to 5.7 ng for an 8 min and 6.6 ng for a 10 min collection period.

Absence of inhibition by morphine or ATP. In contrast to its effect on transmurally stimulated ileum (Schaumann, 1956, 1957; Paton, 1957; Paton & Zar, 1968) morphine (0.65-13 μM) did not, in the rectococcygeus, inhibit motor responses elicited by field stimulation of low frequency (0.02 Hz).

Although ATP (0.18-45.4 μM) profoundly inhibited the cholinergic motor transmission in plexus-containing longitudinal muscle preparations from guinea-pig ileum, it did not affect twitches elicited by 1-5 pulses (0.2 ms) in rabbit rectococcygeus preparations.

Effects of drugs acting upon adrenoceptors. No trace of any adrenergic component could be detected in the motor transmission, which was unaffected by guanethidine (5 μM); or by phentolamine (5.3 μM), propranolol (6.8 μM) or pindolol (8.1 μM).

Phenylephrine. The scantiness of motor α-adrenoceptors in the rectococcygeus muscle was indicated by its relative insensitivity to the selective α-adrenoceptor stimulant, phenylephrine: high concentrations (49 μM) were required to induce maintained contractions. With lower concentrations (0.49-4.9 μM) rhythmic movements were initiated or augmented and there was considerable potentiation of electrically induced twitches and of responses to acetylcholine (Figure 9). Pretreatment with the α-blocking agent, phentolamine (2.65-5.3 μM) prevented

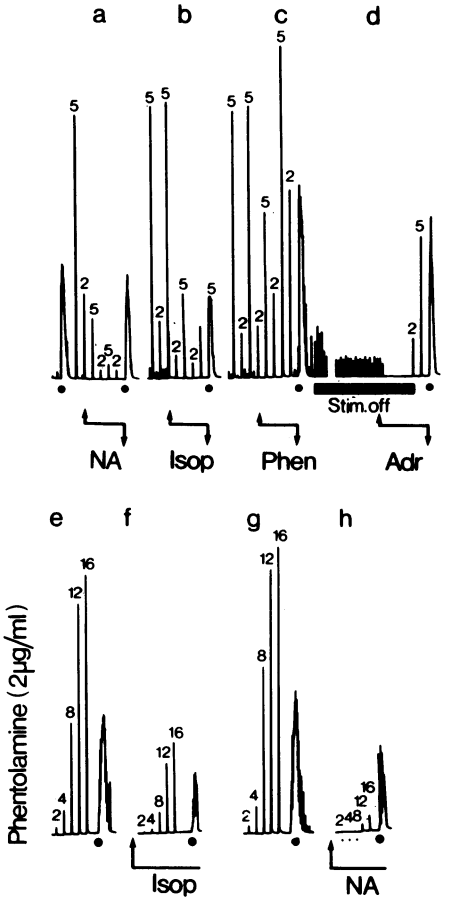

Fig. 9 *(a-d).* Hemi-rectococcygeus with slight spontaneous activity. Response to 2 or 5 pulses, alternating at 1 min intervals. Stimulation interrupted during bar (stim. off) and, at the dots (•), for 30 s exposures to acetylcholine (ACh; 1 µg/ml; 5.5 µM). Between the arrows, 6 min exposures to 1 µg/ml of (a) noradrenaline (NA; 3 µM); (b) isoprenaline (Isop; 3.6 µM); (c) phenylephrine (Phen; 4.9 µM); and (d) adrenaline (Adr; 3 µM). Note increased rhythmic contractions following phenylephrine and their subsequent inhibition by adrenaline.
(e-h). Hemi-rectococcygeus from another rabbit; no rhythmic activity. Phentolamine (5.3 µM; 2 µg/ml) present throughout. Each panel shows the responses to trains of 2-16 pulses delivered at 30 s intervals and, at the dots (•), to 5-methylfurmethide (5-MF; 0.37 µM) injected for 30 seconds. Panels (e) and (g) controls; (f and h) effects of equimolar (15 µM) isoprenaline and noradrenaline, respectively; note that the inhibitory activity of noradrenaline against motor transmission is greater than that of isoprenaline. See text.

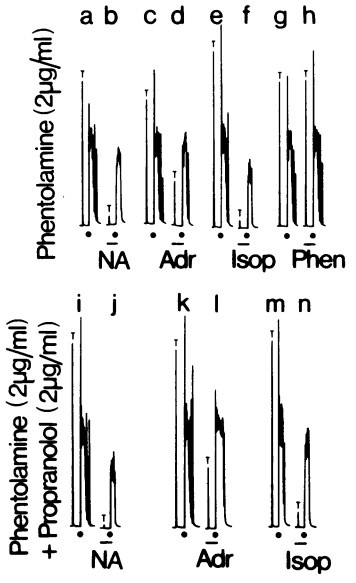

Fig. 10 Rabbit hemi-rectococcygeus preparation free from rhythmic activity. Each panel shows a twitch (T) elicited by a single 0.2 ms pulse and a response to acetylcholine (ACh; 0.3 µg/ml; 1.65 µM) given for 30 s at the dot (•). Phentolamine (5.3 µM; 2 µg/ml) was present throughout and propranolol (6.8 µM; 2 µg/ml) after panel h. At the bars, exposures to 1 µg/ml of noradrenaline (NA; 3 µM), adrenaline (Adr; 3 µM), isoprenaline (Isop; 3.6 µM) or phenylephrine (Phen; 4.9 µM), each administered 2 min before the twitch. Note the marked twitch-inhibitions (without comparable reduction in acetylcholine responses) produced by noradrenaline and by isoprenaline in the presence of both α- and β-blockers; the phenylephrine was inactive at h.

these effects, but phenylephrine-induced rhythmic activity could not be suppressed by subsequent addition of phentolamine.

Isoprenaline. In concentrations of 0.36-36 µM isoprenaline produced dose-related inhibitions of spontaneous rhythmic activity, of motor transmission and of contractions elicited by muscarinic drugs (Figures 9-11). In phentolamine-treated preparations, propranolol (6.8 µM) or pindolol (12.1-16.1 µM) reduced the inhibitory effect of isoprenaline on contractions elicited by muscarinic drugs (Figs 10 and 11); pindolol also reduced the isoprenaline inhibition of motor transmission (Fig. 11), but propranolol hardly at all (Figure 10).

Noradrenaline. Contrary to McKirdy's (1972) finding it was not possible to evoke contractions

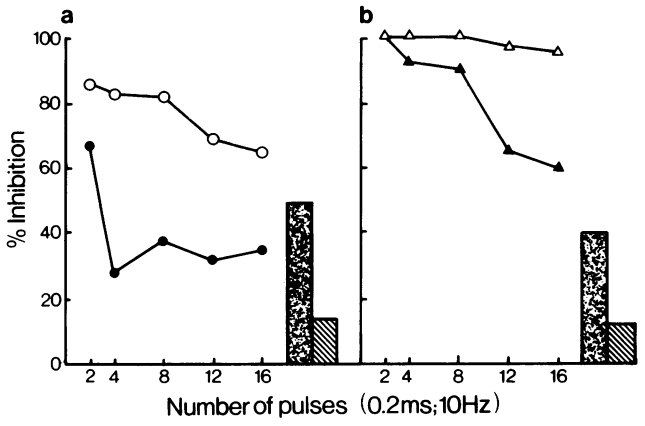

Fig. 11 Pindolol partially antagonizes noradrenaline- and isoprenaline-inhibitions of motor transmission and of contractions induced by 5-methylfurmethide (5-MF; 0.37 μM). Rabbit hemi-rectococcygeus preparations, free from rhythmic activity, stimulated transmurally at 30 s intervals with 2-16 pulses (0.2 ms; 10 Hz); phentolamine (5.3 μM) present throughout. Curves show the percentage inhibitions of motor transmission which were produced by equimolar concentrations (15 μM) of (a) isoprenaline or of (b) noradrenaline before (○,△) and after (●,▲) >0.5 h exposures to pindolol (16.1 μM; 4 μg/ml); despite a 1.1 to 1 molar ratio of antagonist to agonist, the inhibitory effect of isoprenaline on motor transmission was only partially antagonized by pindolol, and that of the more active noradrenaline even less so. Histograms show the percentage inhibitions of 5-MF contractions, produced by noradrenaline and isoprenaline before (stippled columns), and in the presence of (hatched columns), pindolol.

with noradrenaline (0.3-30 μM), which invariably produced dose-related inhibitions of motor transmission, of spontaneous movements, and to a lesser extent, of contractions elicited by muscarinic drugs; of these, acetylcholine contractions were the least inhibited, sometimes not at all.

Phentolamine (5.3 μM) did not prevent the inhibitory action of noradrenaline. In phentolamine-treated preparations it was noticed that the inhibition of motor transmission by noradrenaline was often, but not always, more marked with short than with long trains of pulses and was disproportionately greater than the inhibition of contractions elicited by muscarinic drugs, particularly by acetylcholine (Figures 9 and 10). This suggested that part of the effect of noradrenaline could be due to a presynaptic action. The following observations would support such a view.

Twitch-inhibition produced by noradrenaline remained virtually unchanged after β-adrenoceptor blockade with propranolol (6.8 μM) although it was reduced by pindolol (8.1-40.3 μM) (Figure 11). Moreover, both in the absence and in the presence of β-blockers, the inhibitory potency of noradrenaline was always greater than that of isoprenaline (e.g. Fig. 11), which is the reverse of the usual order of potency at β-adrenoceptors; for instance, the ratio between the molar concentrations of (−)-noradrenaline and (−)-isoprenaline required to produce a 50% inhibition of 2-pulse twitches was 1 : 2.6.

Adrenaline. Variable results were obtained because this drug appeared to possess the contrasting actions of, on the one hand, phenylephrine and, on the other, of noradrenaline and isoprenaline. Thus, the effect on motor transmission could consist either of a potentiation or an inhibition. Spontaneous rhythmic movements were suppressed during exposure to adrenaline but reappeared, considerably accentuated, after the drug was washed out. Acetylcholine contractions were either unaffected (Fig. 10) or, rarely, potentiated (Fig. 9), or reduced to a variable extent in different preparations; the reductions, when present, were antagonized by propranolol. The preparations were contracted only by high concentrations of adrenaline (30 μM). As with phenylephrine, the motor and potentiating actions of adrenaline were abolished by phentolamine (2.65-5.3 μM) and thereafter adrenaline resembled noradrenaline and isoprenaline in its inhibitory effects.

Tyramine. It was of interest to examine whether the profound inhibitory action of noradrenaline upon the motor transmission in the

rectococcygeus muscle could be reproduced by an indirect sympathomimetic drug such as tyramine, which acts by releasing endogenous noradrenaline. But muscle tone and motor transmission remained unaffected during 6 min contacts with tyramine (5.8-288 μM).

(B) Nerve-muscle preparations

Nerve twigs can be seen travelling towards the rectococcygeus muscle through the adjoining mesentery. The retention of a large flap of this mesentery, the free end of which could be threaded into the perspex electrode chamber, provided preparations suitable for stimulation through the extrinsic nerve supply as well as transmurally; the external electrodes were placed as far away from the muscle as the stretched mesentery would allow, usually at a distance of 1-2 cm. Stimulation of these nerves with 0.1-0.5 ms pulses elicited contractions which were pharmacologically indistinguishable from the responses to transmural stimulation. Several tests showed that the muscle was being excited through its extrinsic nerve supply: (a) responses were abolished either by crushing or by cutting the mesentery between the external electrodes and the muscle; (b) the voltage required was considerably lower for the mesenteric nerve than for transmural stimulation; (c) reversing electrode polarity did not affect the responses to transmural stimulation but had a pronounced effect on the effectiveness of mesenteric nerve stimulation, a sign of anodal block.

It was noticed that with short trains of pulses transmural stimulation invariably elicited bigger tension responses than supramaximal nerve stimulation, indicating that only a proportion of the motor nerve twigs could be picked up by the external electrodes at the apex of the mesenteric flap and confirming the visual observation that the nerve supply to the muscle consisted of two or more nerve twigs, often widely separated in the mesentery.

In eight experiments in which such preparations were stimulated either through their extrinsic nerves or transmurally, in alternation at intervals of 1 min, most of the pharmacological results already obtained on desheathed preparations and described in Section A of this paper were confirmed. In addition, there was no evidence for the presence of ganglionic relays between the external electrodes and the muscle, i.e. in the last 1-2 cm of the extrinsic motor nerve pathway. Thus, motor transmission was not depressed by ganglion-blocking concentrations of pentolinium, dimethyltubocurarine or nicotine, although it could be paralyzed by tetrodotoxin. Physostigmine had a very marked potentiating effect. With atropine there was a complete extinction of motor transmission: no response could be obtained even with 99 pulses (10 Hz). The transmission was not affected by morphine, phentolamine or propranolol; it was depressed by noradrenaline and by isoprenaline.

Pronounced rhythmic activity was present in two of these experiments.

(C) Some properties of the adjacent caudo-anal muscle

During the dissection of the rabbit rectococcygeus an adjacent, thin slip of smooth muscle was found crossing it laterally and running from the fourth sacral or first coccygeal vertebra to the anal region. Langley & Anderson (1896) refer to a muscle in this situation as the 'caudo-anal'; in our experience this muscular band was usually narrower and less well defined than shown in Plate 3, Fig. 1 of their paper.

Isolated caudo-anal preparations could be made to contract by drugs; but, on transmural stimulation with trains of 5 or 10 pulses of 0.5-1 ms width delivered at 10 Hz, there was no motor response in seven preparations from different animals and only feeble contractions in four others. The results suggest that this muscle receives a poor motor innervation in the rabbit, unlike the anococcygeus in the rat (Gillespie, 1972), to which it corresponds.

The preparations were contracted by noradrenaline (0.15-1.5 μM) and by histamine (0.54-1.1 μM). In the contracted state produced by either of these drugs the previously ineffective electrical stimulation evoked large relaxations, which persisted in the presence of propranolol (6.8 μM) but were abolished by tetrodotoxin (0.63 μM). Contractions produced by noradrenaline or by field stimulation were blocked by phentolamine (5.3 μM), suggesting that what motor nerve fibres are present are adrenergic; after phentolamine field stimulation produced large relaxations, if tone was present.

Acetylcholine was virtually inactive; when administered at the height of a noradrenaline contraction it produced a slight relaxation in doses of 27.5-55 μM.

Discussion

There is abundant evidence that the rabbit rectococcygeus muscle is free from imbedded autonomic ganglion-cells. Firstly, the preparations could not be made to contract by nicotine, or by $BaCl_2$, in doses which are adequate to excite autonomic ganglion-cells in the intestine and

elsewhere. The fact that the small contraction induced by much larger doses of nicotine could not be abolished by tetrodotoxin shows that this response occurs independently of conducted action potentials and is therefore not due to ganglionic excitation; its potentiation by physostigmine and reduction by atropine suggest that it is another example of the action of nicotine at nerve terminals. Secondly, the ganglion-blocking agents, hexamethonium, pentolinium and dimethyltubocurarine, did not produce any diminution in responses elicited either by transmural stimulation or, more significantly, by stimulation of the motor nerve fibres in the mesentery. The experiments in which the stimulating electrodes were applied to the mesentery at a distance of 1-2 cm from the muscle indicate that the ganglionic relays in this parasympathetic nerve are situated well away from the muscle itself. These observations are consistent with McKirdy's (1972) negative histological finding, when looking for imbedded ganglion-cells in this muscle. As mentioned in the introductory section, Langley & Anderson (1896) described the presence of a 'considerable ganglion' in the pelvic nerve branch supplying the rectococcygeus; it would appear from our results that most, if not all, of the preganglionic fibres running to this muscle relay in that relatively distant ganglion.

The rectococcygeus is therefore exceptional among parasympathetically innervated smooth muscles in possessing a reasonably long post-ganglionic nerve supply, analogous only to the innervation of the sphincter pupillae. Technical difficulties have so far precluded the making of isolated ciliary nerve-iris preparations. The easier accessibility and greater robustness of the rectococcygeus would seem to render it the preparation of choice for the study of parasympathetic post-ganglionic transmission to smooth muscle. The interpretation of pharmacological results is greatly simplified by the absence of ganglion-cells.

Acetylcholine and cholinesterases were found in extracts of this tissue. The release of acetylcholine from preparations treated with physostigmine was barely detectable in the resting state but was greatly augmented by electrical stimulation. Our other results leave no doubt that the motor transmission at these post-ganglionic endings is, in fact, cholinergic: transmission was potentiated by anticholinesterase drugs and was abolished by atropine or by botulinum toxin. It is interesting to note that this muscle is almost insensitive to the wide range of smooth muscle spasmogens tested by us, with the exception of acetylcholine, its motor transmitter, and related muscarinic drugs.

The manometric determinations showed that both acetyl- and butyryl-cholinesterases are present, as in the longitudinal muscle coat of the small and large intestine (Ambache *et al.*, 1971). The absence, from the rectococcygeus muscle, of Auerbach's plexus and of imbedded sacral ganglionic relays makes it possible to investigate, for the first time in a pure state, the mechanism of termination of motor transmission at a parasympathetic post-ganglionic neuromuscular junction. Our results suggest a complementary role for AChE and BuChE in this process: the motor transmission remained virtually unaffected even after 87-90% inhibition of one or other of these enzymes, and the massive potentiation of single-pulse twitches, seen in Fig. 8, occurred only when both cholinesterases were substantially inhibited. It could be argued that this twitch potentiation was due to increased inhibition of only one enzyme when the second anticholinesterase was added. The results exclude such a possibility: it can be estimated from Table 1 that the administration of the second anticholinesterase would increase the 87-90% inhibition of AChE or of BuChE by an insignificant amount.

It is interesting to note that the combined treatment with these two anticholinesterases did not reproduce the tonic effect of physostigmine illustrated in Figure 7a. This could mean either that there is a difference in influence on the resting release of acetylcholine between the combination BW 284C51 plus *iso*-OMPA, on the one hand, and physostigmine on the other; or, alternatively, that physostigmine is capable of exerting some direct action on muscarinic receptors.

Although it is likely that atropinesterase was present in the serum of many of these 64 unselected rabbits, transience of atropine blockade was never observed in these isolated muscles. The traces of blood remaining in the excised tissue are probably insignificant and would soon be expelled from the cut vessels by the first few electrically induced contractions of the muscle in the organ-bath.

The unfailing effectiveness of atropine in abolishing completely the motor transmission at the sacral post-ganglionic endings in the rectococcygeus muscle contrasts sharply with the well-known atropine-resistance of bladder contractions evoked by pre- or post-ganglionic stimulation, a phenomenon which has been attributed to the presence of non-cholinergic post-ganglionic neurones in the sacral nerve pathway to the bladder (Ambache & Zar, 1970). The full susceptibility of the motor transmission in the rectococcygeus muscle to atropine blockade indicates that, in its sacral motor pathway, such

non-cholinergic neurones are not present.

The rectococcygeus appears to be a continuation of the longitudinal muscle layer of the intestine. Ambache, Verney & Zar (1970) have shown that tetanic field stimulation of intestinal longitudinal muscle sheets gives rise to two types of atropine-resistant neurogenic contractions obtainable in plexus-containing but not in plexus-free preparations. The failure to elicit such atropine-resistant neurogenic responses in the rectococcygeus preparation can again be correlated with the absence of Auerbach's plexus and is consistent with the earlier findings.

With catecholamines three different effects were discerned: (i) Activation by phenylephrine of scanty α-receptors in the muscle fibres, resulting in a potentiation of responses to motor nerve stimulation and to muscarinic drugs. This effect was antagonized by phentolamine. (ii) An inhibition by isoprenaline of motor transmission and of responses to muscarinic drugs, which was partly antagonized by β-blockers and therefore presumably due to β-receptors in the muscle. (iii) Additional inhibition, by noradrenaline, affecting motor transmission to a greater extent than muscarinic contractions, and therefore suggestive of an additional action on motor nerve terminals. In contradistinction to Paton & Vizi's (1969) results, this presynaptic effect could not be designated an α-action because it was not abolished by phentolamine and because the inhibitory potency of the specific α-stimulant, phenylephrine, was low. Nor could it be designated a β-effect because the potency of noradrenaline was here greater than that of isoprenaline and because this action was not blocked by propranolol and only weakly antagonized by pindolol (Saameli, 1967).

These effects of exogenous catecholamines do not imply the presence of an adrenergic innervation in this muscle since tyramine, which is known to release endogenous noradrenaline, had no action.

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