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Relaxations mediated by adrenergic and non-adrenergic nerves in human isolated taenia coli

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In human taenia coli electrical field stimulation after cholinergic blockade excited mainly non-adrenergic inhibitory nerves. Adrenergic relaxations during stimulation were demonstrated only with short electrical pulses at high frequencies or with ganglion stimulants. Reduction by tetrodotoxin of relaxations to indirectly acting sympathomimetics supports the histochemical finding that colonic adrenergic nerves are mainly preganglionic, and possibly synapse with non-adrenergic inhibitory nerves. When the latter undergo direct maximal stimulation, responses to adrenergic nerve excitation are masked.

Relaxations of human taenia coli produced by ganglion-stimulating drugs seem mainly adrenergic, whereas those elicited by electrical stimulation have usually proved resistant to conventional adrenergic blocking drugs (Bucknell & Whitney, 1964; Bucknell, 1965, 1966; Wright & Shepherd, 1966; Crema, del Tacca & others, 1968; Stockley & Bennett, 1974). However, Bucknell (1965) reported that relaxations elicited at 8–15 Hz in longitudinal muscle strips from both rabbit and human colon (in the presence of hyoscine to prevent cholinergic contractions) were reduced by bretylium, guanethidine or a combination of dibenamine and pronethalol. Bucknell's results were obtained with 0·2 or 0·3 ms pulses whereas in the more recent studies 1 ms pulses were employed. The importance of short pulses to detect the responses of the adrenergic nerves to electrical field stimulation is demonstrated in this paper and an explanation suggested.

METHODS

Macroscopically normal specimens of human colon (ascending, transverse and sigmoid) were obtained at operation for colonic carcinoma or Crohn's disease and placed in Krebs solution as soon as possible. Strips 1–2 mm wide and 10–20 mm long were cut from the full thickness of the taeniae in the direction of the longitudinal muscle fibres as described previously (Stockley & Bennett, 1974; Bennett & Stockley, 1975). They were set up either immediately or after overnight storage (Krebs solution, 4°) in 10 ml isolated organ baths containing Krebs solution at 37° bubbled with 5% CO_2 in O_2. The load on the tissue was 1 g, and isotonic contractions were registered on pen recorders after 10–50 fold magnification. When electrically-induced responses had been obtained using 1 ms pulses at 4Hz, hyoscine 1 or 2 μg ml^{-1} was added to the Krebs solution to prevent cholinergic contractions.

Square-wave pulses of alternating polarity were delivered across the tissue suspended vertically between pairs of 5 mm^2 silver electrodes 1 cm apart (20 s trains every 4 min, voltage drop of 17 V measured in Krebs solution). The stated frequency of stimulation is the total number of positive and of negative pulses of 0·1, 0·3, 0·5 or 1 ms duration. Responses elicited in the absence and presence of adrenergic blockers were compared. We distinguished between neuronal and non-neuronal components of adrenergic relaxations to tyramine, nicotine and noradrenaline by using tetrodotoxin to inhibit nerve activity.

Drugs used were adenosine 5′-triphosphate (sodium salt; ATP), dihydroergotoxine mesylate (Hydergine, Sandoz), guanethidine sulphate, (−)-hyoscine hydrobromide, nicotine acid tartrate, (−)-noradrenaline bitartrate, oxprenolol hydrochloride, tetrodotoxin (TTX), tranylcypromine sulphate and tyramine hydrochloride. Concentrations are expressed in terms of free acid or base. The Krebs solution contained (g $litre^{-1}$): NaCl, 7·1; $CaCl_2 \cdot 6H_2O$, 0·55; KH_2PO_4, 0·16; KCl, 0·35; $MgSO_4 \cdot 7H_2O$, 0·29; $NaHCO_3$, 2·1; dextrose, 1·0. Results are expressed as median % of control with semiquartile ranges and analysed statistically by Wilcoxon's matched-pairs signed-ranks test. All probability values refer to two-tailed tests.

RESULTS

Experiments with adrenergic blocking drugs were carried out on 9 strips of taenia from 7 colons. The strips responded to electrical stimulation at 4, 8 and 16 Hz with 0·3, 0·5 and 1 ms pulses (Fig. 1), but most

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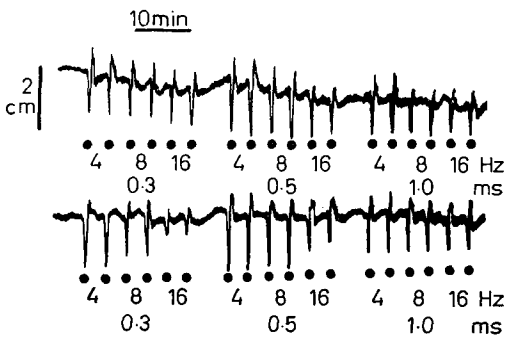

FIG. 1. Responses of a strip of human isolated taenia coli to electrical field stimulation (20 s trains, 17V cm^{-1}, pulse width and frequency as indicated), in the presence of hyoscine (2 $\mu g\ ml^{-1}$) to prevent cholinergic contractions. The responses consisted of relaxation followed by an after-contraction. Stimulation with each set of parameters was given twice before (top trace) and twice after oxprenolol (4 $\mu g\ ml^{-1}$; bottom trace). Oxprenolol reduced the tone (17 mm fall) and substantially reduced relaxations at 16 Hz, 0·3 ms, but not at other frequencies and pulse widths. Despite the fall in tone which occurred during equilibration with oxprenolol (1 h between traces), the ability of the tissue to relax was not reduced; in fact the non-adrenergic relaxations (particularly at 4 Hz) were slightly bigger. In this strip 0·5 ms pulses appeared optimal for producing relaxation.

were unaffected at 0·1 ms. In the presence of hyoscine (2 $\mu g\ ml^{-1}$) electrical excitation caused relaxation followed by a rapid 'after-contraction' immediately stimulation ceased. A small delayed relaxation sometimes ensued. Relaxations at 16 Hz were often smaller than those at 4 Hz with the same pulse duration, possibly due to non-cholinergic excitation. Noradrenaline (0·2 to 1 $\mu g\ ml^{-1}$) or nicotine (1–40 $\mu g\ ml^{-1}$) always caused relaxation; ATP (90–1440 $\mu g\ ml^{-1}$) relaxed 5 out of 7 strips, contracted one, and gave contraction followed by relaxation in another. The order of magnitude of the maximal relaxations differed as follows: noradrenaline > nicotine $\approx$ ATP > electrical stimulation. The concentrations of oxprenolol (0·6–6 $\mu g\ ml^{-1}$, n = 6) and guanethidine (20–30 $\mu g\ ml^{-1}$, n = 3) were selected in each experiment to prevent previously maximal relaxations to noradrenaline and nicotine respectively. Relaxations to ATP in the presence of the blocking drugs increased during 3 and decreased in 1 out of 5 experiments (140% (55–270%) of initial responses). Similar increases occur without oxprenolol or guanethidine (unpublished). During the experiments, tone fell slowly in 3 strips and rose gradually in 2.

Relaxations to nicotine (n = 8) or 0·3 ms pulses at 16 Hz were prevented or greatly reduced by adrenergic blockade with oxprenolol (n = 6, Table 1, Fig. 1) or guanethidine (n = 3), thus confirming the results of Bucknell (1965, 1966). Relaxations at other frequencies and pulse widths were not significantly affected as found previously (Crema & others, 1968; Stockley & Bennett, 1974). The delayed relaxations which occurred in 6 of the 9 strips at 16 Hz with pulses of 1 ms and sometimes less, and at 8 Hz with 1 ms pulses, were prevented by oxprenolol or guanethidine at the above concentrations.

Table 1. *The effect of oxprenolol (0·6–6 $\mu g\ ml^{-1}$) on relaxation induced by electrical field stimulation at various frequencies and pulse widths in strips of human taenia (n = 6).* Relaxation was significantly reduced only with 0·3 ms pulses at 16 Hz ($P = 0.05$ compared with pre-treatment controls).

Frequency (Hz) / Pulse width (ms)	4	8	16
	Medians and semiquartile range, % control		
0·3	99(95–105)	97(14–106)	31(0–89)
0·5	97(76–112)	106(64–123)	88(63–98)
1·0	90(63–100)	93(47–118)	89(48–90)

To examine the possible interactions between adrenergic and non-adrenergic inhibitory nerves, we studied the indirectly acting sympathomimetics tyramine and nicotine (see Discussion). Tyramine (0·8 to 4 mg ml^{-1}; 0·5-5 min contact) caused contraction, followed by a relaxation after wash out, or only a delayed relaxation. The contractions were unaffected by TTX (1 $\mu g\ ml^{-1}$, n = 2), α-adrenoceptor blockade with dihydroergotoxine (1 $\mu g\ ml^{-1}$, n = 4) or β-adrenoceptor blockade with oxprenolol (5 $\mu g\ ml^{-1}$, n = 2), and thus resemble contractions due to a direct action of tyramine in the rabbit ear artery (Farmer, 1966). They were enhanced by the monoamine oxidase inhibitor tranylcypromine (0·8–800 $\mu g\ ml^{-1}$, 3 experiments). A reproducible inhibitory component of responses to tyramine was obtained in 5 strips (from 3 patients) out of 15 strips (from 6 patients). In two of the relaxing strips used as controls, tyramine doses were alternated with noradrenaline and showed no tachyphylaxis. In the other 3 preparations with TTX (1 $\mu g\ ml^{-1}$) relaxations to tyramine (4 mg ml^{-1}) were 78, 60 and 48% of initial controls, whereas relaxations to noradrenaline (50 or 100 ng ml^{-1}) were 210, 63 and 140% respectively.

Relaxations to nicotine (4 $\mu g\ ml^{-1}$) increased with contact time up to at least 4 min; at 0·5, 1 and 2 min, the median relaxations were 45% (30 to 49%), 64% (55 to 70%) and 84% (71 to 85%) of the responses after 4 min contact (n = 6, $P = 0.05$ between each

contact period). Relaxations elicited by electrical field stimulation (10 s trains, 1 ms pulses, 4 Hz) were reduced to less than 5% of controls by TTX (1 μg ml^{-1}) and the relaxations during contact with nicotine were reduced to 1% (0 to 37%), 2% (0 to 5%), 6% (4 to 8%) and 10% (5 to 22%) of controls with 0·5, 1, 2 and 4 min contact periods; and the total relaxations (including a component which continued during and briefly after wash out) were 4% (2 to 37%), 12% (3 to 29%), 37% (14 to 77%) and 27% (14 to 73%) of controls respectively.

As above, relaxations to noradrenaline (0·4 μg ml^{-1}) increased in 4 out of 5 strips in the presence of TTX (83 to 560% of controls, n = 5). Nicotine-induced relaxations which persisted in the presence of TTX seemed mainly adrenergic since 3 μg ml^{-1} oxprenolol reduced them to 21% (11 to 32%), and 15 μg ml^{-1} prevented them (5 and 1 experiments respectively).

DISCUSSION

Adrenergic relaxation to intrinsic nerve stimulation in human isolated taenia coli seems demonstrable only with relatively high frequencies (> 10 Hz) or with ganglion stimulating drugs, whereas low frequencies (1 — 10 Hz) cause non-adrenergic inhibition. This agrees with other findings (Gillespie & Mackenna, 1960; Burnstock, Campbell & others, 1964; Bucknell, 1965; Holman & Hughes, 1965; Akubue, 1966; Burnstock, Campbell & Rand, 1966; Bianchi, Beani & others, 1968; Goldenberg, 1968; Aberg, Andersson & Wellin-Fogelberg, 1969). However, the unexpected decrease in adrenergic contribution with increased pulse duration does not seem to have been reported previously; it might be peculiar to human colonic muscle, although in many other ways human gastrointestinal muscle behaves like tissue from other mammals. Drugs given to the patient and hypoxia during and after the operation do not seem to impair tissue function (Fishlock & Parks, 1963; Bennett & Stockley, 1975), and overnight storage does not consistently modify the neurogenic responses. In several other species colonic adrenergic inhibitory responses were of similar magnitude in fresh preparations and after overnight storage in cold Krebs solution (Holman & Hughes, 1965).

We arranged the stimulating electrodes to avoid direct muscle excitation which at 8 Hz and above might mask small neurogenic relaxations elicited with 1 ms pulses (Bennett & Stockley, 1973, 1974). Previously, following Bucknell's (1966) technique of sewing the electrodes into each end of the strip (so that the electric field passed along rather than across the tissue) we were still unable to demonstrate adrenergic responses with 1 ms pulses (Stockley & Bennett, 1974). Thus, the field direction appears unimportant in determining the type of nerve excited.

Although inhibitory α-adrenoceptor activity has been demonstrated in human taenia coli (Bucknell & Whitney, 1964), oxprenolol alone seems adequate to block adrenergic inhibitory responses: it prevented relaxations to noradrenaline and adrenaline (Belisle & Gagnon, 1971; Gagnon, Devroede & Belisle, 1972; Stockley, 1974; present experiments). Furthermore, nicotine-induced relaxations were prevented by β-adrenoceptor antagonists alone (Bucknell & Whitney, 1964; Goldenberg, 1968; Stockley & Bennett, 1974). The greater effect of adrenergic blocking drugs at higher frequencies, when more transmitter is released per second (Brown, Davies & Gillespie, 1958), also argues against inadequate adrenergic blockade. The tendency for the ATP-induced relaxations to increase showed that the gradual fall in tone which sometimes occurred did not reduce the ability of the tissue to relax, and indicated no substantial unselective antagonism of inhibitory responses. The drug-induced reduction of relaxations at 4 Hz in some experiments might indicate either a blockade of a small adrenergic contribution or a non-selective action.

The significant reduction of relaxation found with 0·3 ms pulses at 16 Hz, and the prevention by adrenergic blockade of delayed relaxations confirm the results of Bucknell (1965, 1966). The failure to reduce significantly the relaxations to 1 ms pulses, agrees with Crema & others (1968) and Stockley & Bennett (1974). Martinson & Muren (1963) related differences in the response of cat stomach to vagal excitation at various voltages and pulse durations to the calibre of different nerve types. We cannot entirely explain our results in this way since 1 or 2 ms pulses excite extrinsic postganglionic sympathetic nerves (Burnstock & others, 1966; Bianchi & others, 1968; Aberg & others, 1969), and 1 ms pulses at 16 Hz caused delayed adrenergic relaxation. Why then is there no effect of adrenergic transmitter released at lower frequencies which summates with the non-adrenergic relaxations? If both do act together, submaximal responses should be reduced by adrenergic blockade as seen with 0·3 ms pulses at 16 Hz. Histological evidence indicates that non-adrenergic transmitter, but probably little noradrenaline, acts directly on the muscle. The adrenergic nerves surround myenteric ganglion cells, but few reach the muscle layers (Baumgarten, 1967; Bennett,

Garrett & Howard, 1968; Capurso, Friedmann, & Parks, 1968; Howard & Garrett, 1970). Many of these ganglia might be the cell bodies of non-adrenergic inhibitory nerves: they stain only weakly for cholinesterase (normally associated with cholinergic ganglia) and do not contain catecholamines (Garrett, Howard & Nixon, 1969; Howard & Garrett, 1970). Adrenergic nerves might therefore act indirectly by stimulating non-adrenergic nerves, and an adrenergic effect will only be demonstrable if direct electrical stimulation of non-adrenergic nerves is submaximal. Thus, with 0·3 ms pulses at 16 Hz, the substantial adrenergic contribution might indicate submaximal direct stimulation of non-adrenergic nerves. With 1 ms pulses the non-adrenergic responses were much larger, appearing maximal at 4 Hz, and would mask the adrenergic component. Perhaps the delayed relaxation after strong nerve stimulation was caused by noradrenaline which diffused from around the ganglia to the muscle.

Relaxation to the ganglion stimulants nicotine and dimethylphenylpiperazinium (DMPP) appears to be almost entirely adrenergic (Bucknell & Whitney, 1964). Because no colonic myenteric ganglia appear to be adrenergic (Bucknell, 1965, 1966), the drugs presumably act by releasing noradrenaline from the adrenergic nerve-endings; nicotine releases noradrenaline from cat nictitating membrane (Thompson, 1958) and rabbit pulmonary artery (Su & Bevan, 1970) which contain only postganglionic adrenergic nerves, and this action is selectively inhibited by hexamethonium (Haefely, 1972). The noradrenaline might act on non-adrenergic inhibitory nerves and/or after diffusing to the gut muscle. Release of noradrenaline directly from nerve terminals should be unaffected by TTX (Haefely, 1972), so the considerable reduction by TTX of relaxations to short exposure to nicotine suggests conduction in non-adrenergic nerves. The residual relaxations with more prolonged nicotine treatment might result from diffusion of noradrenaline to muscle. The results with tyramine, which liberates intraneuronal noradrenaline by a different mechanism (Trendelenburg, 1972) support a smilar conclusion. Although adrenergically-mediated relaxations to tyramine tended to be masked, and thus delayed during the muscle contraction elicited by tyramine, they were reduced by TTX.

The physiological significance of adrenergic inhibitory fibres remains undefined because relatively high frequencies seem necessary for their stimulation. Folkow (1952) and more recent studies (see Burnstock & Costa, 1975) show that the physiological frequency in sympathetic nerves rarely exceeds 10 Hz and that normal vasomotor tone is maintained by 1–2 Hz. Because blood flow was reduced by frequencies below those which relaxed the gut, Celander (1959) proposed that inhibition of cat small intestine to preganglionic sympathetic stimulation might be due to reduction of intestinal blood flow and/or to local overflow of vasoconstrictor transmitter. There is no blood flow in human isolated taenia, and with short impulses the adrenergic relaxations seem immediate. Sympathetic nerves might therefore have a role in controlling motility, even though it may be pronounced only in extreme circumstances when frequencies above 10 Hz occur. Although single impulses applied to periarterial nerves cause no response (Gillespie, 1962), low frequency activity might help modulate non-adrenergic inhibitory nerve activity. Perhaps the unselective effect of field stimulation does not allow such an interaction to be demonstrated.

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REFERENCES

ABERG, G., ANDERSSON, R. & WELLIN-FOGELBERG, I. (1969). *Eur. J. Pharmac.*, **7**, 296–299.
AKUBUE, P. I. (1966). *J. Pharm. Pharmac.*, **18**, 625–628.
BAUMGARTEN, H. G. (1967). *Z. Zellforsch. mikrosk. Anat.*, **83**, 133–146.
BELISLE, S. & GAGNON, D. J. (1971). *Br. J. Pharmac.*, **41**, 361–366.
BENNETT, A., GARRETT, J. R. & HOWARD, E. R. (1968). *Br. med. J.*, **1**, 487–489.
BENNETT, A. & STOCKLEY, H. L. (1973). *J. Physiol., Lond.*, **233**, 34P–35P.
BENNETT, A. &. STOCKLEY, H. L. (1974). *Br. J. Pharmac.*, **50**, 453P–454P.
BENNETT, A. & STOCKLEY, H. L. (1975). *Gut*, **16**, 443–453.
BIANCHI, C., BEANI, L., FRIGO, G. M. & CREMA, A. (1968). *Eur. J. Pharmac.*, **4**, 51–61.
BROWN, G. L., DAVIES, B. N. & GILLESPIE, J. S. (1958). *J. Physiol., Lond.*, **143**, 41–54.
BUCKNELL, A. (1965). *Ibid.*, **177**, 58P–59P.
BUCKNELL, A. (1966). Ph.D. Thesis, University of London.

BUCKNELL, A. &. WHITNEY, B. (1964). *Br. J. Pharmac. Chemother.*, **23**, 164–175.
BURNSTOCK, G., CAMPBELL, G., BENNETT, M. & HOLMAN, M. E. (1964). *Int. J. Neuropharmac.*, **3**, 163–166.
BURNSTOCK, G., CAMPBELL, G. & RAND, M. J. (1966). *J. Physiol., Lond.*, **182**, 504–526.
BURNSTOCK, G. & COSTA, M. (1975). *Adrenergic transmission. London:* Chapman & Hall.
CAPURSO, L., FRIEDMANN, C. A. & PARKS, A. G. (1968). *Gut*, **9**, 678–682.
CELANDER, O. (1959). *Acta physiol. scand.*, **47**, 299–309.
CREMA, A., DEL TACCA, M., FRIGO, G. M. & LECCHINI, S. (1968). *Gut*, **9**, 633–637.
FARMER, J. B. (1966). *Br. J. Pharmac. Chemother*, **28**, 340–347.
FISHLOCK, D. J. & PARKS, A. G. (1963). *Br. med. J.*, **2**, 1528.
FOLKOW, B. (1952). *Acta physiol. scand*, **25**, 49–76.
GAGNON, D. J., DEVROEDE, G. & BELISLE, S. (1972). *Gut*, **13**, 654–657.
GARRETT, J. R., HOWARD, E. R. & NIXON, H. H. (1969). *Arch. Dis. Childh.*, **44**, 406–417.
GILLESPIE, J. S. (1962). *J. Physiol. Lond.*, **162**, 54–75.
GILLESPIE, J. S. & MACKENNA, B. R. (1960). *Ibid.*, **152**, 191–205.
GOLDENBERG, M. M. (1968). *Archs int. Pharmacodyn. Thér.*, **175**, 347–364.
HAEFELY, W. (1972). *Catecholamines.* Editors: Blaschko H. & Muscholl, E. pp. 611–725, Heidelberg: Springer.
HOLMAN, M. E. & HUGHES, J. R. (1965). *Aust. J. exp. Biol. med. Sci.*, **43**, 277–290.
HOWARD, E. R. & GARRETT, J. R. (1970). *Proc. Soc. Med.*, **63**, 1264–1266.
MARTINSON, J. & MUREN, A. (1963). *Acta physiol. scand.*, **57**, 309–316.
STOCKLEY, H. L. (1974). Ph.D. Thesis, University of London.
STOCKLEY, H. L. & BENNETT, A. (1974). Proc. IV Internat. Symposium on Gastrointestinal Motility, pp. 165–176, Vancouver: Mitchell Press.
SU, C. & BEVAN, J. A. (1970). *J. Pharmac. exp. Ther.*, **175**, 533–540.
THOMPSON, J. W. (1958). *J. Physiol., Lond.*, **141**, 46–72.
TRENDELENBURG, U. (1972). *Catecholamines.* Editors: Blaschko, H. & Muscholl, E., pp. 336–362, Hiedelberg: Springer.
WRIGHT, P. G. & SHEPHERD, J. J. (1966). *Gut*, **7**, 41–51.