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Effect of electrode positions on contractions of guinea-pig isolated ileum to electrical stimulation

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Tetrodotoxin (TTX) reportedly abolishes contractions of guinea-pig isolated intestine to electrical field stimulation, but we recently obtained TTX-resistant contractions apparently due to direct muscle stimulation (Bennett & Stockley, 1973). We had a different electrode arrangement (one above and one below the tissue—'longitudinal' field stimulation—somewhat analogous to electrodes tied into each end of the tissue; Bucknell, 1965), so we compared responses with four other arrangements: uninsulated platinum (0.46 mm gauge) wires, one each side of the ileum (Ambache & Freeman, 1968) or wires insulated on entry to the bath but not opposite the tissue (similar to Crema, del Tacca, Frigo & Lecchini, 1968) ('transverse' stimulation); intraluminal and extraluminal electrodes ('transmural' stimulation, Paton, 1955); ring electrodes 2 mm apart around one end of the segment (similar to Furness, 1970).

Segments of guinea-pig ileum in Krebs solution (37°C, 5% CO₂ in O₂, load 0.5 g) were stimulated with 'longitudinal' field stimulation, at a voltage giving just-supramaximal stimulation of longitudinal muscle isotonic contractions at 1 or 2 Hz (10 s trains of alternating square wave pulses). Voltages for the other electrodes were those matching responses to 'longitudinal' stimulation at 1 or 2 Hz, 1 ms duration. Approximately equivalent voltages were: 'longitudinal', 100 V \equiv 17 V/cm; 'transverse' insulated, 10 V \equiv 20 V/cm; 'transverse' uninsulated 50 V \equiv 100 V/cm; 'transmural' 10 V \equiv 20 V/cm; ring electrodes 100 V \equiv 500 V/cm.

Frequency-response curves were obtained at 0.1-64 Hz with 'longitudinal' and another type of stimulation, first without and then with TTX 0.5 μ g/ml. Reduction of responses to 'longitudinal'

stimulation with TTX was less than with other electrodes (Figure 1). Threshold frequencies for TTX-resistant contractions were sometimes as low as 0.1, 2, 4, 4 and 16 Hz respectively for 'longitudinal', 'transmural', ring electrodes, uninsulated

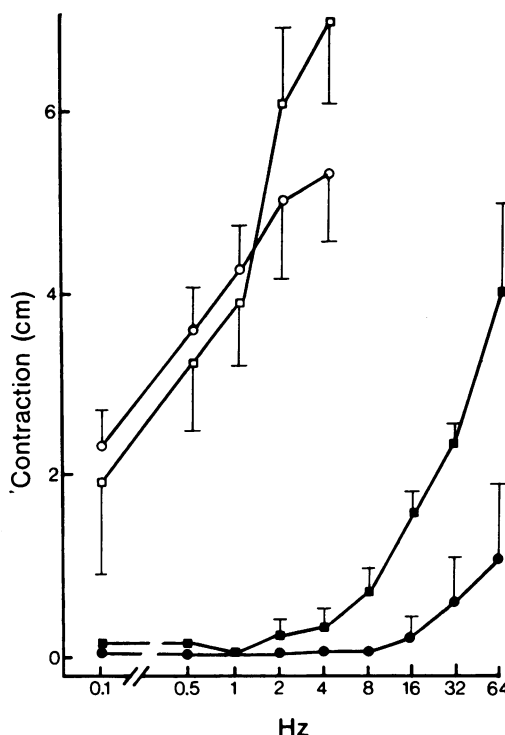


Fig. 1 TTX-resistant contractions were obvious at 2-64 Hz with 'longitudinal' field stimulation (squares) and sometimes occurred at even lower frequencies, but they occurred only at 16-64 Hz with 'transverse' stimulation (circles) using part-insulated electrodes (see text). Each tissue was studied with both electrode arrangements. Open symbols, before TTX; filled symbols, in the presence of TTX 0.5 μ g/ml. Vertical bars, 1 s.e. mean. The other electrode arrangements (results not shown) also had less tendency than 'longitudinal' stimulation to produce TTX-resistant contractions.

'transverse' and insulated 'transverse' stimulation (3-6 experiments each). 'Longitudinal' stimulation therefore appeared least satisfactory for exciting nerves alone, but all methods could produce TTX-resistant contractions at higher frequencies. These contractions were increased by higher voltages.

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The noradrenaline concentration and the cholinesterase activity of the separated longitudinal and circular layers of muscle of the guinea-pig vas deferens

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The vas deferens of the guinea-pig consists of two distinct layers of smooth muscle. The outer layer, here referred to as longitudinal muscle, has fibres disposed along the long axis of the vas deferens. The inner layer, referred to as circular muscle, is composed of fibres which encircle the mucosa.

Histochemical studies have shown that the two layers each have a dense plexus of varicose adrenergic nerve fibres which fluoresce when examined by the Falck (1962) technique but another population of nerve fibres exhibiting specific staining for acetylcholinesterase is found almost exclusively in the circular layer (Gosling & Dixon, 1972; Furness & Iwayama, 1972).

We have now supplemented these histochemical observations by measuring the noradrenaline con-

centration and the cholinesterase activity of separated longitudinal and circular muscle layers. Vasa deferentia were removed from freshly killed guinea-pigs and the longitudinal and circular muscle layers were dissected apart. Conventional histological methods were used to validate the division of longitudinal from circular muscle.

Noradrenaline content of each layer was measured by the method of Häggendal (1963) and cholinesterase activity of each layer by the method of Ellman, Courtney, Andres & Featherstone (1961) using acetylthiocholine (ASCh) as substrate. Different preparations were used for noradrenaline and cholinesterase measurements.

The results are shown in Table 1.

Other measurements of cholinesterase activity made in the presence of the acetylcholinesterase inhibitor BW284 C51 (1×10^{-5} M) or the pseudo-cholinesterase inhibitor TIPA (tetra isopropylpyrophosphoramide, 3×10^{-5} M) suggested that the difference between the activity of the two layers could largely be attributed to acetylcholinesterase.

Our results taken with the histochemical findings emphasize the differences in innervation of the two layers; and support the suggestion that the longitudinal layer has a sparse cholinergic innervation whereas the circular layer has a dense choli-

Table 1 The noradrenaline concentration and cholinesterase activity of the separated circular and longitudinal muscle layers of the guinea-pig vas deferens. The values shown are means for the numbers of guinea-pigs used for each measurement (*n*) and include the standard errors of the means (s.e. mean). Probability values (*P*) for the significance of the differences (paired *t*-test) between circular and longitudinal layers are also given

	Circular	Longitudinal	Ratio C/L
Noradrenaline concentration in $\mu\text{g/g}$ (\pm s.e. mean) (<i>n</i> = 6)	16.46 \pm 2.32	13.54 \pm 1.96	1.22 \pm 0.06
		0.02 $> P >$ 0.01	
Cholinesterase activity in <i>n</i> moles of ASCh hydrolysed/min/mg protein (\pm s.e. mean) (<i>n</i> = 20)	18.57 \pm 2.06	8.13 \pm 1.07	2.62 \pm 0.27
		$P <$ 0.001	