'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.

References

AMBACHE, N. & FREEMAN, M.A. (1968). Atropine-resistant longitudinal muscle spasm due to excitation of non-cholinergic neurones in Auerbach's plexus. *J. Physiol., Lond.*, **199**, 705-727.

BENNETT, A. & STOCKLEY, H.L. (1973). Electrically induced contractions of guinea-pig isolated ileum resistant to tetrodotoxin. *Br. J. Pharmac.*, **48**, 359-360.

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

CREMA, A., DEL TACCA, M., FRIGO, G.M. & LECCHINI, S. (1968). Presence of a non-adrenergic inhibitory system in the human colon. *Gut*, **9**, 633-637.

FURNESS, J.B. (1970). An examination of nerve-mediated, hyoscine-resistant excitation of the guinea-pig colon. *J. Physiol., Lond.*, **207**, 803-821.

PATON, W.D.M. (1955). The response of the guinea-pig ileum to electrical stimulation by coaxial electrodes. *J. Physiol., Lond.*, **27**, 40-41P.

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 concentration 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 pseudocholinesterase 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 μg/g (±s.e. mean) (n = 6)	16.46 ± 2.32	13.54 ± 1.96	1.22 ± 0.06
		$0.02 > P > 0.01$	
Cholinesterase activity in $n$moles of ASCh hydrolysed/min/mg protein (±s.e. mean) (n = 20)	18.57 ± 2.06	8.13 ± 1.07	2.62 ± 0.27
		$P < 0.001$	

nergic innervation. Furthermore, the two layers may not have an equally dense noradrenergic innervation although the difference would seem to be small.

References

ELLMAN, G.L., COURTNEY, K.D., ANDRES, V. & FEATHERSTONE, J.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, 7, 88-95.

FALCK, B. (1962). Observations on the possibilities of the cellular localisation of monoamines by a fluorescent method. *Act Physiol. Scand.*, **56**, Supplement 197, 1-25.

FURNESS, J.B. & IWAYAMA, T. (1972). The arrangement and identification of axons innervating the vas deferens of the guinea-pig. *J. Anat.*, **113**, 129-196.

GOSLING, J.A. & DIXON, J.S. (1972). Differences in the manner of autonomic innervation of the muscle layers of the guinea-pig ductus deferens. *J. Anat.*, **112**, 81-91.

HÄGGENDAL, J. (1963). An improved method for fluorimetric determination of small amounts of adrenaline and noradrenaline in plasma and tissues. *Acta Physiol. Scand.*, **59**, 242-254.

The *in vitro* effects of vinblastine on the nerve-mediated responses of the guinea-pig vas deferens

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It has recently been shown that an intravenous injection of vinblastine causes degeneration of noradrenergic nerves after about 48 h (Bennett, Cobb & Malmfors, 1973). Thoa, Wooten, Axelrod & Kopin (1972) have suggested that an immediate *in vitro* effect of vinblastine on the guinea-pig vas deferens is the inhibition of noradrenaline release from nerves. In the present investigation we have examined the acute effects of vinblastine and vincristine on the mechanical responses of the guinea-pig vas deferens to stimulation of pre and post-ganglionic nerve trunks and nerve terminals.

Vasa deferentia and the attached hypogastric nerves were removed from guinea-pigs weighing 200-400 g. The tissues were set up in 100 ml organ baths, containing Kreb's solution at 37° C, bubbled with 95% oxygen and 5% carbon dioxide. Stimulating electrodes were arranged so that the preganglionic nerve trunks, the postganglionic nerve trunks or the intramural nerve terminals could be stimulated separately. Postganglionic nerve trunk stimulation was confirmed by the addition of a mixture of pentolinium, mecamylamine and hexamethonium (1×10^{-5} M) to the organ bath. Longitudinal contractions of the vasa deferentia in response to nerve stimulation were recorded isotonically.

With the stimulus parameters used (10 s trains of pulses at 10 Hz, 40 V strength, 0.2 ms pulse duration, applied to pre or postganglionic nerve trunks; 10 s trains of pulses at 10 Hz, 140 V strength, 0.2 ms pulse duration applied to the intramural nerve terminals), reproducible responses were obtained over a period of 6 to 8 hours.

When pre or postganglionic nerve trunks and intramural nerve terminals were stimulated alternately (every 2 min), vinblastine or vincristine (1×10^{-4} M) caused an enhancement of both responses that lasted about 30 minutes. Subsequently the response to nerve trunk stimulation (pre or postganglionic) was reduced and eventually extinguished after about 180 min when the responses to stimulation of nerve terminals were little affected. With more prolonged drug contact times (4 h or more) the responses to both nerve trunk and nerve terminal stimulation were irreversibly abolished.

The results provide little evidence for blockade of transmission through the hypogastric ganglion by vinblastine; this is in contrast to its effects on the cat superior cervical ganglion (Trifaró, Collier, Lastowecka & Stern, 1972). The responses to stimulation of nerve terminals persisted when the responses to stimulation of preterminal nerve trunks were blocked; one possible explanation for this finding is that vinblastine and vincristine interfere with action potential propagation in preterminal nerve trunks.

References

BENNETT, T., COBB, J.L.S. & MALMFORS, T. (1973). Fluorescence histochemical and ultrastructural observations on the effects of intravenous injections of vinblastine on noradrenergic nerves. *Z. Zellforsch. Mikroskop. Anat.*, **141**, 517-527.

THOA, N.B., WOOTEN, G.F., AXELROD, J. & KOPIN, I.J. (1972). Inhibition of release of dopamine-β-hydroxylase and norepinephrine from sympathetic nerves by colchicine, vinblastine, or cytochalasin-B. *Proc. Nat. Acad. Sci. U.S.A.*, **69**, 520-522.

TRIFARO, J.M., COLLIER, B., LASTOWECKA, A. & STERN, D. (1972). Inhibition by colchicine and by vinblastine of acetylcholine-induced catecholamine release from the adrenal gland: an anticholinergic action, not an effect upon microtubules. *Molec. Pharmac.*, **8**, 264-267.