In the present work, we have examined mechanical responses of the longitudinal muscle of isolated segments of the proximal colon. Stimulation of intrinsic nerves with transmural electrodes resulted in complex responses to which at least three nerve types contributed, these being, 1, cholinergic nerves which gave primary contractions, 2, inhibitory, but not adrenergic, nerves which gave primary relaxations followed by rebound contractions of the muscle, 3, excitatory nerves which gave slow contractions after long latencies. There was generally little contribution of adrenergic nerves to the response to transmural stimulation. After blockade of muscarinic receptors by hyoscine (10^{-7} g/ml) there was occasionally evidence of a brief, apparently non-cholinergic, contraction during short (10 s) bursts of stimuli.

The slow contraction (Fig. 1) was observed at stimulation frequencies from 5 to 50 Hz. It began 10–15 s after the beginning of trains of stimuli lasting 10–30 s. The contractions lasted 0·5–4 min. Slow contractions were very susceptible to blockade by guanethidine and were readily restored following the washout of this drug (Fig. 1). The contraction is considered to be nerve-mediated in that it was blocked by tetrodotoxin (10^{-7} g/ml) and non-cholinergic in that it was unaffected by hyoscine or atropine in sufficient concentration to block the primary cholinergic contraction elicited by transmural stimulation. It is apparently not due to the release of noradrenaline, because directly acting sympathomimetic amines all relax the muscle and none of these relaxations could be reversed by α- or β-adrenoceptor blockade. The slow contraction can be distinguished from the rebound contraction following stimulation of intrinsic inhibitory neurones by the selective blocking action of guanethidine and by the fact that it occurs during, rather than after, an extended period of stimulation (see Furness, 1970).

REFERENCES

ELLIOTT, T. R. & BARCLAY-SMITH, E. (1904). Antiperistalsis and other muscular activities of the colon. *J. Physiol., Lond.*, **31**, 272–304.

FURNESS, J. B. (1970). An examination of nerve-mediated, hyoscine-resistant excitation of the guinea-pig colon. *J. Physiol., Lond.*, **207**, 803–822.

FURNESS, J. B. & COSTA, M. (1971). The morphology and distribution of intrinsic adrenergic neurones of the guinea-pig proximal colon. *Z. Zellforsch. Mikr. Anat.*, **120**, 346–363.

HUKUHARA, T. & NEYA, T. (1968). The movements of the colons of rats and guinea-pigs. *Jap. J. Physiol.*, **18**, 551–562.

Effects of metformin on glucose uptake by the isolated rat diaphragm

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The biguanide antidiabetic drugs do not produce significant hypoglycaemia in non-diabetic subjects. In diabetic patients phenformin increases glucose uptake into muscle in the human forearm (Butterfield, 1968). Previous *in vitro* studies on the effects of buformin and phenformin on glucose uptake by the isolated rat diaphragm preparation (Vallance-Owen & Hurlock, 1954) have been inconclusive due to the use of high drug concentrations (Daweke & Bach, 1963).

The effects of metformin on the isolated rat diaphragm preparation have been studied under normal and diabetic conditions (Table 1). Metformin at a therapeutic concentration (10 μg/ml) did not affect glucose uptake by this preparation under normal conditions. A tenfold increase in the concentration of metformin, however, caused a significant depression of uptake. This emphasizes the danger of extrapolation from results obtained with high drug concentrations. Diaphragms taken

from alloxan-diabetic rats show diminished glucose uptake and responsiveness to insulin when compared with those from normal rats. Diaphragms incubated in a medium containing free fatty acids (FFA) show a metabolic pattern similar to that seen in diabetes, in which a high rate of FFA oxidation causes inhibition of glycolysis and hence of glucose uptake (Randle, Garland, Hales & Newsholme, 1963). Metformin (10 μg/ml) produced a significant increase in glucose uptake in the presence of insulin (100 μU/ml) by diaphragms from alloxan-diabetic rats and also by normal diaphragms incubated with sodium butyrate (25 mg/100 ml).

In normal muscle tissue the rate of glucose utilization is limited by membrane transport, whereas in diabetes transport is limited by the rate of intracellular glucose metabolism, which is impaired as described above. It is suggested that metformin increases the rate of intracellular glucose metabolism, thus enhancing membrane transport in the diabetic state but having no effect on transport in normal muscle.

TABLE 1. *Effect of metformin on glucose uptake by isolated rat diaphragm*

	Normal diaphragm								Diaphragm in sodium butyrate (25 mg/100 ml)		Diaphragm from alloxan-diabetic rat	
Insulin concentration (μU/ml)	0		100				1,000		100		100	
Metformin hydrochloride concentration (μg/ml)	0	10	0	10	0	100	0	10	0	10	0	10
Mean glucose uptake*	5·7	6·1	10·0	10·7	10·6	9·6	12·0	12·1	8·7	9·7	5·9	6·9
Mean percentage change ± S.E.M.†	+27±27		+8±6		−10±2		+3±8		+12±4		+27±12	
t	1·0		1·4		4·4		0·4		3·4		2·3	
Number of experiments	8		12		8		8		10		10	
Significance	N.S.		N.S.		$0{\cdot}01>P>0{\cdot}002$		N.S.		$0{\cdot}01P>>0{\cdot}002$		$0{\cdot}05>P>0{\cdot}02$	

* Results are expressed in mg glucose taken up per g fresh weight of tissue in 90 min.
† All comparisons are made on a "within rats" basis.
N.S.=Not significant.

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REFERENCES

BUTTERFIELD, W. J. H. (1968). The effects of phenformin on peripheral glucose utilization and insulin action in obesity and diabetes mellitus. *Ann. N.Y. Acad. Sci.*, **148**, 724–733.

DAWEKE, H. & BACH, I. (1963). Experimental studies on the mode of action of biguanides. *Metabolism*, **12**, 319–322.

RANDLE, P. J., GARLAND, P. B., HALES, C. N. & NEWSHOLME, E. A. (1963). The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet*, **1**, 785–789.

VALLANCE-OWEN, J. & HURLOCK, B. (1954). Estimation of plasma-insulin by the rat diaphragm method. *Lancet*, **1**, 68–70.

Evidence that the potentiation by neostigmine of constrictor responses of the rabbit ear artery is not due to anticholinesterase activity at adrenergic nerve endings

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The finding that anticholinesterases, including physostigmine and neostigmine, potentiate responses to electrical stimulation of adrenergic nerves has been used as evidence for the involvement of acetylcholine in such transmission at various sites

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