Inhibitory responses to transmural stimulation in isolated intestinal preparations

SIR,—Transmural electrical stimulation of guinea-pig isolated ileum elicits contractile responses due to activation of parasympathetic nerve elements within the muscle wall (Paton, 1955, 1957). During the course of experiments to determine the nature of the cholinergic fibres associated with the periarterial nerves in the rabbit intestine (Gillespie & Mackenna, 1961; Day & Rand, 1961; Bentley, 1962) we used transmural stimulation in segments of rabbit isolated intestine. We were surprised to note that in most of the preparations transmural stimulation caused a complex response consisting of immediate inhibition of spontaneous activity followed by a marked contractile response. In about half of the preparations the contractile response was followed by a second inhibitory phase. In Fig. 1 the effects of sympathetic (periarterial) and transmural stimulation are compared in a segment of rabbit isolated ileum suspended in Tyrode solution at 37°. Sympathetic stimulation produced a complete inhibition of the pendular movements which outlasted the stimulation period. Complete recovery of the spontaneous movements occurred after several minutes. In contrast, when the same stimulus was applied transmurally, an inhibitory response occurred which changed during the stimulus to a contractile response outlasting the stimulus period by several minutes.

We have attempted to analyse this complex response to transmural stimulation by means of blocking drugs. The inhibitory phase of the response was prolonged, or in those preparations where it was absent initially, it was revealed after the addition of atropine or hyoscine (10^{-7} to 10^{-4} g/ml) to the bath (Fig. 2B). These drugs did not affect the excitatory phase and produced either no effect on the responses to sympathetic stimulation, or caused only a slight impairment. The initial inhibitory effect of transmural stimulation was unaffected, or in some preparations partly blocked by guanethidine in concentrations (10^{-6} to 10^{-5} g/ml) which completely abolished the responses to sympathetic stimulation (Fig. 2C). The response to transmural stimulation was markedly altered when the bath temperature was lowered, the inhibitory phase being prolonged and the excitatory phase reduced or abolished (Fig. 2D). Both phases of the response to transmural stimulation were unaffected, or occasionally slightly reduced by the anti-adrenaline agents phentolamine and propranolol, added to the bath individually or simultaneously in concentrations (10^{-7} to 5×10^{-7} g/ml) which abolished the responses to added catecholamines and to sympathetic nerve stimulation.

From the results obtained with anti-adrenaline agents and with guanethidine we conclude that the inhibitory responses to transmural stimulation are unlikely to be due entirely to activation of sympathetic adrenergic nerve elements within the muscle wall. However, the following preliminary observations suggest to us that the inhibitory responses are nervously mediated.

The local anaesthetic agent cocaine abolished both phases of the response to transmural stimulation in concentrations (2×10^{-5} to 6×10^{-5} g/ml) similar to those which abolished the responses to sympathetic nerve stimulation.

All phases of the response to transmural stimulation were present when pulse widths as low as 0·1 msec, which are unlikely to affect smooth muscle directly, were used. Moreover, it was shown that the optimal frequency for the inhibitory component was lower (10 to 20 pulses/sec) than the optimal frequency for sympathetic relaxations (30 to 50 pulses/sec).

The complex responses to transmural stimulation were strikingly similar to the effects of the automatic ganglion stimulants nicotine and tetramethyl ammonium in isolated preparations of rabbit ileum.

The characteristics of the inhibitory responses to transmural stimulation

described here are essentially similar to those recently described in the cat stomach (Martinson, 1965a,b), in the guinea-pig isolated taenia coli (Burnstock, Campbell & Rand, 1966), and in the guinea pig isolated stomach (Campbell, 1966) and suggest the presence of non-adrenergic inhibitory neurons in the gastrointestinal tract.

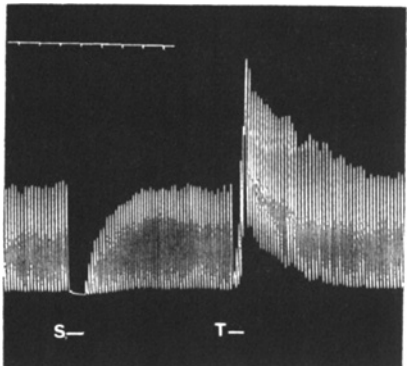

FIG. 1. Longitudinal contractions of rabbit isolated ileum suspended in aerated Tyrode solution at 37°. Sympathetic nerve stimulation (at S) and transmural stimulation (at T) each applied for 20 sec periods with 2 msec 20 V rectangular pulses at a frequency of 50 pulses/sec. Time: 30 sec intervals.

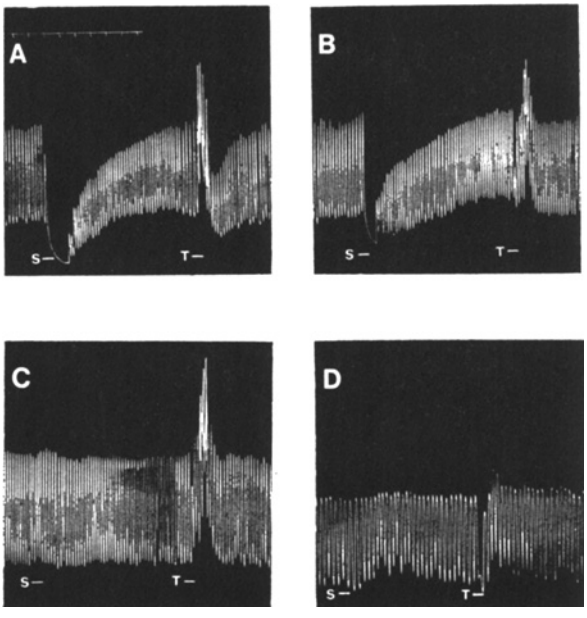

FIG. 2. Rabbit isolated ileum preparations; sympathetic stimulation (at S) and transmural stimulation (at T) applied as in Fig. 1. Control responses in A, 20 min after adding hyoscine (10^{-6} g/ml) in B, 30 min after adding guanethidine (10^{-6} g/ml) in C. In D, in the presence of guanethidine and hyoscine, responses repeated after reducing bath temperature from 37 to 31°.

We have repeated our experiments using transmural stimulation in intestinal preparations taken from duodenum, ileum and colon of the rabbit and cat. In all these preparations, transmural stimulation produced initial inhibitory responses. In some preparations of cat intestine transmural stimulation produced only inhibition which was not abolished by guanethidine. In those preparations of cat intestine showing a mixed response of inhibition and excitation, the excitatory phase was abolished by low concentrations of hyoscine or atropine. The atropine sensitivity of the motor component in cats, and the lack of sensitivity in rabbits, is consistent with the hypothesis that this part of the response is due to activation of parasympathetic nerve elements within the myenteric plexus, since the parasympathetic nerves to rabbit intestine are relatively insensitive to atropine (Ambache, 1951; Ambache & Edwards, 1951) whilst those of the cat are readily susceptible (Gillespie & Mackenna, 1960; Ambache, 1951).

The work of Martinson (1965a,b) and Campbell (1966) using stomach preparations, suggests that the connections of these inhibitory neurons with the central nervous system may be via the vagus nerves. However, since we have obtained inhibitory responses to transmural stimulation in colon preparations it may be that the sacral parasympathetic outflow also contains preganglionic fibres forming connections with non-adrenergic inhibitory fibres within the muscle wall.

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March 3, 1967.

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