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A PHARMACOLOGICAL ANALYSIS OF THE RESPONSES TO TRANSMURAL STIMULATION IN ISOLATED INTESTINAL PREPARATIONS

BY

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Inhibition of gastro-intestinal motility by stimulation of the vagus nerves was first described towards the end of the nineteenth century by Langley (1898) and by Bayliss & Starling (1899), and has since been reported at intervals (for example, Auer & Meltzer, 1907; Veach, 1925; McSwiney & Wadge, 1928; Harrison & McSwiney, 1936; Martinson & Muren, 1960, 1963; Paton & Vane, 1963; Campbell, 1966a).

Harrison & McSwiney (1936) suggested that the inhibitory responses they recorded were caused by adrenergic fibres in the vagus nerves, and this view was tentatively supported by Greeff, Kasperat & Osswald (1962) and by Paton & Vane (1963). Recent studies, however, both in whole animals (Martinson & Muren, 1963; Martinson, 1964, 1965) and in isolated intestinal preparations (Burnstock, Campbell & Rand, 1966; Bennett, Burnstock & Holman, 1966; Campbell, 1966a) do not support this hypothesis. Thus the inhibitory responses to transmural stimulation in the taenia coli persisted in the presence of guanethidine or bretylium in concentrations which abolished the effects of sympathetic stimulation (Burnstock, Campbell & Rand, 1966). Moreover, the electrophysiological studies of Bennett, Burnstock & Holman (1966) on the taenia suggest that the inhibitory responses to sympathetic and to transmural stimulation are mediated by different sets of nerves.

Recently, Holman & Hughes (1965) have obtained biphasic responses consisting of inhibitory and motor components after transmural stimulation of isolated intestinal preparations taken from mice, rats, guinea-pigs and rabbits. We have obtained similar responses in isolated intestinal preparations taken from rabbits and kittens, and have attempted a pharmacological analysis of the components of the response. In most experiments we have used the isolated ileum of the rabbit because its responses to sympathetic stimulation and its susceptibility to blocking drugs have been well characterized, and the preparation shows regular activity for many hours.

A preliminary account of some of this work has already been published (Day & Warren, 1967).

METHODS

Rabbits weighing 1–3 kg were killed by a blow on the head and bled. Sections of intestine about 3 cm long were removed together with their mesenteric attachments. The tissues were set up in aerated Tyrode solution and the longitudinal contractions recorded with isotonic frontal writing levers writing on a smoked drum. The bath temperature varied between 28° and 37° C in different experiments; details are given in RESULTS. Preparations of kitten intestine were set up in the same way.

In some experiments with rabbit intestine, Krebs bicarbonate or McEwen (1956) solution gassed with 5% carbon dioxide in oxygen was used, but the results were the same as in aerated Tyrode solution. Preparations of kitten intestine gave poor results in Tyrode solution, however, and McEwen solution with 5% carbon dioxide in oxygen was used for most of these.

The periarterial (sympathetic) nerves were stimulated with an electronic stimulator delivering rectangular pulses through bipolar platinum electrodes of the type described by Burn & Rand (1960). Transmural stimulation was effected with bipolar intraluminal electrodes of the type described by Paton (1955).

In most experiments sympathetic or transmural stimulation was applied for 20 sec periods, repeated at not less than 3 min intervals, with pulses of supramaximal strength (usually 20 V). Details of frequency and pulse width are given in the RESULTS section.

Reserpine pretreatment

Rabbits were pretreated with reserpine by injecting the commercial preparation (Serpasil, CIBA) into the marginal ear vein in a dose of 0.3 mg/kg on each of 3 days and using the animals on the fourth day.

RESULTS

Transmural stimulation of rabbit isolated intestine

The response to transmural stimulation in segments of rabbit isolated ileum set up at 37° C usually consisted of mixed inhibitory and motor components. There was some variation between different preparations, but in general there were four main types of response. These are illustrated in Fig. 1. The most usual was a rapid and complete inhibition of spontaneous activity which changed, during the stimulation period, into a contraction which subsided at the end of the stimulus and was followed by inhibition of variable extent and duration (Fig. 1a). The second type of response commonly seen was identical, except that the secondary inhibition was absent or very slight (Fig. 1b).

The third type, seen in a few preparations, consisted of an immediate contraction followed, after the stimulation, by inhibition (Fig. 1c). The fourth type, seen in only about ten of more than two hundred preparations, consisted solely of a contraction lasting throughout the stimulation period, followed by the return of normal spontaneous activity (Fig. 1d).

Basically similar responses to transmural stimulation were obtained in preparations taken from all regions of the intestine from duodenum to terminal colon of rabbits, guinea-pigs and kittens. In general, however, the spontaneous movements of rabbit ileum were the most regular and were therefore used in most experiments.

Effect of local anaesthetics

A comparison was made between the action of local anaesthetic agents on the response to transmural and to sympathetic (periarterial) stimulation to determine whether the response to transmural stimulation was likely to be nervously mediated. The local

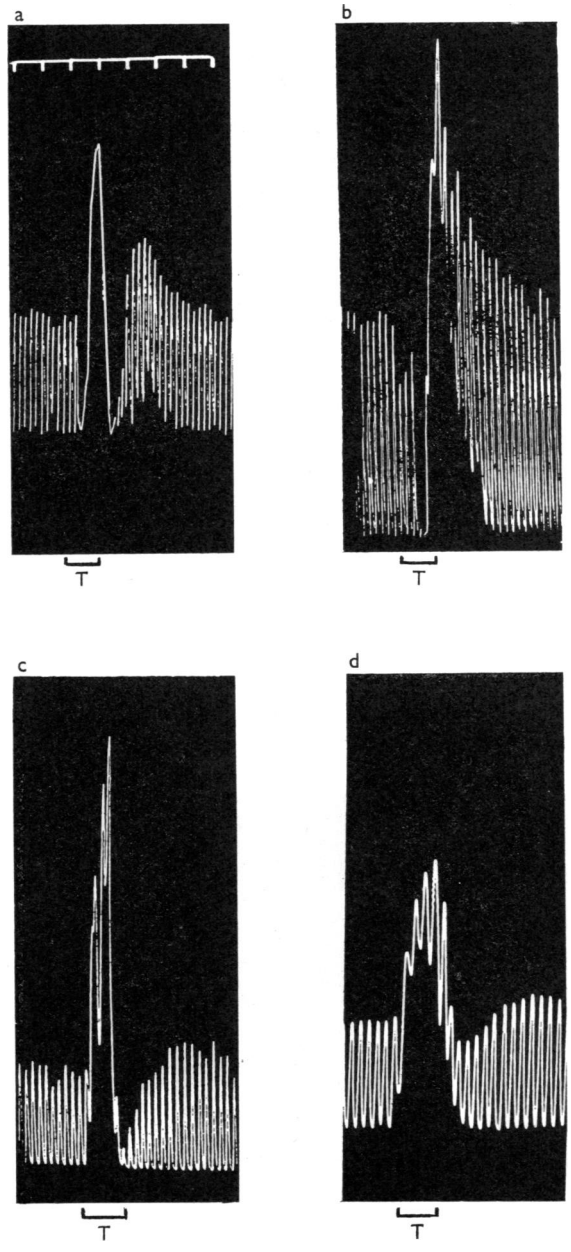

Fig. 1. Four main types of response to transmural stimulation (T) in different segments of rabbit isolated ileum in aerated Tyrode solution at 37° C. Stimulation applied for 20 sec with 1 msec 20 V pulses at a frequency of 20 pulses/sec. Time marker in 30 sec intervals.

anaesthetics used were lignocaine, procaine, cinchocaine and cocaine. These, with the occasional exception of cocaine, depressed the spontaneous activity of the ileum in relatively low concentrations (4 to 50 μg/ml.) thus making it impossible to establish with certainty whether the responses were impaired. In six experiments out of twelve in which cocaine (50 μg/ml.) was used there was little impairment of the pendular movements, but the inhibitory and motor components of the transmural response were abolished as was the inhibition to sympathetic stimulation. The impairment of the responses caused by cocaine was partially reversed by washing.

Effect of altering the bath temperature

When the bath temperature was progressively lowered from 37° C to 28° C the response to transmural stimulation changed; the inhibitory phase became more prolonged while the motor phase was greatly reduced. In many experiments at 28° C the response to transmural stimulation was pure inhibition whereas at 37° C the motor component was more marked. This is illustrated in Fig. 2. At 28° C transmural stimulation produced an inhibitory response which outlasted the stimulation period. At 33° C a small motor component appeared in the response and the inhibitory phase was less prolonged, while at 37° C the main response during stimulation was contraction followed by inhibition. The motor responses to acetylcholine (0.01 to 0.04 μg/ml.) were altered in the same way by lowering the bath temperature as was the motor component of the transmural response. Thus the responses were reduced by lowering the temperature from 37° C and were sometimes almost abolished at 28° C. This depression was completely reversed by returning the bath temperature to 37° C.

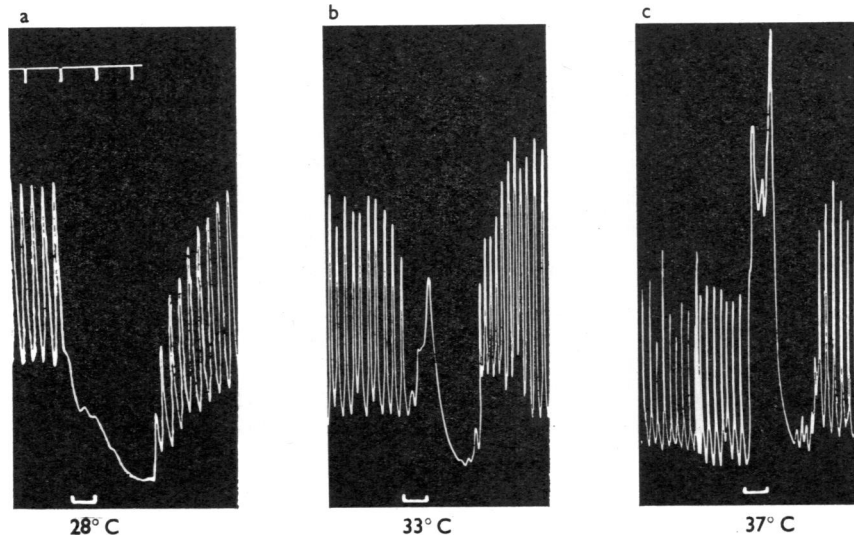

Fig. 2. Effect of raising the bath temperature on the response to transmural stimulation in a segment of rabbit isolated ileum suspended in aerated Tyrode solution. Transmural stimulation (at ⎵) applied for 20 sec with 2 msec pulses of supramaximal strength at a frequency of 20 pulses/sec. Time marker in 30 sec intervals.

Comparison between transmural and sympathetic inhibition

Stimulus parameters

In some preparations the relaxations obtained by stimulating the sympathetic nerves were compared with those obtained by transmural stimulation in order to investigate the possibility of a common origin for these responses. It was found that the optimal frequency of stimulation for transmural inhibition was lower (10 to 20 pulses/sec) than for sympathetic inhibition (50 pulses/sec). It was, however, difficult to obtain a valid comparison between the two responses because the motor component of the transmural response varied in different preparations and at different frequencies and may therefore have influenced the inhibitory response in a variable manner.

In several preparations the relaxation to transmural stimulation was larger at a stimulus frequency of 50 pulses/sec than it was at 20 pulses/sec. In these experiments the addition of guanethidine (1–10 μg/ml.) to the bath abolished the effects of sympathetic stimulation and reduced the optimal frequency for transmural inhibition to 20 pulses/sec. Thus, in some experiments at least, there may have been a sympathetic component to the transmural inhibition. Submaximal inhibitions to transmural stimulation could be elicited by frequencies of stimulation (1 to 5 pulses/sec) which in most experiments were too low to cause inhibition to sympathetic stimulation.

The motor component of the response to transmural stimulation was fully developed usually at a frequency of 20 pulses/sec. In most experiments increasing the frequency to 50 pulses/sec did not increase this part of the response.

The threshold pulse-width for both sympathetic and transmural inhibition was of the order of 0.1 msec. Pulse-width of 0.5 msec–0.1 msec tended to increase the inhibitory component and decrease the motor component of the transmural response. In most preparations a pulse width of 1 msec was used for transmural stimulation because this was supramaximal for both inhibitory and motor components of the response.

To obtain maximal responses at any given pulse width and frequency for both sympathetic and transmural stimulation the necessary voltage was usually between 5 and 10. A supramaximal voltage (20 V) was used in all experiments for both sympathetic and transmural stimulation.

Anti-adrenaline substances

Inhibition of the isolated intestine by sympathetic stimulation and by added noradrenaline is caused by the activation of both α and β receptors. Thus, a mixture of both α and β receptor blocking substances is necessary to abolish these effects (Furchgott, 1960). An experiment in which a mixture of anti-adrenaline substances was tested on the inhibitory responses to transmural and sympathetic stimulation and to added noradrenaline is shown in Fig. 3. In this experiment the bath temperature was maintained at 32° C and the response to transmural stimulation was predominantly inhibitory. The addition to the bath of a mixture of phentolamine (1 μg/ml.) and propranolol (2 μg/ml.) abolished the inhibitory responses to sympathetic stimulation and to added noradrenaline, but the transmural inhibition was unaffected in size and slightly more prolonged in duration than before the blocking drugs.

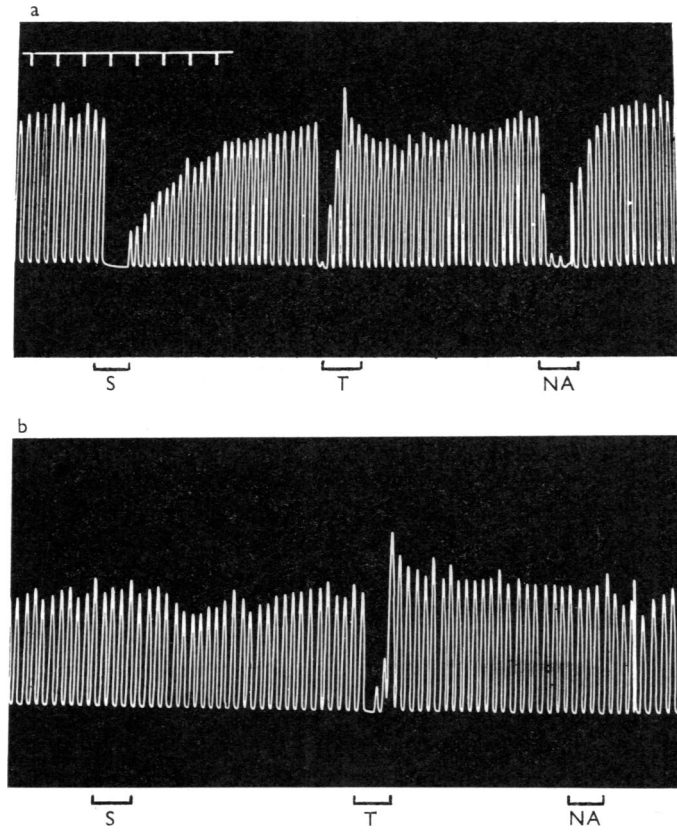

Fig. 3. Rabbit isolated ileum in aerated Tyrode solution at 32° C. a: Control responses to sympathetic stimulation (S) applied for 20 sec with 2 msec 20 V pulses at a frequency of 50 pulses/sec, transmural stimulation (T) with 0.5 msec 20 V pulses at 20 pulses/sec and added noradrenaline (NA) in a concentration of 0.02 μg/ml. left in contact for 30 sec. b: Same responses repeated 30 min after adding a mixture of propranolol (2 μg/ml.) and phentolamine (1 μg/ml.) to the bath. Time marker in 30 sec intervals.

Adrenergic neurone blocking agents

Guanethidine (1–10 μg/ml.) or xylocholine (3–20 μg/ml.) when added to the bath abolished the inhibitory responses to sympathetic stimulation while in most preparations producing little or no impairment of the inhibitory component of the transmural response. This is shown in the experiment illustrated in Fig. 4 which is of a preparation maintained at 32° C in order to enhance the inhibitory component and depress the motor component of the transmural response.

Between Fig. 4a and 4b the preparation was left in contact with a high concentration (10 μg/ml.) of guanethidine for 45 min. In Fig. 4b the sympathetic response was abolished while the inhibition to transmural stimulation was only slightly reduced. In preparations maintained at 37° C the motor response to transmural response was more prominent than at lower temperatures and it was not significantly altered by even high concentrations of guanethidine or xylocholine.

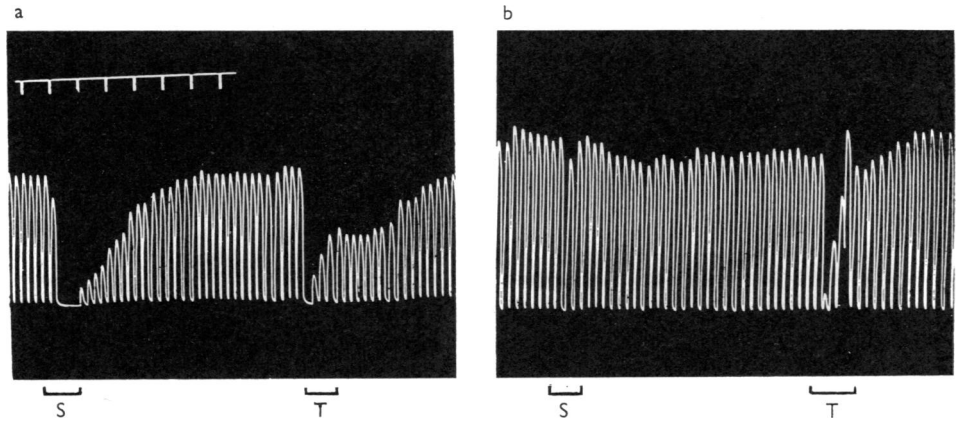

Fig. 4. Rabbit isolated ileum at 32° C. a: Sympathetic stimulation (S) with 2 msec 20 V pulses at a frequency of 50 pulses/sec applied for 20 sec, and transmural stimulation (T) for 20 sec with 0.5 msec 20 V pulses at a frequency of 20 pulses/sec. b: Same responses 45 min after adding guanethidine (10 μg/ml.) to the bath. Time marker in 30 sec intervals.

In a few preparations at 37° C, guanethidine caused a marked impairment of the inhibitions to transmural stimulation. The block could, however, be distinguished from the sympathetic nerve blockade by the fact that it could be reversed by lowering the bath temperature by 4°–7° C, whereas this did not reverse the sympathetic blockade.

Reserpine treatment

Reserpine was either administered intravenously to rabbits for several days before the experiment, or added to the bath containing the tissues.

In twenty preparations of ileum set up at 32° C or 37° C and taken from six rabbits treated with reserpine, the inhibitory responses to sympathetic stimulation were impaired but not abolished. The inhibitory component of the transmural response also seemed to be impaired, but this could have been caused by an enhancement of the motor component masking the inhibition.

Clearer results were obtained in ten other preparations taken from untreated rabbits and set up at 32° C in order to reduce the motor phase of the response. In these experiments reserpine (0.5–1.0 μg/ml.) was added to the bath and caused a slowly developing impairment of the inhibitory responses to both sympathetic and transmural stimulation which were both usually completely abolished after contact with the drug for 3–4 hr. In these experiments dopamine (50 μg/ml.) partially reversed the sympathetic nerve block but produced little or no enhancement of the inhibitory component of the transmural response. This observation is illustrated in Fig. 5. In this experiment the inhibitory component of the transmural response and the sympathetic inhibition were abolished after contact for 225 min with reserpine (0.5 μg/ml.). Dopamine (50 μg/ml.) was added to bath and left for 45 min after which time the sympathetic inhibition but not the transmural inhibition was largely restored.

The results so far described show that the inhibitory responses to sympathetic and to transmural stimulation are affected differently by varying the conditions of stimulation by α- and β-receptor blocking agents, by adrenergic neurone blocking drugs, and by

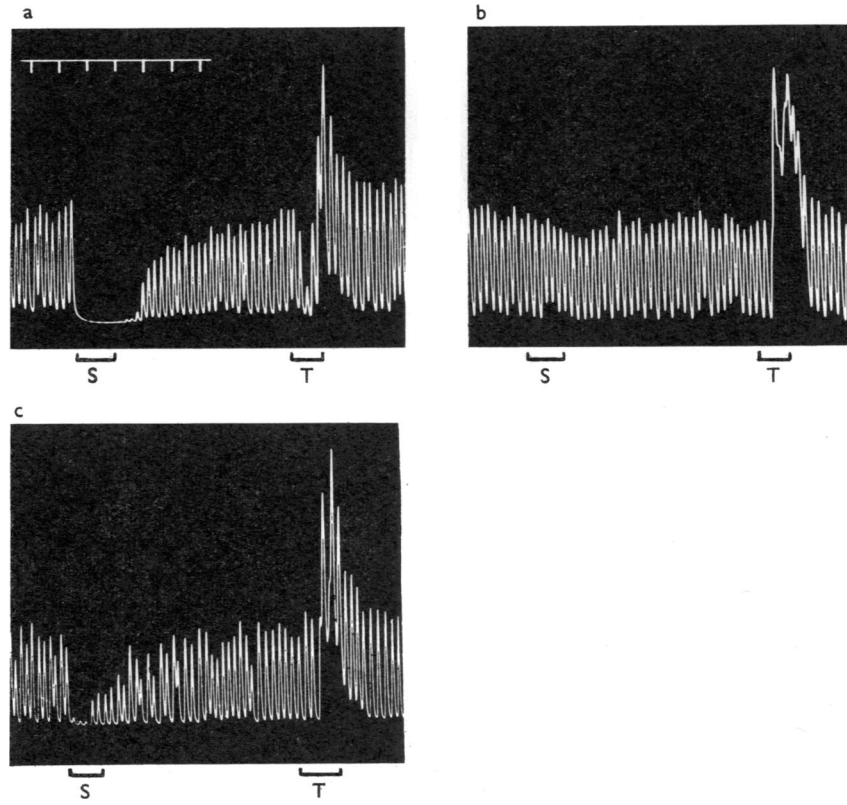

Fig. 5. Rabbit ileum at 32° C. a: Control responses to sympathetic stimulation (S) with 1 msec 20 V pulses at a frequency of 50 pulses/sec and transmural stimulation (T) with 1 msec 20 V pulses at 20 pulses/sec each applied for 20 sec periods. b: The same responses repeated 225 min after the addition of reserpine (0.5 μg/ml.) to the bath. Between b and c dopamine (50 μg/ml.) was added to the bath and the responses were repeated 45 min later in c. Hyoscine (1 μg/ml.) was present in the bath throughout the experiment. Time marker in 30 sec intervals.

depletion of sympathetic transmitter by reserpine. The evidence, therefore, seems to indicate a different origin for the two responses.

The next part of the investigation was designed to determine the nature of the motor component of the response to transmural stimulation.

Effect of atropine and hyoscine

The effects of both atropine and hyoscine in concentrations ranging from 0.1 to 100 μg/ml. were tested on the responses to transmural stimulation. Atropine in concentrations above 0.1 μg/ml. frequently inhibited spontaneous activity of the gut as noticed by Holman & Hughes (1965). Hyoscine rarely caused this effect and was therefore used in most experiments. In preparations maintained at 37° C in which the motor component of the transmural response was well marked, hyoscine potentiated the initial inhibitory phase of the response but usually had little effect on the motor component. In those preparations in which an initial inhibition was absent (Fig. 1c), or in which the response was entirely motor (Fig. 1d), hyoscine revealed an initial inhibitory component. This

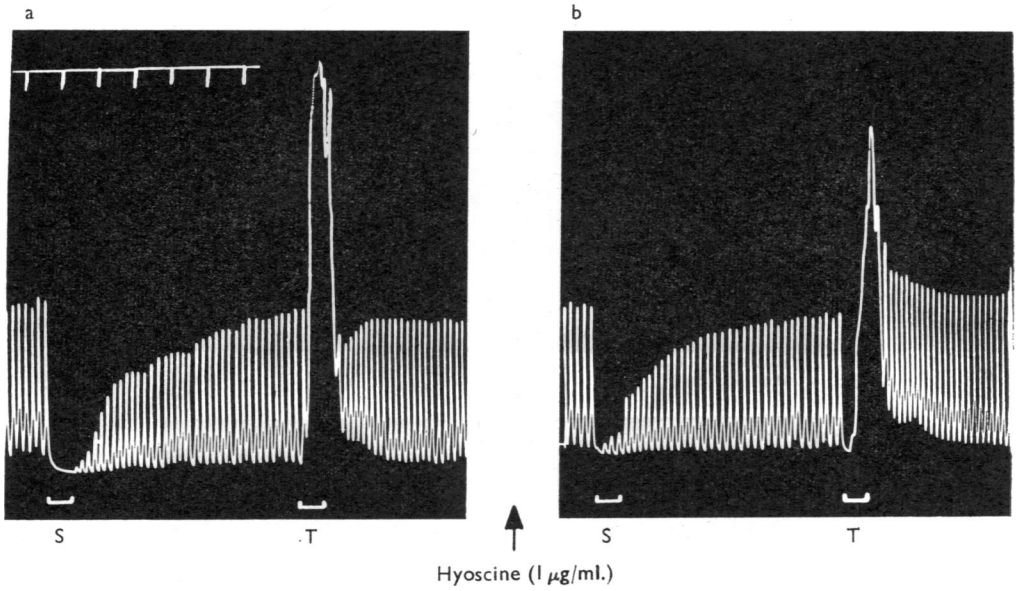

Fig. 6. Rabbit ileum at 37° C. a: Control responses to sympathetic stimulation (S) with 2 msec 20 V pulses at a frequency of 50 pulses/sec and transmural stimulation (T) with 2 msec 20 V pulses at a frequency of 20 pulses/sec each applied for 20 sec. b: Responses repeated 15 min after adding hyoscine (1 μg/ml.) to the bath. Time marker in 30 sec intervals.

observation is illustrated in Fig. 6 where the transmural response was converted by hyoscine (1 μg/ml.) from pure motor to initial inhibition followed by a reduced motor effect. In this experiment there was a slight impairment of the response to sympathetic stimulation after hyoscine.

In preparations maintained below 37° C the motor component of the transmural response was usually much less well marked and was relatively more inhibited by hyoscine. The experiment illustrated in Fig. 7 shows the effect of hyoscine on the response to added acetylcholine and to transmural stimulation in a preparation maintained at 32° C. In Fig. 7a the stimuli for the transmural response were altered in order to get graded motor effects. In Fig. 7b after contact for 15 minutes with hyoscine (1 μg/ml.) the response to added acetylcholine was abolished and transmural stimulation then produced only inhibition. In preparations of ileum taken from kittens, transmural stimulation produced initial motor effects which were followed by long lasting guanethidine-insensitive inhibitions. In preparations at 37° C the motor component of the transmural response in kitten ileum was abolished by low concentrations of hyoscine (0.01 μg/ml.).

Anticholinesterases

The addition of physostigmine (eserine) or neostigmine to the bath in concentrations of 0.05 to 0.1 μg/ml. markedly enhanced the motor component of the transmural response with a consequent masking of the inhibitory component. Figure 8 illustrates an experiment in which the response to transmural stimulation was either purely inhibitory or

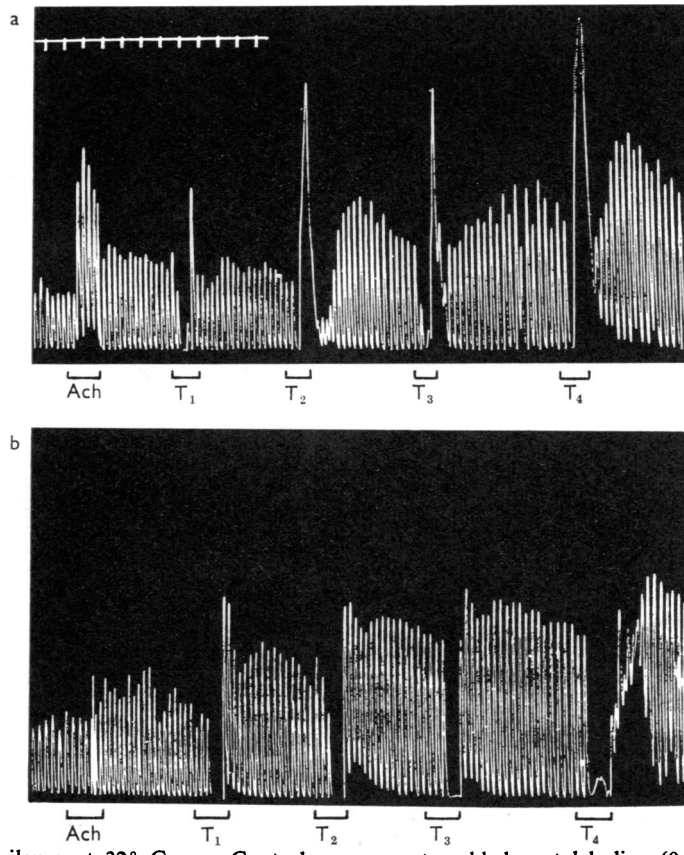

Fig. 7. Rabbit ileum at 32° C. a: Control responses to added acetylcholine (0.02 μg/ml.) (Ach) and transmural stimulations applied for 20 sec periods with supramaximal strength pulses: T_1, pulse width of 0.5 msec and a frequency of 20 pulses/sec; T_2, 0.5 msec and 50 pulses/sec; T_3, 2 msec and 20 pulses/sec; T_4, 2 msec and 50 pulses/sec. b: Same responses repeated in the presence of hyoscine (1 μg/ml.). Time marker in 30 sec intervals.

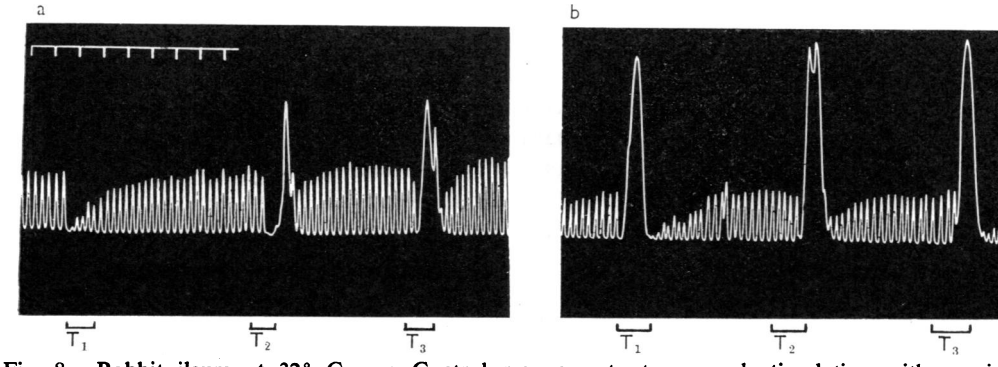

Fig. 8. Rabbit ileum at 32° C. a: Control responses to transmural stimulation with varying stimuli. T_1, pulse width 0.5 msec, frequency 50 pulses/sec; T_2, 2 msec and 20 pulses/sec; T_3, 2 msec and 50 pulses/sec. Each pulse of supramaximal strength and with a stimulus period of 20 sec. b: The same responses are repeated 14 min after the addition of physostigmine (0.1 μg/ml.) to the bath. Time marker in 30 sec intervals.

biphasic according to the intensity of the stimuli used. The addition of physostigmine (0.1 μg/ml.) to the bath revealed a large motor component to the response which had previously been purely inhibitory and the motor components of the other two responses were enhanced and the inhibitory components inhibited.

DISCUSSION

Our results suggest that transmural stimulation of segments of rabbit and kitten isolated intestine activates at least three distinct nervous pathways. The transmural response consists of inhibitory and motor components, and the appearance of the response varies according to the relative predominance of one component over the other. The inhibitory component seems to be caused chiefly by activation of non-adrenergic neurones whose presence in various intestinal preparations has recently been described (Burnstock, Campbell, Bennett & Holman, 1964 ; Holman & Hughes, 1965 ; Burnstock, Campbell & Rand, 1966 ; Campbell, 1966a). It seems likely, however, that activation of sympathetic nerve endings within the muscle wall may also contribute to the inhibitory component. Thus the response is usually slightly impaired by guanethidine in concentrations sufficient to cause sympathetic nerve block.

The evidence in favour of a non-adrenergic mechanism to explain the major part of the transmural inhibition is substantial. Thus the inhibitory response to transmural stimulation is slightly enhanced in the presence of a mixture of α- and β-receptor blocking agents in concentrations which abolish the inhibitory effects of sympathetic stimulation and of added noradrenaline. Holman & Hughes (1965) reported that pronethalol depresses the tone of intestinal preparations and reduces the responses to transmural stimulation. We have used propranolol, a more specific and more potent β-receptor blocking drug than pronethalol, which neither effected intestinal tone nor depressed either component of the transmural response. It may be that the reduction of the transmural responses caused by pronethalol in the experiments of Holman & Hughes (1965) was not specific because, as these authors pointed out, pronethalol is a potent local anaesthetic agent.

In agreement with other workers (Holman & Hughes, 1965 ; Burnstock, Campbell & Rand, 1966 ; Bennett, Burnstock & Holman, 1966) we found the threshold as well as the optimal frequency for transmural inhibition to be lower than that for sympathetic inhibition. Several nervous pathways seem to be activated by transmural stimulation, however, and may therefore alter the characteristics of the non-adrenergic inhibition.

The only substance, apart from cocaine, which convincingly impaired the transmural inhibition was reserpine. The action of reserpine was best demonstrated by adding the drug to the preparation in the bath. The time course of the blocking action of reserpine on the transmural inhibition was similar to that for the sympathetic responses, but could be distinguished from it by the fact that dopamine restored only the sympathetic responses. Thus, the blocking action of reserpine on the transmural inhibitory response seems to be independent of its catecholamine depleting action which causes the sympathetic blockade. Gillespie & Mackenna (1960) found that the inhibitory action of nicotine on rabbit isolated colon persisted in the presence of the adrenergic neurone blocking drug xylocholine, but was reduced or abolished in preparations taken from rabbits which had been pretreated with reserpine. These observations are consistent with our own using guanethidine and reserpine, because nicotine and other ganglion stimulants are known to activate the

non-adrenergic inhibitory neurones in isolated intestinal preparations (Holman & Hughes, 1965; Burnstock, Campbell & Rand, 1966). It therefore seems likely that reserpine depletes the stores of neuro-humoral transmitter from both the sympathetic and the non-adrenergic inhibitory nerves in the intestine.

The motor component of the response to transmural stimulation in isolated intestinal preparations has been described by other workers as " rebound " contraction (Holman & Hughes, 1965; Campbell, 1966b; Bennett, 1966). These workers suggest that because the contraction persists in the presence of high concentrations of atropine it is not mediated by cholinergic nerves but occurs as a direct result of the inhibitory phase of the transmural response. The inhibitory response causes hyperpolarization of the smooth muscle membrane which is replaced at the end of stimulation by an increase in rate of firing of action potentials with a consequent increase in muscular tension (Bennett, 1966; Campbell, 1966b). This interpretation does not explain the major part of the motor response in our experiments for four main reasons. First, the motor component, like the response to added acetylcholine, was depressed by lowering the bath temperature while the inhibitory component was enhanced. Second, in some experiments the motor component preceded the inhibition or, in a few experiments at 37° C, occurred in the absence of an inhibitory phase. Third, the motor component was partly blocked by hyoscine, particularly at low bath temperatures, and the inhibition was potentiated by hyoscine at all bath temperatures. Finally, the motor component was potentiated by anticholinesterases. These observations taken together strongly suggest that the inhibitory and motor components of the response to transmural stimulation are separate phenomena and are probably mediated through different nervous pathways. Despite the relative insensitivity of the motor response to hyoscine in rabbit intestine, the evidence suggests that the motor response is cholinergic in nature. In the kitten intestine the motor response to transmural stimulation was abolished by low concentrations of hyoscine. The relative insensitivity of the cholinergic nerves in rabbit intestine as compared with those in the kitten intestine to the blocking action of atropine-like drugs has been described previously by Ambache & Edwards (1951). These workers showed that the motor response to nicotine in kitten isolated ileum was converted to inhibition in the presence of atropine while the motor effect of nicotine in rabbit ileum persisted in the presence of high concentrations of atropine. Botulinum toxin, a selective cholinergic nerve blocking drug, however, reverses the nicotine motor response to inhibition in both species (Ambache, 1951).

In our view the most likely mechanism to explain the actions of atropine-like drugs and anticholinesterases on the response to transmural stimulation is that these drugs alter the time-course of the motor component. An anticholinesterase, by preserving the acetylcholine released by transmural stimulation, may reduce the latency of the motor component and consequently obscure the inhibitory phase of the response. A similar mechanism may explain the apparent block of the " rebound " contraction by neostigmine in Campbell's (1966b) experiments, because after inhibition of cholinesterase the motor response may be fully developed during the stimulation period and therefore not persist afterwards. Conversely, atropine-like agents, by partly blocking the effects of acetylcholine released by transmural stimulation, would increase the latency of the contraction thus enhancing the initial inhibition and making most of the motor component occur after the stimulation with an apparent potentiation of the " rebound " contraction.

Our evidence suggests that the motor response to transmural stimulation is mediated by cholinergic fibres—presumably of parasympathetic origin—and that the inhibition results chiefly from activation of non-adrenergic inhibitory neurones within the muscle wall.

SUMMARY

1. Transmural stimulation of segments of isolated intestine taken from kittens and rabbits and maintained at 37° C produced biphasic responses consisting of initial inhibition of pendular movements followed by a marked increase in tone of the preparations.

2. Progressive reduction of the bath temperature from 37° C to 28° C markedly enhanced the inhibitory component of the transmural response but impaired the motor component and the contractions to added acetylcholine.

3. That the inhibitory responses to transmural stimulation were not of sympathetic origin was shown by their differing optimal stimulus parameters, the resistance of the transmural response to guanethidine and to mixtures of α- and β-receptor blocking drugs.

4. Reserpine treatment impaired both sympathetic and transmural inhibitions but only the former responses were restored by adding dopamine to the bath.

5. Hyoscine and atropine enhanced the inhibitory component of the transmural response in rabbit ileum at 37° C but had little or no effect on the motor component at this temperature. At lower bath temperatures these drugs were relatively more effective at inhibiting the motor component. The motor component of the transmural response in kitten intestine was abolished by a low concentration of hyoscine even at 37° C.

6. The motor component of the transmural response was markedly enhanced by anticholinesterase agents.

7. The evidence suggests that the major part of the inhibitory component of the transmural response is caused by activation of non-adrenergic inhibitory neurones within the muscle wall. On the other hand, the motor component seems chiefly to result from activation of cholinergic nerve endings and is apparently not a " rebound " phenomenon resulting from the initial inhibition as has been suggested by other workers.

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REFERENCES

AMBACHE, N. (1951). Unmasking, after cholinergic paralysis by botulinum toxin, of a reversed action of nicotine on the mammalian intestine, revealing the probable presence of local inhibitory ganglion cells in the enteric plexuses. *Br. J. Pharmac. Chemother.*, **6**, 51–67.

AMBACHE, N. & EDWARDS, J. (1951). Reversal of nicotine action on the intestine by atropine. *Br. J. Pharmac. Chemother.*, **6**, 311–317.

AUER, J. & MELTZER, S. J. (1907). Peristalsis of the rabbit caecum. *Am. J. Physiol.*, **18**, xiv–xv.

BAYLISS, W. M. & STARLING, E. H. (1899). The movements and innervation of the small intestine. *J. Physiol., Lond.*, **24**, 99–143.

BENNETT, M. R. (1966). Rebound excitation of the smooth muscle cells of the guinea-pig taenia coli after stimulation of intramural inhibitory nerves. *J. Physiol., Lond.*, **185**, 124–131.

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1966). Transmission from intramural inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **182**, 541–558.

BURN, J. H. & RAND, M. J. (1960). The relation of circulating noradrenaline to the effect of sympathetic stimulation. *J. Physiol., Lond.*, **150**, 295–305.

BURNSTOCK, G., CAMPBELL, G., BENNETT, M. & HOLMAN, M. E. (1964). Innervation of the guinea-pig taenia coli: are there intrinsic inhibitory nerves which are distinct from sympathetic nerves? *Int. J. Neuropharmac.*, **3**, 163–166.

BURNSTOCK, G., CAMPBELL, G. & RAND, M. J. (1966). The inhibitory innervation of the taenia of the guinea-pig caecum. *J. Physiol., Lond.*, **182**, 504–526.

CAMPBELL, G. (1966a). The inhibitory nerve fibres in the vagal supply to the guinea-pig stomach. *J. Physiol., Lond.*, **185**, 600–612.

CAMPBELL, G. (1966b). Nerve-mediated excitation of the taenia of the guinea-pig caecum. *J. Physiol., Lond.*, **185**, 148–159.

DAY, M. D. & WARREN, P. R. (1967). Inhibitory responses to transmural stimulation in isolated intestinal preparations. *J. Pharm. Pharmac.*, **19**, 408–410.

FURCHGOTT, R. F. (1960). Receptors for sympathomimetic amines. *Adrenergic Mechanisms*, pp. 246–252, Ciba Symposium, ed. Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M. London: Churchill.

GILLESPIE, J. S. & MACKENNA, B. R. (1960). The inhibitory action of nicotine on the rabbit colon. *J. Physiol., Lond.*, **152**, 191–205.

GREEFF, K., KASPERAT, H. & OSSWALD, W. (1962). Paradoxe Wirkungen der elektrischen Vagusreizung am isolierten Magen—und Herzvorhofpräparat des Meerschweinchens, sowie deren Beeinflussung durch Ganglienblocker, Sympathicolytica, Reserpin und Cocain. *Arch. exp. Path. Pharmak.*, **243**, 528–545.

HARRISON, J. S. & MCSWINEY, B. A. (1936). The chemical transmitter of motor impulses to the stomach. *J. Physiol., Lond.*, **87**, 79–86.

HOLMAN, M. E. & HUGHES, J. R. (1965). Inhibition of intestinal smooth muscle. *Aust. J. exp. Biol. Med. Sci.*, **43**, 277–290.

LANGLEY, J. N. (1898). On inhibitory fibres in the vagus to the end of the oesophagus and the stomach. *J. Physiol., Lond.*, **23**, 407–414.

MARTINSON, J. & MUREN, A. (1960). Studies on vagal excitation and inhibition of gastric motility. *Acta physiol. scand.*, **50**, Suppl. 175, 103–104.

MARTINSON, J. & MUREN, A. (1963). Excitatory and inhibitory effects of vagus stimulation on gastric motility in the cat. *Acta physiol. scand.*, **57**, 309–316.

MARTINSON, J. (1964). The effect of graded stimulation of efferent vagal nerve fibres on gastric motility. *Acta physiol. scand.*, **62**, 256–262.

MARTINSON, J. (1965) Vagal relaxation of the stomach. Experimental re-investigation of the concept of the transmission mechanism. *Acta physiol. scand.*, **64**, 453–462.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

MCSWINEY, B. A. & WADGE, W. J. (1928). Effects of variations in intensity and frequency on the contractions of the stomach obtained by stimulation of the vagus nerve. *J. Physiol., Lond.*, **65**, 350–356.

PATON, W. D. M. (1955). The response of the guinea-pig ileum to stimulation by co-axial electrodes. *J. Physiol., Lond.*, **127**, 40–41*P*.

PATON, W. D. M. & VANE, J. R. (1963). An analysis of the responses of the isolated stomach to electrical stimulation and to drugs. *J. Physiol., Lond.*, **165**, 10–46.

VEACH, H. O. (1925). Studies on the innervation of smooth muscle, I. Vagus effects on the lower end of the esophagus, cardia and stomach of the cat, and the stomach and lung of the turtle in relation to Wedensky inhibition. *Am. J. Physiol.*, **71**, 229–264.