Br. J. Pharmac. (1971), **41**, 213–226.

Secondary excitation of intestinal smooth muscle

J. B. FURNESS

Department of Zoology, University of Melbourne, Parkville 3052, Victoria, Australia

Summary

1. A period of stimulation of intrinsic or extrinsic nerves to intestinal muscle is often followed by a secondary contraction. In the present work, the basis for such secondary contractions in the longitudinal muscle of the large bowel of guinea-pigs and rabbits was examined.

2. In general, the action of cholinergic nerves did not contribute significantly to the secondary contractions. Concentrations of atropine or hyoscine, which completely blocked primary cholinergic contractions, potentiated or did not significantly reduce secondary contractions.

3. Atropine resistant, nerve mediated primary contractions of the guinea-pig ileum were inhibited by anticholinesterases, although these drugs potentiated secondary contractions in other segments of the gut.

4. The occurrence of a secondary contraction following inhibition of smooth muscle activity did not depend on the nature of the initial inhibition. Thus, secondary contractions were observed following the responses to both non-adrenergic and adrenergic inhibitory nerves, adenosine triphosphate, noradrenaline and brief periods of anoxia. The secondary contractions following hyperpolarizing drugs or anoxia were not prevented by tetrodotoxin in a concentration sufficient to paralyse all nerves.

5. It is concluded that cholinergic nerves do not contribute significantly to secondary contractions except at frequencies of stimulation higher than about 50 Hz or after the inhibition of cholinesterases. In the segments of gut examined, the secondary contractions were principally myogenic.

Introduction

Secondary excitation or 'rebound' of gut muscle follows a variety of inhibitory stimuli. The secondary excitation has been considered by some to be due to the action of excitatory nerves (Langley, 1898; Day & Warren, 1968) and by others to be a reaction of the muscle to preceding inhibition (Barry, 1932; Bennett, 1966; Campbell, 1966). Results which indicate that the secondary excitation observed in the guinea-pig colon after blockade of muscarinic receptors is myogenic were presented in detail in a recent publication (Furness, 1970). In the present work, the possible contribution of excitatory nerves to secondary contractions has been examined. Further evidence which suggests that a myogenic rebound following inhibition is a general feature of gut muscle is presented.

Methods

Mechanical responses recorded longitudinally from isolated segments from the caecal taenia, the proximal and distal colon and the non-terminal ileum of the

guinea-pig, and from the transverse colon of the rabbit were examined under conditions similar to those previously described (Furness, 1970). The movements of the tissues, suspended in a 50 ml organ bath, were recorded by a frontal-point writing lever on a smoked drum. The tension on the preparation was 0·5–3 g and the magnification of the lever was 4–8 times. The tissue was suspended in modified Krebs solution of 35–36° C (Furness, 1969) which was bubbled with 95% O_2 and 5% CO_2.

The intrinsic nerves were stimulated with platinum rings, 3 cm apart, one around each end of the tissue. Platinum ring electrodes were used to stimulate the pelvic nerves to the colon. Arteries supplying the gut were drawn through similar electrodes which were used to stimulate the accompanying nerve trunks. In the **Results** section, these have been referred to as perivascular electrodes and the nerves to the gut which are stimulated, paravascular, a name which indicates their relationship to the blood vessels. In all cases, pulse widths between 0·1 and 0·5 ms, usually 0·2 ms, were used to stimulate the nerves. Extrinsic nerves, pelvic and paravascular, were stimulated supramaximally. The strength of the stimulus applied to intramural nerves depended on the particular experiment. In most cases, supramaximal stimuli were used.

Animals of both sexes were used. The guinea-pigs weighed between 150 and 800 g and the rabbits between 2 and 2·5 kg. The animals were stunned and bled out and the tissue dissected immediately.

The following drugs, their concentrations given in terms of the salt at the final concentration in the bath, were used: adenosine triphosphate-5-disodium tetrahydrate, atropine sulphate, bretylium tosylate, hyoscine hydrobromide, neostigmine methylsulphate, noradrenaline bitartrate, physostigmine sulphate and tetrodotoxin (Sankyo).

Results

Primary contractions in response to nerve stimulation

Two situations were examined in which primary cholinergic contractions were elicited, little complicated by the simultaneous stimulation of other nerves. These were the response of the distal colon of the guinea-pig to stimulation of the pelvic nerves and the response of the guinea-pig ileum to transmural stimulation. In the guinea-pig ileum, the frequency of stimulation was kept at 10 Hz or below and the strength was not sufficient to cause non-cholinergic excitation (Ambache & Freeman, 1968). In both situations, the response was blocked by hyoscine (6×10^{-7} g/ml), without revealing the action of any other nerve fibres on the muscle. Cholinergic primary contractions were rapid in onset and offset. If the stimulation was stopped after 5–10 s, during the contraction, the gut immediately relaxed. If the stimulation period was extended beyond 10–15 s, the contraction usually declined even though stimulation was continued. Similar primary contractions were sometimes observed in response to transmural stimulation of the colon or taenia caeci (see below). Anticholinesterases, neostigmine and physostigmine (10^{-7}–10^{-5} g/ml) potentiated the primary cholinergic contractions of the colon and ileum and extended their time course. In the presence of an anticholinesterase, the primary contraction often continued to develop after the cessation of short trains of stimuli (5–10 s). With longer

trains of stimuli, the contraction was sometimes maintained after the stimulation was stopped. Pieces of gut took up to 5 min to regain their original tone after primary contractions in the presence of anticholinesterases.

Non-cholinergic primary contractions were examined in the guinea-pig ileum in the presence of hyoscine (6×10^{-7} g/ml). The contractions were elicited by trains of transmural stimuli at 10–50 Hz, usually lasting 10 seconds. The non-cholinergic spasms declined immediately after stimulation was stopped in 80% of the experiments. In the other examples, the contraction continued to develop for about 1–3 s after the end of stimulation and then rapidly declined. Neostigmine or physostigmine ($1–2{\cdot}5 \times 10^{-6}$ g/ml) added in the presence of hyoscine always decreased the amplitude of the non-cholinergic primary contractions (Fig. 1). The responses quickly returned to their original, or greater, amplitude when the bath was circulated with fresh, drug-free solution. Anticholinesterases had no detectable effect on the duration of the non-cholinergic contraction, but they often increased the delay between the beginning of a period of stimulation and the start of the contraction.

Responses of the colon and taenia caeci to transmural stimulation

The responses of these preparations to brief periods (about 10 s) of transmural stimulation could be divided into four types. This division of the responses has been described in detail by Campbell (1966). Type 1 consisted of a contraction during stimulation and a relaxation to the normal level of tone once stimulation was stopped. In the second type (type 2), a contraction during stimulation and a further contraction on cessation of stimulation were observed. In this type there

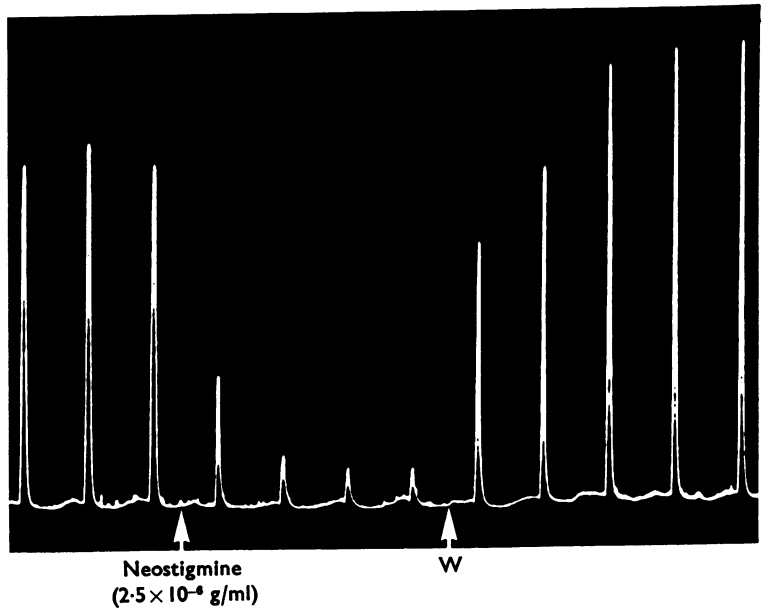

FIG. 1. Effect of anticholinesterase on the non-cholinergic contraction of the guinea-pig ileum. Responses to equal transmural stimuli at 50 Hz for 10 s every 3 min in the presence of hyoscine (6×10^{-7} g/ml) were markedly reduced by the addition of neostigmine ($2{\cdot}5 \times 10^{-6}$ g/ml, first arrow) to the organ bath. After fresh solution was run into the bath (W), the response was quickly re-established.

was generally an increase in the rate of contraction when the stimulation was stopped. The type 3 response consisted of an initial relaxation which weakened and was followed by a contraction during stimulation. When the stimulation was stopped, there was further contraction of the preparation. With responses of type 4, the only change during stimulation was a relaxation, the amplitude of which depended on the tone of the preparation. After the end of stimulation, the preparation contracted, slightly in preparations of high tone and considerably in preparations of low tone.

In type 2 and 3 responses, where two phases of contraction occurred, these could be separated by extending the period of stimulation (Fig. 2). When this was done, the contraction which occurred during stimulation did not persist and the preparation usually regained its resting tone after about 60 seconds. However, when the stimulus was stopped, the preparation again rapidly contracted. Except in the proximal colon of the guinea-pig, the first contraction was nearly always blocked by atropine (10^{-8}–5×10^{-7} g/ml) or hyoscine (10^{-7} 6×10^{-7} g/ml), but the contraction following stimulation remained the same, or was potentiated, by these drugs (Fig. 2). In the proximal colon, a strong contraction was sometimes observed during stimulation in the combined presence of hyoscine and atropine. This atropine resistant contraction also declined during stimulation and a second contraction was observed when stimulation was stopped. Tetrodotoxin (1.2×10^{-7} g/ml) completely blocked both the atropine resistant primary contraction and the secondary contraction of the proximal colon (Fig. 3).

In the taenia caeci or distal colon of the guinea-pig and in the colon of the rabbit, the effect of hyoscine (6×10^{-7} g/ml) or atropine (10^{-7} g/ml) on responses of type 1 was examined. In all cases the contraction was reduced to less than 10% of its original amplitude by either of these drugs and in most cases the blockade was complete. The response during stimulation was often reversed to a relaxation by muscarinic blockade and this relaxation was followed by a secondary contraction

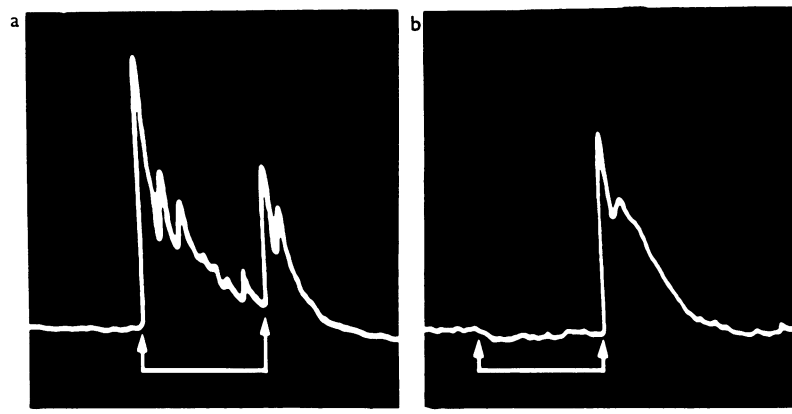

FIG. 2. Differential effect of muscarinic blockade on primary and secondary contractions to transmural stimulation in the rabbit transverse colon. Intramural nerves were stimulated at a frequency of 2 Hz for 60 s (indicated by the horizontal lines between the arrows). In normal solution (a), there was a primary contraction which was quickly established and declined with continued stimulation. When stimulation was stopped, a rapid secondary contraction was observed. The same preparation in the presence of atropine (5×10^{-7} g/ml) (b) gave a slight relaxation during stimulation and a potentiated secondary contraction when stimulation was stopped.

when the stimulus was stopped (Fig. 4). In many cases, hyoscine revealed a contraction, following stimulation, where none had previously been observed and this secondary contraction was larger than the primary cholinergic contraction originally obtained during the period of stimulation (Fig. 4).

The sensitivity to muscarinic blockade of the secondary contractions following the inhibitory action of intramural nerves depended on the frequency of stimulation (Figs. 5 and 6). Secondary contractions following a single pulse or trains of stimuli at 0·2–10 Hz were never reduced by hyoscine and at frequencies of 20 or 50 Hz there was sometimes enhancement and sometimes reduction of the secondary contraction by hyoscine or atropine. The secondary contraction following a train of stimuli at 100 Hz was usually reduced by muscarinic blockade. The most common effect of hyoscine, which occurred in 80% of preparations, was a potentiation of the response at frequencies less than 50 Hz and a depression or little change at higher frequencies (Fig. 5). Usually, the potentiation was most marked at low frequencies. The effects of atropine were similar to those of hyoscine, except that, in one experiment, a depression of the secondary contraction at low frequency (2–10 Hz) was observed in the presence of atropine (10^{-6} g/ml). In many experiments, atropine or hyoscine caused an increase in the amplitude of the initial inhibition which occurred during stimulation.

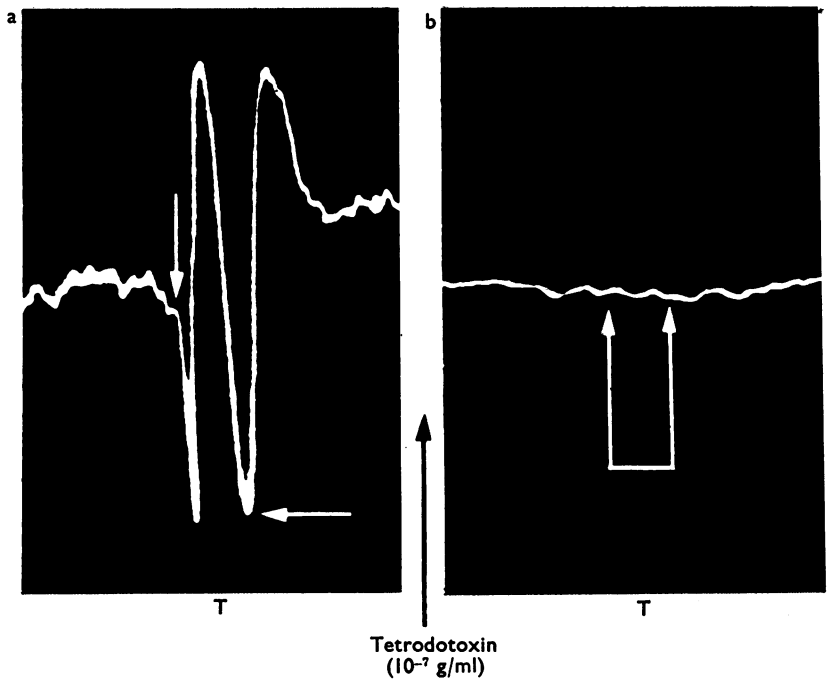

FIG. 3. Non-cholinergic primary contraction of the proximal colon of the guinea-pig blocked by tetrodotoxin (10^{-7} g/ml). In (a) the response of the colon to stimulation at a frequency of 10 Hz for 20 s in the presence of both atropine (10^{-7} g/ml) and hyoscine (6×10^{-7} g/ml). Stimulation (begun at the first arrow) gave an immediate relaxation. The relaxation soon reversed into a contraction which declined before stimulation was stopped (second arrow). After the cessation of stimulation the preparation contracted beyond its original level of tone. All phases of the response were blocked by tetrodotoxin (b).

The overall tone of segments of gut muscle was sometimes reduced by hyoscine or atropine. Such a reduction in tone could lead directly to an enhancement of the secondary contraction (Burnstock, Campbell & Rand, 1966; Furness, 1970). However, in the present work, the potentiation of secondary contractions by muscarinic blockade was observed in preparations in which tone was unchanged as well as in those in which tone was reduced by muscarinic blockade.

Sometimes, after stimulation at 50 Hz, and more often after stimulation at 100 Hz, two secondary contractions were observed. The first was fast and insensitive to atropine or hyoscine, similar to secondary contractions at lower frequencies, and the second was slow, reaching a peak 3–10 min after the end of stimulation and taking up to 15 min to decline. Hyoscine (6×10^{-7} g/ml) completely blocked the second after-contraction (Fig. 6). In general, the two contractions were quite distinct; the first after-contraction almost completely subsided before the second one occurred.

The effects on the biphasic response to transmural stimulation (type 3 or 4 responses) of two anticholinesterases, physostigmine and neostigmine, in concentra-

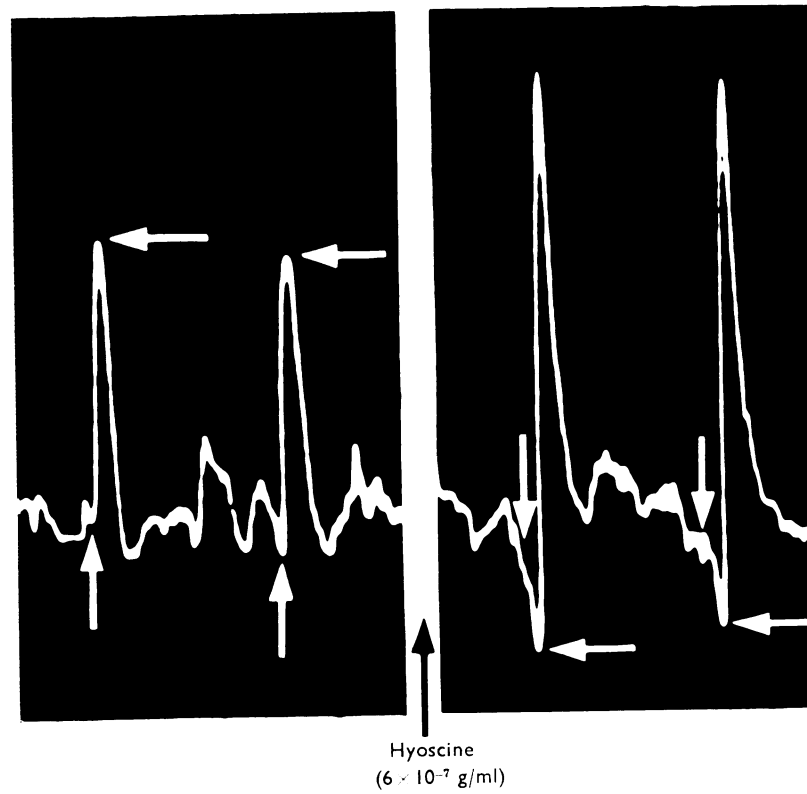

FIG. 4. Effect of hyoscine (6×10^{-7} g/ml) on a type 1 response of the taenia caeci of the guinea-pig. The two records are from the one preparation with equal stimuli at 40 Hz given for 10 s at intervals of 3 minutes. There was no change in tone between the two records. Before hyoscine was added, the taenia contracted immediately on stimulation (vertical arrows) and relaxed immediately stimulation was stopped (horizontal arrows). In the presence of hyoscine, there was a slight relaxation during stimulation (between the arrows) and the preparation contracted as soon as the stimulus was withdrawn. Note that the primary contraction was converted to a relaxation and that a secondary contraction of greater amplitude than the original primary contraction appeared where no contraction was previously observed.

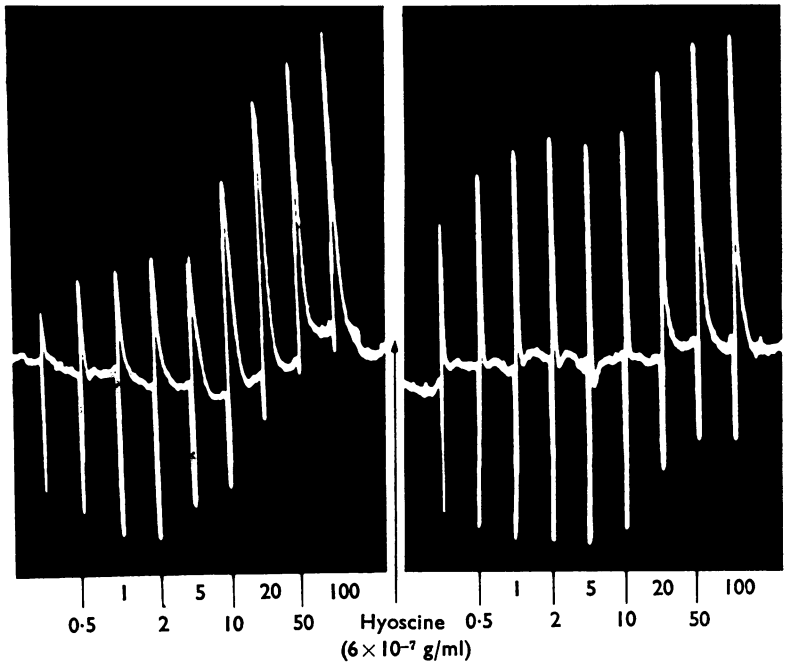

FIG. 5. The effect of hyoscine on the response of the guinea-pig distal colon to frequencies of stimulation up to 100 Hz. The first response is to a single pulse and subsequent responses are to trains of 10 s duration at the indicated frequencies (Hz). The stimulus always resulted in an initial inhibition and a secondary contraction after stimulation was stopped. Muscarinic block potentiated the secondary contraction at low frequencies. Stimulation at 3 min intervals. There was no change in the tone of the colon following the addition of hyoscine.

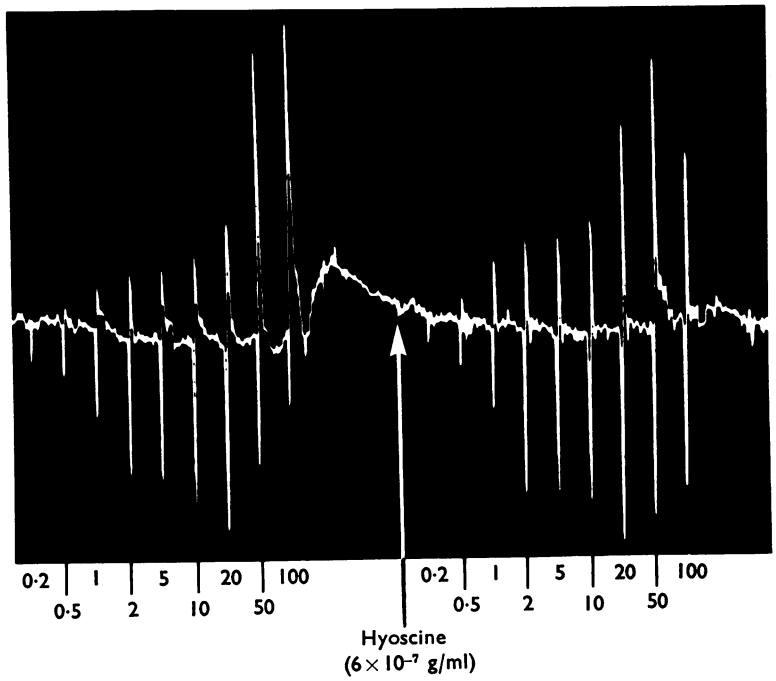

FIG. 6. Effect of muscarinic blockade on the late secondary contraction to transmural stimulation in the guinea-pig colon. In normal solution, there was a fast secondary contraction following all stimuli from 0·5 to 100 Hz. However, after the stimulation at 100 Hz, a slow contraction developed following the initial fast after-contraction. Hyoscine (6×10^{-7} g/ml, at the arrow) potentiated the secondary contractions at frequencies up to 50 Hz. At 100 Hz, the fast secondary contraction was reduced and the slow after-contraction was abolished. Stimulation for 10 s at 3 min intervals.

tions from 10^{-6} to 5×10^{-5} g/ml, were examined. Anticholinesterases often reversed the initial inhibition to a primary contraction. In those preparations in which an initial relaxation was still observed in the presence of an anticholinesterase, the inhibition usually became less and was replaced by a contraction during periods of stimulation of 10 s or longer at frequencies above 5 Hz. Secondary contractions to transmural stimulation were generally increased in both amplitude and duration by anticholinesterases. An example of the action of cholinesterase inhibition is shown in Fig. 7. This preparation had a high tone and, before neostigmine was added, transmural stimulation gave only a relaxation. However, in the presence of neostigmine, the initial relaxation was not sustained and a large and extended contraction was observed which continued after the end of stimulation. The reversal of initial inhibition and the potentiation of the secondary contraction by physostigmine or neostigmine were completely reversed by the addition of hyoscine (6×10^{-7} g/ml) after the anticholinesterase had been washed out.

Responses to stimulation of paravascular nerves

Secondary contractions, which were never blocked but were sometimes potentiated by atropine or hyoscine, were also observed in response to stimulation of the paravascular sympathetic nerves. These contractions were observed in about 30% of preparations of the proximal or distal colon of the guinea-pig and in the taenia

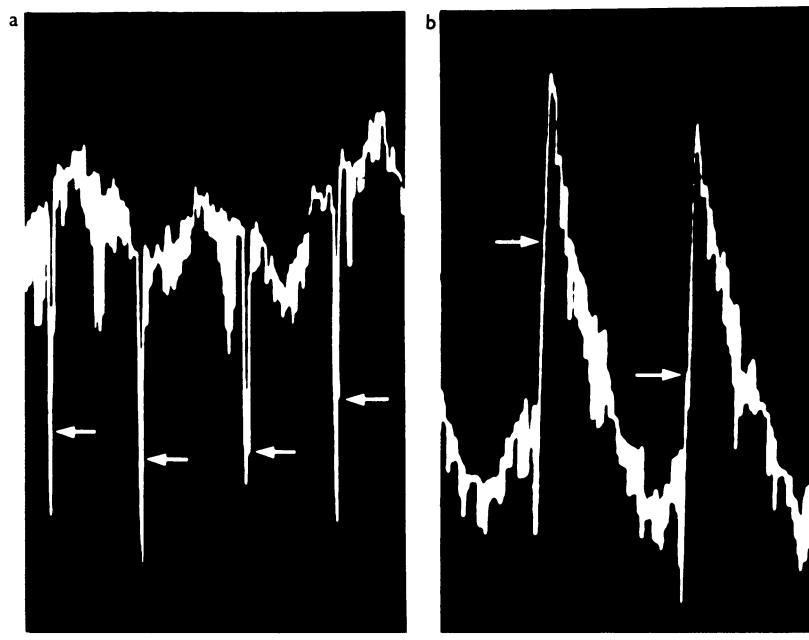

FIG. 7. Action of anticholinesterase on the response of the guinea-pig taenia caeci to transmural stimulation. The stimuli were for 10 s at 10 Hz, at 3 min intervals. The end of stimulation is indicated by the horizontal arrows. Before the application of neostigmine ($2{\cdot}5 \times 10^{-6}$ g/ml), the preparation relaxed in response to the stimuli and there was no secondary contraction beyond the normal level of tone (a). Twenty minutes after the addition of neostigmine (b), the preparation relaxed initially and then contracted beyond the resting tone during stimulation. The gut continued to contract after stimulation was stopped. As the secondary contractions declined only slowly, the interval between stimuli was extended. There was no change in the tone of the preparation after the addition of neostigmine to the bath.

caeci in about 10% of the preparations. When the primary inhibition was blocked by bretylium, the secondary contraction was also blocked (Fig. 8). In the proximal colon, bretylium sometimes caused a rise in tone. Simultaneous blockade of both phases of the response to perivascular stimulation in the distal colon of the guinea-pig has previously been reported by Holman & Hughes (1965), who used guanethidine, and by Furness (1970), who used propranolol as a blocking agent. Blockade of the secondary contraction in response to paravascular stimulation usually caused little change in the initial relaxation or the secondary contraction in response to stimulation of the intramural inhibitory nerves, except where a change in tone accompanied blockade. These results suggest that the secondary excitation following stimulation of paravascular nerves is dependent on the existence of the initial inhibition.

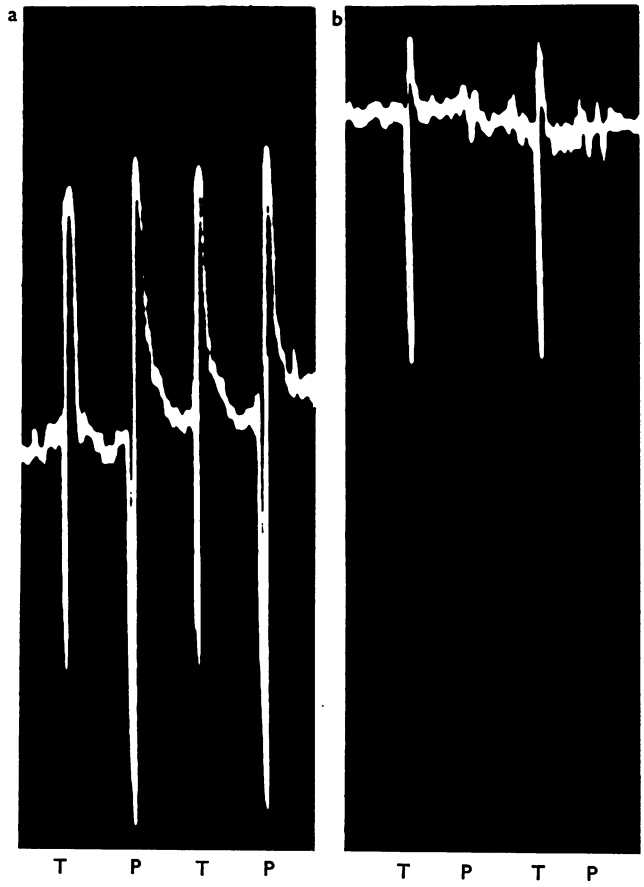

FIG. 8. Selective blockade by bretylium of the initial inhibition and the secondary contraction following stimulation of paravascular nerves to the guinea-pig proximal colon. Stimulation of paravascular nerves at 20 Hz for 10 s (P) and transmural stimulation at 2 Hz for 10 s (T). Stimulus interval 3 minutes. Before the application of bretylium (10^{-6} g/ml), both perivascular and transmural stimulation gave a relaxation during stimulation and a contraction when stimulation was turned off (a). Bretylium raised the tone of the preparation and blocked both the initial relaxation and the secondary contraction due to stimulation of paravascular nerves. The biphasic response to transmural stimulation was reduced but not blocked.

Secondary contractions to non-nervous stimuli

Three inhibitory agents whose removal results in a secondary excitation of the gut have been examined in the colon and taenia. These are adenosine triphosphate (Drury & Szent-Györgyi, 1929; Gillespie, 1934), noradrenaline or adrenaline (Templeton & Lawson, 1932) and brief anoxia (Bayliss & Starling, 1899; Job, Schaumann & Schmidt, 1955). The present experiments confirmed these authors' observations (see also Furness, 1970). In addition, it has been found that the secondary contractions persist in the combined presence of hyoscine (6×10^{-7} g/ml) and tetrodotoxin (2×10^{-7} g/ml). The secondary contraction which followed the washout of adenosine triphosphate (10^{-6} g/ml) was the most prominent. It occurred almost immediately after the bath was flushed with fresh solution and persisted in the presence of either hyoscine (6×10^{-7} g/ml) or tetrodotoxin (2×10^{-7} g/ml). The relaxation caused by noradrenaline (5×10^{-9} g/ml) was more resistant to the washout of the drug than was that of adenosine triphosphate. Even so, the washout of noradrenaline was often followed by secondary excitation. The excitation following the action of noradrenaline was sometimes reduced by hyoscine (6×10^{-7} g/ml). However, secondary contractions following the action of noradrenaline were still

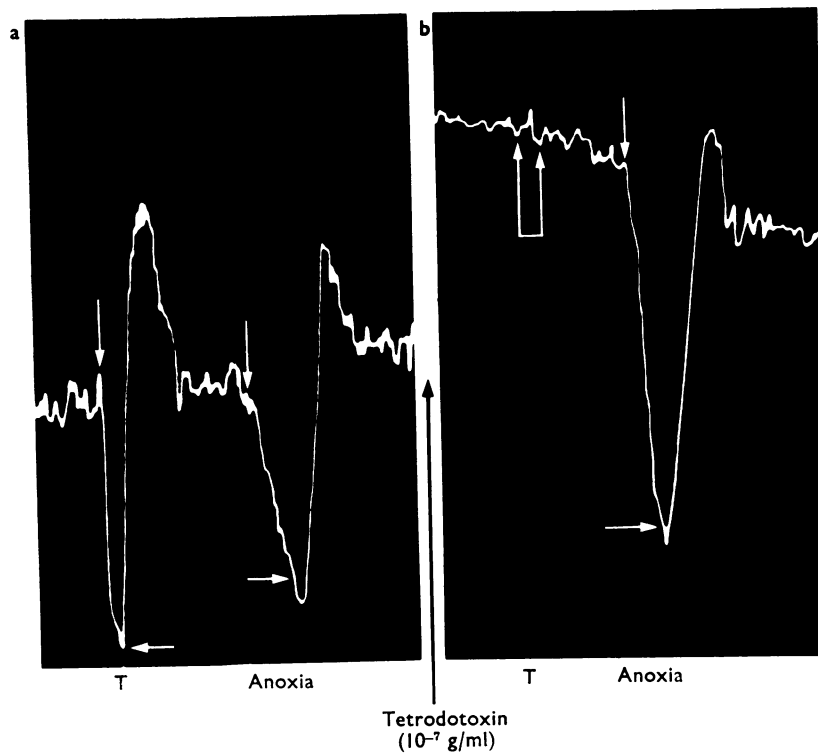

FIG. 9. Secondary contraction following inhibition of the guinea-pig proximal colon by anoxia and transmural stimulation. In (a), transmural stimulation for 15 s at 5 Hz (T, between the arrows) caused relaxation. When the stimulus was stopped, the colon contracted beyond its resting level. A similar response was obtained by a brief period of anoxia. The record in b, taken 4 min after the addition of tetrodotoxin (10^{-7} g/ml) to the organ bath, shows that the response to transmural stimulation (T) was blocked, while that to brief anoxia was not significantly changed. The tone of the preparation rose slightly after the application of tetrodotoxin.

observed in the presence of hyoscine (6×10^{-7} g/ml) or tetrodotoxin (2×10^{-7} g/ml).

Relaxations in response to transmural stimulation and to brief anoxia in the guinea-pig proximal colon are shown in Fig. 9. Both these relaxations were followed by similar secondary contractions, but the biphasic response to transmural stimulation was blocked by tetrodotoxin (10^{-7} g/ml) without significantly changing either the initial relaxation or the after-contraction in response to a brief period of anoxia. As it occurred sometimes, the proximal colon increased in tone when the nervous conduction was blocked by tetrodotoxin. The response to anoxia varied. Inhibition was most commonly seen in the proximal colon, but for other segments there was often little change during brief anoxia. In some preparations of distal colon and taenia coli a contraction occurred during anoxia which was depressed by hyoscine (6×10^{-7} g/ml).

Discussion

If secondary contractions were due to the persistence of an excitatory transmitter, one would expect primary contractions also to be maintained after the end of stimulation. However, primary cholinergic responses to transmural stimulation or to stimulation of the pelvic nerves to the colon are not sustained during periods of stimulation longer than about 10 seconds. With shorter periods of stimulation, primary cholinergic contractions generally decline as soon as stimulation is stopped. Other workers have reported that continued stimulation does not maintain the cholinergic response (Garry & Gillespie, 1955 ; Rand & Ridehalgh, 1965 ; Bianchi, Beani, Frigo & Crema, 1968 ; Hukovic & Somogyi, 1969 ; Kottegoda, 1969). Beani, Bianchi & Crema (1969) have shown that the rate of release of acetylcholine declines during extended stimulation of the intramural nerves of the guinea-pig colon at moderate frequencies. These results make it seem unlikely that secondary contractions are due to the persistence of the action of acetylcholine. Moreover, the observation that a block of muscarinic receptors generally potentiates the secondary contractions suggests that normally there is no cholinergic basis for the secondary contractions. However, when the duration of action of cholinergic nerves is extended by inhibition of cholinesterase or after stimulation at very high frequency, a cholinergic contribution to the secondary contraction is revealed. Other investigations also have found a potentiation of secondary excitation by muscarinic blocking agents (Campbell, 1966 ; Lund & Christensen, 1969 ; Furness, 1970), although a depression has been reported by Kuriyama, Osa & Toida (1967) and Day & Warren (1968). Additional evidence for a non-cholinergic rebound is contained in the work of Hobbiger, Mitchelson & Rand (1969) who found that the primary contraction of the taenia of the guinea-pig caecum caused by nicotine or dimethylphenylpiperazinium is reversed by hyoscine and that the relaxation so revealed is followed by a contraction greater than that before the blockade of cholinoceptors.

In the present work, non-cholinergic spasms of the guinea-pig ileum never persisted for more than 3 s after the end of a 10 s burst of stimuli. In addition, the non-cholinergic contraction of the ileum is depressed by cholinesterase inhibitors, whereas the secondary contractions in other areas of the gut are potentiated by these drugs. Recently, Ambache & Zar (1970) compared the non-cholinergic excitation of the guinea-pig ileum with that in the distal colon. The non-cholinergic excitation observed by them in the colon is probably a rebound phenomenon, for their records of the contractions of the colon show a clear initial inhibition. These

authors have shown that the non-cholinergic spasms of the ileum are inhibited by histamine, but that there is little effect of this drug on the secondary contractions of the colon. Because the non-cholinergic spasms of the ileum respond differently to anticholinesterases and to histamine and because generally they do not persist after electrical stimulation, it seems unlikely that non-cholinergic nerves of this type contribute to the secondary contractions that have been observed in the regions of the gut used in the present work. However, in situations other than those examined, for example the response to vagal stimulation of the proventriculus of the chicken (Nakazato, Sato & Ohga, 1970), a neurogenic, non-cholinergic secondary contraction may occur.

The present work provides additional evidence for a myogenic basis of secondary contractions. Rebound contractions sometimes follow the stimulation of sympathetic nerves (Pflüger, 1857 ; Bayliss & Starling, 1898). In the distal colon of the guinea-pig the rebound was previously shown to depend on the presence of a preceding inhibition ; if this inhibition was blocked, the secondary excitation also disappeared (Holman & Hughes, 1965 ; Furness, 1970). The same phenomenon has now also been observed in the proximal colon and in the taenia caeci. In these situations, stimulation of the paravascular nerves, in the presence of atropine or hyoscine, results in an adrenergic hyperpolarization of the gut muscle (Bennett, Burnstock & Holman, 1966 ; Furness, 1969). The hyperpolarization of the gut by non-adrenergic inhibitory fibres is also followed by rebound excitation (Bennett, 1966 ; Kuriyama *et al.*, 1967). In addition, rebound follows hyperpolarization of the muscle membrane by current passed from large external electrodes when all the nerves have been paralysed by tetrodotoxin (Furness, 1970).

Secondary excitation following intestinal inhibition caused by anoxia (Bayliss & Starling, 1899 ; Job *et al.*, 1955) and by various chemical agents (Drury & Szent-Györgyi, 1929 ; Templeton & Lawson, 1932 ; Gillespie, 1934 ; Axelsson & Holmberg, 1969 ; Bowman & Hall, 1970 ; Furness, 1970) has been reported. It has now been found that the secondary excitation following the action of hyperpolarizing drugs can also be observed in the presence of tetrodotoxin (see also Bowman & Hall, 1970). Brief periods of anoxia in tetrodotoxin poisoned preparations give rise to an initial relaxation followed by rebound excitation when the oxygen supply is restored. It therefore seems that myogenic excitation is a general phenomenon that follows a great variety of inhibitory stimuli.

Only the secondary contraction following the action of noradrenaline is consistently depressed by hyoscine. It has been suggested that the action of exogenous noradrenaline in inhibiting transmitter release from cholinergic nerve endings in the gut is due to a hyperpolarization of the terminals (Paton & Vizi, 1969). If this were the case, the termination of the hyperpolarizing influence of noradrenaline on cholinergic nerve terminals might cause an enhanced release of acetylcholine. Thus, an initial inhibition of the gut, due to the action of noradrenaline on the gut muscle, might be followed by a secondary contraction, partly due to an enhanced release of acetylcholine from intrinsic nerve terminals.

It is concluded that any cholinergic contribution to the secondary contraction of mammalian gut is generally insignificant. However, if the time of action of acetylcholine is extended, for example, by anticholinesterases or stimulation at very high frequencies, a cholinergic contribution to the rebound excitation is possible. The

evidence discussed above suggests that a myogenic reaction to the withdrawal of an inhibitory stimulus contributes the major part to the secondary excitation of gut muscle. However, the release of a non-cholinergic spasmogen from the tissue could also be involved.

I should like to thank Professor G. Burnstock for his continued support and encouragement. This work was supported by a grant from the Australian Research Grants Committee.

REFERENCES

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

AMBACHE, N. & ZAR, M. A. (1970). An inhibitory action of histamine on the guinea-pig ileum. *Br. J. Pharmac.*, **38**, 229–240.

AXELSSON, J. & HOLMBERG, B. (1969). The effects of extracellularly applied ATP and related compounds on electrical and mechanical activity of the smooth muscle taenia coli from the guinea-pig. *Acta physiol. scand.*, **75**, 149–156.

BARRY, D. T. (1932). The functions of the great splanchnic nerves. *J. Physiol., Lond.*, **75**, 480–490.

BAYLISS, W. M. & STARLING, E. H.[1] (1898). A preliminary note on the innervation of the small intestine. *J. Physiol., Lond.*, **23**, IX–XI.

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

BEANI, L., BIANCHI, C. & CREMA, A. (1969). The effect of catecholamines and sympathetic stimulation on the release of acetylcholine from the guinea-pig colon. *Br. J. Pharmac.*, **37**, 1–17.

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

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1966). Transmission from perivascular inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **182**, 527–540.

BIANCHI, C., BEANI, L., FRIGO, G. M. & CREMA, A. (1968). Further evidence for the presence of non-adrenergic inhibitory structures in the guinea-pig colon. *Eur. J. Pharmac.*, **4**, 51–61.

BOWMAN, W. C. & HALL, M. T. (1970). Inhibition of rabbit intestine mediated by α- and β-adrenoreceptors. *Br. J. Pharmac.*, **38**, 399–415.

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

DAY, M. D. & WARREN, P. R. (1968). A pharmacological analysis of the responses to transmural stimulation in isolated intestinal preparations. *Br. J. Pharmac. Chemother.*, **32**, 227–240.

DRURY, A. N. & SZENT-GYÖRGYI, A. (1929). The physiological activity of adenine compounds with especial reference to their action on the mammalian heart. *J. Physiol., Lond.*, **68**, 213–237.

FURNESS, J. B. (1969). An electrophysiological study of transmission to the smooth muscle of the colon. *J. Physiol., Lond.*, **205**, 549–562.

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

GARRY, R. C. & GILLESPIE, J. S. (1955). The response of the musculature of the colon of the rabbit to stimulation *in vitro*, of the parasympathetic and of the sympathetic outflows. *J. Physiol., Lond.*, **128**, 557–576.

GILLESPIE, J. H. (1934). The biological significance of the linkages in adenosine triphosphoric acid. *J. Physiol., Lond.*, **80**, 345–359.

HOBBIGER, F., MITCHELSON, F. & RAND, M. J. (1969). The actions of some cholinomimetic drugs on the isolated taenia of the guinea-pig caecum. *Br, J. Pharmac.*, **36**, 53–69.

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

HUKOVIĆ, S. & SOMOGYI, G. (1969). An observation on isolated and innervated recta of rats and mice. *Pharmac. Res. Comm.*, **1**, 271–275.

JOB, C., SCHAUMANN, O. & SCHMIDT, H. (1955). Die Wirkung der Anoxie auf den isolierten Meerschweinchendarm. *Arch. exp. Path. Pharmak.*, **226**, 130–139.

KOTTEGODA, S. R. (1969). An analysis of possible nervous mechanisms involved in the peristaltic reflex. *J. Physiol., Lond.*, **200**, 687–712.

KURIYAMA, H., OSA, T. & TOIDA, N. (1967). Nervous factors influencing the membrane activity of intestinal smooth muscle. *J. Physiol., Lond.*, **191**, 257–270.

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

LUND, G. F. & CHRISTENSEN, J. (1969). Electrical stimulation of esophageal smooth muscle and effects of antagonists. *Am. J. Physiol.*, **217,** 1369–1374.

NAKAZATO, Y., SATO, H. & OHGA, A. (1970). Evidence for a neurogenic 'rebound' contraction of the smooth muscle of the chicken proventriculus. *Experientia*, **26,** 50–51.

PATON, W. D. M. & VIZI, E. S. (1969). The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmac.*, **35,** 10–28.

PFLÜGER, E. (1857). Ueber das Hemmungs-Nervensystem für die peristaltischen Bewegungen der Gedärme. Berlin: Verlag von Agust Hirschwald.

RAND, M. J. & RIDEHALGH, A. (1965). Actions of hemicholinium and triethylcholine on responses of guinea-pig colon to stimulation of autonomic nerves. *J. Pharm. Pharmac.*, **17,** 144–156.

TEMPLETON, R. D. & LAWSON, H. (1932). Studies in the motor activity of the large intestine. IV. Response to autonomic drugs. *Am. J. Physiol.*, **101,** 511–528.

(*Received July* 17, 1970)