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A pharmacological analysis of the responses of the gastrointestinal smooth muscle of the bat to transmural and periarterial nerve stimulation

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1 A comparative study of the responses of the gastrointestinal tract of the guinea-pig and of the fruit-eating bat *Eidolon helvum* to transmural nerve stimulation (TNS) was made.

2 The stomach and rectum of the guinea-pig, the bat and the guinea-pig ileum contracted in response to TNS. These contractions were cholinergic in nature because atropine blocked and physostigmine potentiated them.

3 Tetrodotoxin reversibly abolished these contractions suggesting that they were nerve-mediated.

4 The bat isolated ileum usually responded to TNS with mixed motor and inhibitory components. In some cases, there were only motor or inhibitory components.

5 The motor component was abolished by atropine and potentiated by physostigmine. However, the inhibitory component was non-adrenergic and non-cholinergic (NANC).

6 Tetrodotoxin abolished the motor component without influencing the inhibitory component.

7 Periarterial nerve stimulation of the bat ileum produced a relaxation that was blocked by bretylium, propranolol, phentolamine, reserpine and tetrodotoxin.

8 It is concluded that the bat gastrointestinal smooth muscle, like the guinea-pig, has cholinergic excitatory innervation; however, the bat ileum has both a cholinergic excitatory innervation and a non-adrenergic and non-cholinergic inhibitory component.

Introduction

The straw-coloured fruit-bat, *Eidolon helvum*, is a flying mammal of the order *Pteropidae*. It is the second largest of the West African Megachiroptera, ranking next to the Hammer-headed fruit-bat, *Hypsignathus monstrous*, in size. The head and the body length of *E. helvum* is about 20 cm and the wing-spread about 75 cm (Halstead & Segun, 1975). This flying mammal is very common and widespread along the West African coast from the Cameroons to Senegal. On the campus of the University of Ife, the population is estimated at well over one million. They seem to prefer tall trees, especially those standing singly or in small groups. The sex is readily distinguishable, with the female having two axillary nipples (Halstead & Segun, 1975). The fruit-bat is essentially a gregarious species but the size of the congregation which it forms may vary from a couple of dozen to many thousands according to circumstances. These gatherings are for two fundamentally different purposes; one for feeding by night and the other for roosting by day. Breeding takes place in the dry season, the female bearing usually one but occasionally two young ones.

The pharmacology of the bat is still in its infancy. The alimentary canal is in some ways similar to that of the rainbow lizard, *Agama agama*, which Fabiyi & Okpako (1973) found not to have an ileo-caecal junction. The ileum runs straight into the rectum (Halstead & Segun, 1975). The present work is an attempt to study the pharmacology of the entire gastrointestinal tract smooth muscle in response to transmural nerve stimulation (TNS), periarterial nerve stimulation (PNS) and to common drugs.

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Methods

Fruit-bat

Male bats were captured from large colonies in the wild during the daytime. Those with slight injuries in the wings and those free from wounds were kept in well-ventilated cages for subsequent use. Bats weighing 250–400 g were killed with a blow to the head and the whole of the gastrointestinal tract dissected out and removed. Smooth muscle preparations (3–4 cm long) from the stomach, ileum and rectum were used throughout the studies. These were not separated into longitudinal and circular layers. The bat has no ileocaecal junction and the ileum runs into the rectum which is about 4 cm long (Halstead & Segun, 1975). Each tissue was set up in a 50 ml organ bath containing Tyrode solution of the following composition (mmol l^{-1}): NaCl 138, KCl 2.7, $MgCl_2$ 0.53, $NaHCO_3$ 11.9, NaH_2PO_4 0.34, $CaCl_2$ 1.8 and glucose 5.5. The solution was maintained at 37°C and gassed with air. The pH was kept at 7.40 ± 0.06. Isometric contractions and relaxations were recorded with a force-displacement transducer (UFI) on a Ugo Basile one-channel pen recorder model 7050.

Guinea-pig

For comparative purposes, the gastrointestinal tract of the guinea-pig was used. Male guinea-pigs weighing 450–500 g were killed by a blow to the head. A portion of the stomach, ileum (3–4 cm long), and rectum (1.5 cm) were removed and kept in Tyrode solution. Each tissue was set up as described above. Isometric responses were recorded as under (1) above.

Transmural nerve stimulation (TNS)

The tissue was set in such a way that one of the platinum wires of the electrodes was inserted into the lumen of the tissue and the other wire was immersed in the Tyrode solution. This arrangement is similar to the co-axial electrodes described by Paton (1957). The platinum wires were connected to leads of an SRI stimulator which delivered rectangular wave pulses when the tissues were stimulated electrically. For electrical stimulation of the guinea-pig rectum, the preparation was tied alongside the electrode since the rectum is so short that the base of the electrode could not be inserted in the lumen. The frequency, duration and pulse widths employed when stimulating transmurally were varied and are indicated in the text.

Periarterial nerve stimulation (PNS)

The tissue was prepared for PNS according to Finkelman (1930). The mesentery was threaded through bipolar platinum electrodes (Burn & Rand, 1960) connected to a C.F.P. 8048 stimulator. Pulses of 0.3–0.5 ms at supramaximal voltage (25 V) and varying frequencies were used. The frequency, duration and pulse widths are indicated in the text.

Reserpinization

Reserpine (0.5 mg kg^{-1}) was given intraperitoneally daily for 3 days before the animals were killed.

The following resting tensions were applied to the tissues: stomach 0.5 g, proximal duodenum and distal ileum 0.5 g and rectum 1 g. The preparations were allowed to equilibrate for 45 min during which the solution was changed every 15 min. Stimulation was performed every 5 min. Antagonists were added to the bath 30 min before TNS or PNS after control responses (in the absence of antagonist) had been obtained. In each experiment, another tissue was set up with no drug added to monitor changes in tissue sensitivity which might not be due to drugs.

Statistical analysis

Results are expressed as means ± s.e. The differences between means were determined by Student's t test. These were accepted as significant when $P < 0.05$.

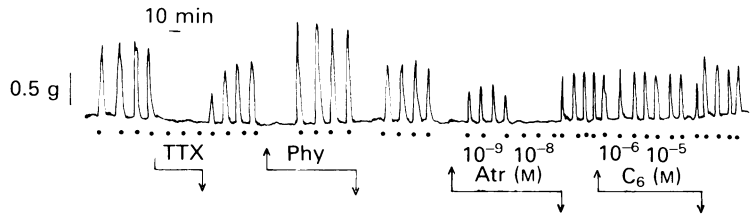

Figure 1 A representative tracing of a record of the response of the bat stomach to transmural nerve stimulation (TNS), in the absence and in the presence of tetrodotoxin (TTX, 10^{-7} M), atropine (10^{-9} M 10^{-8} M) (Atr), physostigmine (Phy) (2.0×10^{-6} M) and hexamethonium (C_6) (10^{-6} M and 10^{-5} M). The preparation was stimulated with 0.5 ms pulse width, 10 Hz and at supramaximal voltage for 10 s. Responses of the guinea-pig duodenum were very similar. ↑ represents point of drug addition, ● the point of TNS and at ↓ the bathing fluid was replaced.

Drugs

The following drugs were used: acetylcholine chloride (Sigma), (−)-noradrenaline (Sigma), phentolamine hydrochloride (Ciba-Geigy), reserpine (Ciba-Geigy), (±)-propranolol hydrochloride (Sigma), tetrodotoxin (Sankyo, Japan), atropine sulphate (BDH), bretylium tosylate (Burroughs Wellcome) and physostigmine salicylate (Burroughs Wellcome). Drug concentrations refer to the base.

Results

Stomach and duodenum

Transmural stimulation of the guinea-pig or the bat isolated stomach and duodenum with 0.2–0.5 ms pulse width, at various frequencies with supra-maximal voltage for 5 s gave contractions. Responses were more consistent at 0.5 ms than at lower pulse widths therefore this was chosen for subsequent work. Figure 1 shows a 'typical' contraction of the bat stomach in response to TNS. Tetrodotoxin (TTX, 10^{-7} M) and atropine (10^{-9} M and 10^{-8} M) reduced and abolished the contractions. Physostigmine (2.0×10^{-6} M) enhanced the contractions but hexamethonium (10^{-6} M and 10^{-5} M) did not influence these responses (Figure 1). Similar responses were obtained in the guinea-pig stomach and in the bat and the guinea-pig duodenum (not shown).

Ileum

Guinea-pig The proximal ileum of the guinea-pig produced single phasic contractions on TNS. Contractions were more consistently obtained with a pulse width of 0.5 ms than 0.3 ms. Also, there was no significant difference ($P > 0.05$) in the magnitude of contractions at 2 Hz to 10 Hz (Table 1). In the guinea-pig ileum, the frequency was kept between 5 Hz and 10 Hz and the strength of stimulation (voltage and pulse width) was not sufficient to cause a non-cholinergic excitation (Ambache & Freeman, 1968). TTX (10^{-7} M) (Figure 2a) and atropine (10^{-8} M) (Figure 2b) abolished the contractions whilst physostigmine (2.0×10^{-6} M) (Figure 2c) produced an enhancement.

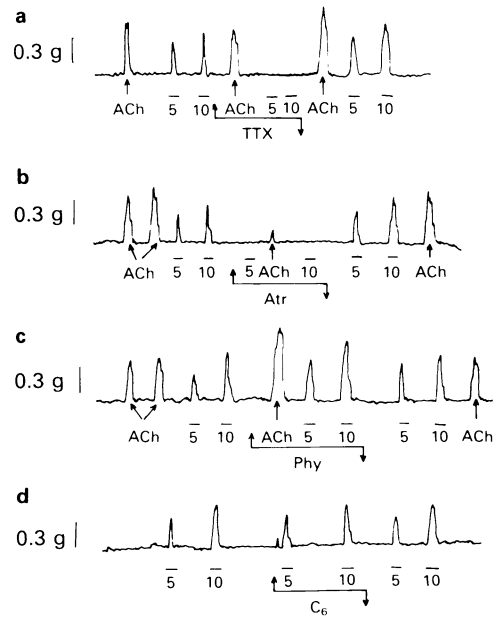

Figure 2 Effects of tetrodotoxin (TTX, 10^{-7} M), atropine (Atr, 10^{-8} M), physostigmine (Phy, 2.0×10^{-6} M) and hexamethonium (C_6, 10^{-5} M) on the responses of the guinea-pig ileum to TNS. Stimulation parameters are as in the text. Numbers represent the frequency (Hz) and vertical bar denotes the stimulation period. (a) At ↑ TTX was added to the bath and washed out of the bath at ↓. ACh = acetylcholine (10^{-8} M). (b) Abolition of the responses of the guinea-pig ileum to TNS and to ACh by atropine. At ↑ atropine was added to the bath and washed out at ↓. (c) Enhancement of the responses of the guinea-pig ileum to TNS and to ACh by physostigmine added to the bath at ↑ and washed out of the bath at ↓. (d) Inability of hexamethonium (C_6) to influence the responses of the guinea-pig ileum to TNS. At ↑ C_6 was added to the bath and washed out at ↓.

Table 1 Effect of various frequencies and pulse widths on contractions of the guinea-pig ileum during transmural nerve stimulation

Pulse width (ms)	0.5	1.0	2.0	4.0
		Responses (g tension)		
Frequency (Hz)				
2	0.70 ± 0.02	0.68 ± 0.02	0.71 ± 0.01	0.70 ± 0.02
4	0.69 ± 0.01	0.69 ± 0.01	0.71 ± 0.02	0.70 ± 0.01
6	0.69 ± 0.01	0.68 ± 0.02	0.71 ± 0.02	0.73 ± 0.01
8	0.68 ± 0.02	0.69 ± 0.02	0.69 ± 0.02	0.70 ± 0.01
10	0.69 ± 0.01	0.67 ± 0.01	0.69 ± 0.02	0.69 ± 0.01

Responses are expressed in g tension (mean ± s.e.mean of at least 6 observations).

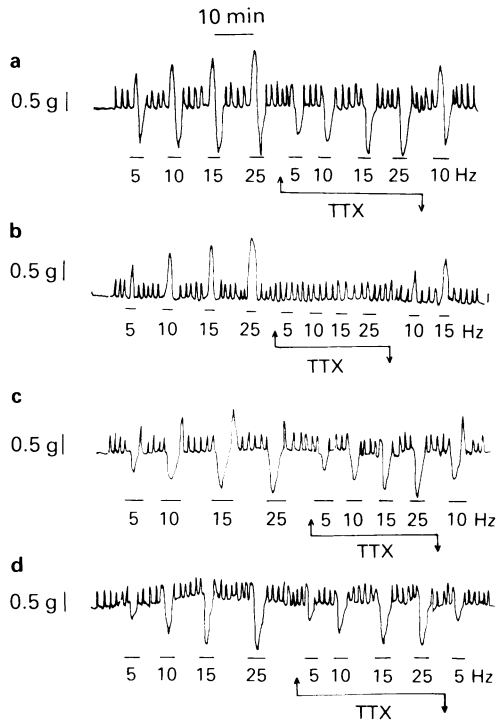

Figure 3 (a–d) Types of responses of the isolated ileum of the bat to TNS and the effects of tetrodotoxin (TTX, 10^{-7} M) on these responses. Stimulation parameters: duration 0.5 ms, various frequencies and at supramaximal voltage for 10 s. TTX was added at ↑ 15 min before TNS and washed out at ↓. Type (a) constitutes 60% of the preparations, Type (b) 20%, Type (c) 12% and Type (d) 8%. Horizontal bars indicate periods of stimulation.

On the other hand, hexamethonium (10^{-5} M) had no effect on TNS-induced contractions (Figure 2d). TTX at this concentration did not influence ACh-induced contractions whilst atropine blocked the effect of ACh (see Figure 2a and b).

Bat The bat ileum has an inherent spontaneous rhythmic contraction similar to that seen in the rabbit ileum. From preliminary experiments, we found that the distal segment (at least, 10 cm from the rectum) was more sensitive to drugs and electrical stimulation than the proximal portion hence the former was used in this work. Responses (contractions and relaxations) were elicited with trains of pulses of 0.5–100 Hz delivered at 0.5–1.0 ms duration and supramaximal voltage (25 V). Stimulation lasted for 10–20 s and was repeated every 5 to 10 min. Responses of the bat ileum to TNS consisted of mixed motor and inhibitory components (Figure 3). As shown in Figure 3, there was variation between different preparations but, in general, the responses could be divided into four groups. This variation in response did not appear to be related to the stimulus parameters in this study. The variation is unlikely to be due to the area from which the tissue was taken because we restricted ourselves to the same area, i.e. 10 cm from the rectum and usually only two segments of the ileum were used from one bat. The responses of the more proximal or more distal of the two segments could be type 1, type 2, type 3 or type 4. Thus, type 1 which was most usual, constituted 60% of the responses. This consisted of a rapid excitatory phase followed by an immediate inhibitory phase during the stimulation period (Figure 3a). It is important to note that both phases were observed before the end of the stimulation, i.e. the inhibitory phase was not due to a post-stimulation phenomenon. To test for a possible tachyphylaxis of any of the phases, ileal preparations were set up and routinely stimulated without drug addition for the duration of the experiment. There was no tachyphylaxis to any of the phases. The tissue returned to baseline at the end of the stimulation. Type 2 consisted of a purely excitatory component (Figure 3b). This was obtained in about

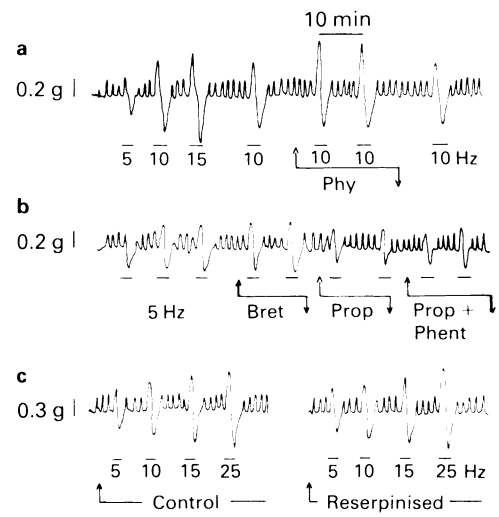

Figure 4 (a) Potentiation by physostigmine (Phy, 2.0×10^{-6} M) of the excitatory phase of the responses of the bat ileum to TNS. ↑ indicates point of addition of physostigmine and ↓ the point of washing out. Horizontal bars indicate periods of stimulation. (b) Effect of bretylium (Bret) 10^{-6} M and 10^{-5} M, propranolol (Prop) 10^{-7} M and 10^{-6} M and phentolamine (Phent) 10^{-6} M and a mixture of Phent and Prop at 10^{-6} M on the responses of the bat ileum to TNS. Drugs were added at ↑, 30 min before TNS at 5 Hz, 0.5 ms at supramaximal voltage. ↓ indicates drug washout. Horizontal line denotes stimulation period. (c) Frequency-response of the bat ileum after TNS in control (untreated) (left panel) and in reserpinized preparation (right hand panel). Pulse width = 0.5 ms at supramaximal voltage for 10 s.

20% of the ilea studied. There was no after-stimulation relaxation. Type 3 was typified by an immediate relaxation followed by a contraction and this accounts for 12% of the responses (Figure 3c). Type 4, seen in less than 8% of the preparations, consisted of a relaxation lasting throughout the stimulation period (Figure 3d). These variations in response to TNS were seen only in the ileal segments. TTX (10^{-7} M) reversibly abolished the excitatory component without influencing the inhibitory component (see Figures 3a,b,c and d). Results used for pharmacological analysis in this work are from Type 1.

Pharmacological analysis of the excitatory and inhibitory components

Cholinergic transmission Physostigmine (2.0×10^{-6} M) increased the tone and the responses of the motor component to TNS (Figure 4a). The inhibitory component was not influenced by physostigmine. Atropine at 10^{-9} M, concentrations that reduced and abolished ACh-induced contractions respectively, reduced the motor component without influencing the inhibitory phase (Figure 5). Atropine also reduced the amplitude of spontaneous contractions of the ileum. Hexamethonium (10^{-6} M and 10^{-5} M) did not influence any of the phases.

Adrenergic transmission Bretylium (10^{-6} M and 10^{-5} M), propranolol (10^{-7} M and 10^{-6} M), phentolamine (10^{-7} M and 10^{-6} M) or a mixture of both phentolamine and propranolol did not influence excitatory and the inhibitory phases (Figure 4b). Pretreatment of bats with reserpine had no effect on the biphasic responses (Figure 4c).

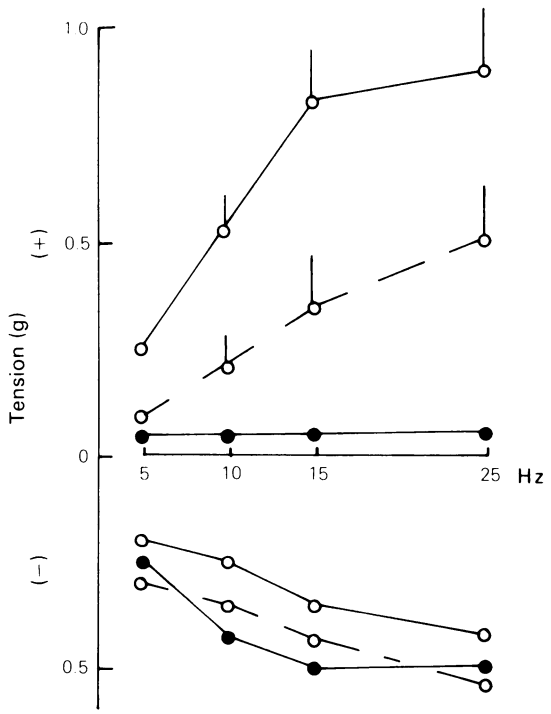

Figure 5 Effect of atropine (O---O) 10^{-9} M and (●———●) 10^{-8} M on the responses of the bat ileum to TNS. Atropine was added to the bath 30 min before TNS. Vertical bar is the s.e.mean of 6 observations. (O———O) represents control responses. Responses are expressed as contraction (+) and relaxation (−) in tension.

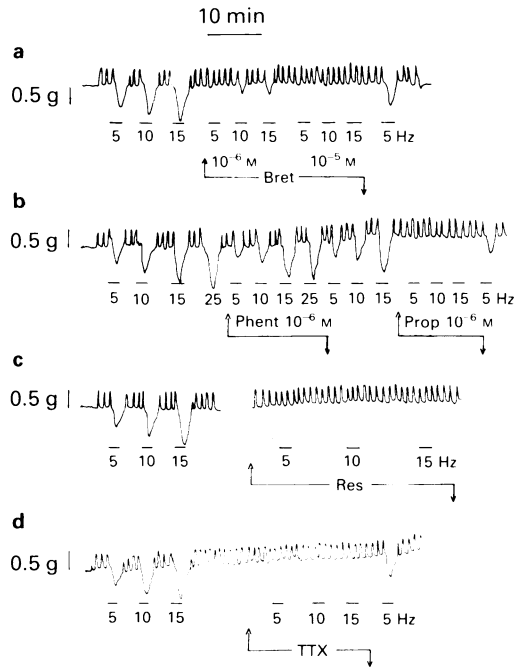

Figure 6 Responses of the bat ileum to periarterial nerve stimulation at 5, 10, 15 and 25 Hz in the absence and in the presence of bretylium (Bret) 10^{-6} M and 10^{-5} M (a); phentolamine (Phent) 10^{-6} M and propranolol (Prop) 10^{-6} M (b). ↑ represents the point of addition of drug and ↓ the point of drug washout. (c) Shows a typical record from control (unreserpinized) bat ileum and from reserpinized (Res) preparations stimulated periarterially at 5, 10, and 15 Hz, 0.5 ms and supramaximal voltage delivered every 15 min for 10 s. (d) Effect of tetrodotoxin (TTX, 10^{-7} M) added at ↑ on the responses of the bat ileum to periarterial nerve stimulation. ↓ denotes drug washout and period of stimulation. In all tracings, a horizontal line indicates period of stimulation.

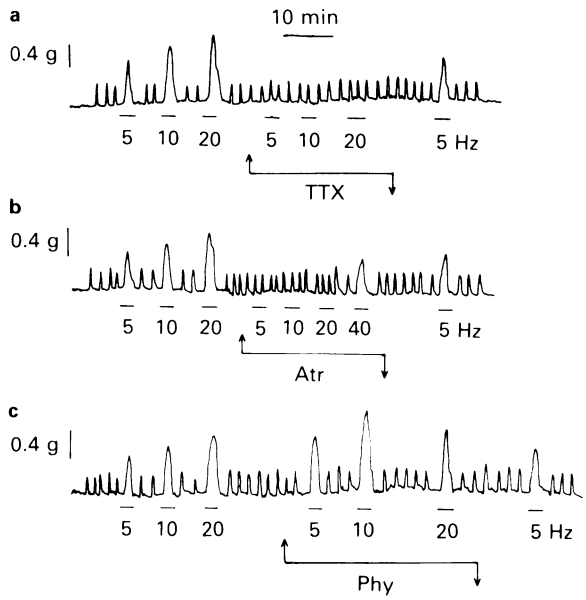

Figure 7 Effects of tetrodotoxin (TTX, 10^{-7} M) (a), atropine (Atr, 10^{-8} M) (b) and physostigmine (Phy, 2.0×10^{-6} M) (c) on the responses of the bat rectum to TNS. Drugs were added to the bath at ↑ and were washed out of the bath at ↓. Horizontal line indicates the period of stimulation. Stimulation parameters: 0.5 ms pulse width at supramaximal voltage (25 V).

Periarterial nerve stimulation of the bat ileum

The periarterial nerves were stimulated at supramaximal voltage (25 V) with a pulse width of 0.5 ms at various frequencies to produce an inhibition of the pendular movements (Figure 6a). Bretylium (10^{-6} M and 10^{-5} M) abolished the inhibitory effects of sympathetic nerve stimulation (Figure 6a). Phentolamine (10^{-6} M) reduced control responses to about 30%; at a higher frequency, 25 Hz, the inhibition was only about 10% (Figure 6b), whilst propranolol (10^{-6} M) abolished the inhibitory responses (Figure 6b). In addition, reserpinization abolished the inhibitory effect of PNS (Figure 6c). TTX (10^{-7} M) abolished the inhibitory responses to 5, 10 and 15 Hz (Figure 6d).

Rectum

When the rectum of the guinea-pig and the bat were transmurally stimulated, only an excitatory response was obtained. These contractions were reversibly blocked by TTX (10^{-7} M) (Figure 7a) and by atropine (10^{-8} M) (Figure 7b); however, they were enhanced by physostigmine (2.0×10^{-6} M). The contractions of the bat rectum were slower in onset than those of the guinea-pig rectum.

Discussion

In the present study, we have shown the effects of transmural nerve stimulation and periarterial nerve stimulation on the gastrointestinal smooth muscle preparations from the fruit-eating bat and the guinea-pig. Thus, the stomach and the rectum of the two animals and the ileum of the guinea-pig responded to TNS with a contraction which was readily and reversibly blocked by atropine and TTX but was resistant to hexamethonium. These results suggest that the contractions were mediated via postganglionic cholinergic neurones. Transmural stimulation of the isolated muscle segments, according to the method of Paton (1957), is a simple way of exciting the nerve fibres within the walls of the gastrointestinal tract. All types of fibres both afferent and efferent and those of interneurones may be stimulated and complex responses may be obtained. Thus, the bat ileum upon transmural nerve stimulation, produced four different responses viz; (a) contraction followed by relaxation, (b) contraction, (c) relaxation followed by a contraction and (d) relaxation. Campbell (1966) in a study on nerve-mediated excitation of the taenia coli of the guinea-pig obtained 3 components of the response to TNS: contraction during stimulation, relaxation during stimulation and contraction after stimulation. In some cases, the motor component of the response to TNS in isolated intestinal preparations has been described as 'rebound' contractions (Holman & Hughes, 1965; Campbell, 1966; Bennett, 1966). These workers suggested that because the contractions persist in the presence of high concentrations of atropine they were not mediated by cholinergic nerves but occur as a direct result of the inhibitory phase of the TNS response. The inhibitory response causes hyperpolarization of smooth muscle membrane which is replaced at the end of stimulation by an increase in muscle tension (Bennett, 1966; Campbell, 1966). This interpretation does not explain the major part of the motor response in our work for the following reasons: (1) the excitatory phase was present only during TNS and not as a post-stimulation contraction, (2) atropine, in low concentrations that blocked the effect of exogenous ACh, abolished completely the excitatory phase, (3) the response was potentiated by physostigmine, an anticholinesterase. Furthermore, the ability of tetrodotoxin (which stabilizes nerve membranes by blocking sodium channels without affecting the activity of the smooth muscle; Gershon, 1966) to abolish these contractions evoked by TNS indicated that the excitatory phase is neuronally mediated. In agreement with Paton & Zar (1967), hexamethonium did not block TNS-induced responses suggesting that the site of action was peripheral to the ganglion. The inhibitory response of the bat ileum to TNS persisted in the presence of adrenergic neurone

blockers, α- and β-adrenoceptor, cholinoceptor and sodium channel blockade. This suggests that the relaxation is non-adrenergic, non-cholinergic and non-neuronal in origin. This is difficult to comprehend because the relaxation occurred only during stimulation. Campbell (1966) suggested that relaxation during stimulation and contraction after stimulation may be due to stimulation of inhibitory nerves (inhibitory in the sense that their action is to hyperpolarize the cell membrane). Burnstock *et al.* (1970) and Burnstock (1972) suggested the presence of non-adrenergic, non-cholinergic (NANC) nerves in various parts of the gastrointestinal tract and other smooth muscle systems. Since TTX did not influence the inhibitory component, it may be that certain muscle cells contain inhibitory substance(s) excitation of which leads to release of the substance(s) which is/are non-adrenergic and non-cholinergic. It is not unlikely that there is conductance from one muscle cell to the other, but, rarely does such an effect occur in mammalian tissues. Thus, at a low pulse width of 0.5 ms, Fabiyi & Okpako (1973) obtained similar results in which TTX had no effect on the tetanic responses of the lizard rectum and the excitatory responses were said to be due to a direct excitation of muscle fibres. This is important in view of the fact that the alimentary canal of the lizard is very similar to that of the bat; the ileo-caecal junction is conspicuously absent in the two species. The present work may reveal the primitive nature of the bat ileum. Stimulation of the periarterial nerves of the bat ileum produced a relaxation which was blocked by bretylium, propranolol, TTX and reduced by phentolamine suggesting an action on adrenergic neurones. Thus, responses of the bat ileum to sympathetic nerve stimulation are very similar to those of the rabbit.

In conclusion, we have shown similarities of the guinea-pig and the bat gastrointestinal smooth muscle to transmural nerve stimulation and the responses of the bat ileum to periarterial nerve stimulation. However, the bat ileum differ considerably by exhibiting an inhibitory phase in addition to the excitatory phase. Whilst the excitatory phase is neurogenic and cholinergically mediated from stomach to the rectum, the inhibitory phase is non-adrenergic, non-cholinergic and non-neuronal following TNS.

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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.* **199,** 705–727.

BENNETT, M.R. (1966). Rebound excitation of the smooth muscle cells of guinea-pig taenia coli after stimulation of intraluminal inhibitory nerves. *J. Physiol.* **195,** 124–131.

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

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.*, **24,** 509–581.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D.G. & SMYTH, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmac.*, **40,** 668–688.

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

FABIYI, J.A. & OKPAKO, D.T. (1973). On the responses of the isolated rectum of the rainbow lizard to drugs and electrical field stimulation. *Comp. Gen. Pharmac.*, **4,** 297–303.

FINKELMAN, B. (1930). On the nature of inhibition in the intestine. *J. Physiol.* **70,** 145–157.

GERSHON, M.D. (1966). Effect of tetrodotoxin on innervated smooth muscle preparations. *J. Physiol.* **186,** 4P.

HALSTEAD, C.B. & SEGUN, A.O. (1976). *Dissection Guides of Common Tropical Animals.* Fruit Bat *Eidolon helvum* 3, p. 15. Nigeria: Ethiope Publishing House.

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

PATON, W.D.M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of co-axially stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **11,** 119–127.

PATON, W.D.M. & ZAR, ABOO, M. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal strips. *J. Physiol.*, **194,** 13–33.

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