Brit. J. Pharmacol. (1961), **17**, 245–260.

EFFECT OF GUANETHIDINE IN REVEALING CHOLINERGIC SYMPATHETIC FIBRES

BY

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(*Received June* 21, 1961)

Guanethidine abolished the inhibitory response of segments of rabbit intestine to stimulation of the sympathetic nerves which accompany the mesenteric arteries. In the majority of experiments a motor response of the intestinal segment was then revealed; it was more readily observed in intestinal segments from young than from adult rabbits. The motor response of the intestine to sympathetic stimulation after guanethidine was blocked by atropine. It was not blocked by hexamethonium and was present in rabbits in which the vagal innervation to the small intestine had been sectioned. In the cat isolated atria, guanethidine blocked the accelerator response to sympathetic nerve stimulation and revealed a response resembling that to stimulation of the vagus. It was concluded that guanethidine blocked the release of noradrenaline and thus revealed the response to the direct action of acetylcholine released from cholinergic sympathetic nerves.

Cholinergic fibres occur so frequently in sympathetic nerves that they may be considered as typical rather than exceptional. Their existence provides a clue to the mechanism by which the impulse arriving at the sympathetic nerve ending may liberate noradrenaline (Burn & Rand, 1959b, 1960b).

The detection of cholinergic sympathetic fibres has been facilitated by the use of reserpine. When animals are injected with reserpine the noradrenaline content of the tissues is considerably reduced (Bertler, Carlsson & Rosengren, 1956; Burn & Rand, 1958a; Burn & Rand, 1959a), and consequently the response to sympathetic nerve stimulation which resembles the response to noradrenaline is reduced or abolished (Bein, 1953; Muscholl & Vogt, 1958; Burn & Rand, 1958b; Burn, Leach, Rand & Thompson, 1959; Trendelenburg & Gravenstein, 1958), and in some cases replaced by a response which can be shown to be due to acetylcholine liberation. Thus, in reserpine-treated rabbits, Gillespie & MacKenna (1959, 1961) found that stimulation of the sympathetic nerves to the isolated colon produced contraction instead of inhibition, and Huković (1959) found that stimulation of the accelerans nerve to the isolated atria produced slowing instead of an increase in rate. Burn & Rand (1960a) demonstrated cholinergic fibres in the sympathetic supply to the nictitating membrane, spleen, and uterus of the cat, and to the rabbit ear.

* Research Fellow supported by the Wellcome Trust.

The report by Boura & Green (1959) on the pharmacology of bretylium contains data which suggest that bretylium not only prevents the release of noradrenaline but also unmasks the direct (muscarinic) action of acetylcholine from cholinergic sympathetic fibres on the innervated tissue. Thus, after bretylium, stimulation of the nervi accelerans to the cat heart no longer increased but decreased the rate, and this slowing of the heart could be blocked by atropine. Similarly, Huković (1960) found that bretylium blocked the vasoconstrictor response to sympathetic nerve stimulation in the perfused rabbit ear and unmasked a dilator response. Blair, Glover, Kidd & Roddie (1960) found that bretylium blocked vasoconstriction in the human forearm caused by sympathetic adrenergic fibres but left unaffected vasodilatation caused by cholinergic sympathetic nerves excited by emotion. The effect of bretylium in blocking adrenergic but not cholinergic fibres in sympathetic nerves can explain syncope on exertion in patients who are receiving bretylium to control hypertension (Brandon, 1960).

Another drug which, like bretylium, blocks the usual response to stimulation of sympathetic "adrenergic" nerves is guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960). This paper deals with some observations on the presence, as revealed by guanethidine, of cholinergic fibres in the sympathetic nerves to the rabbit intestine and to the cat atria.

METHODS

Isolated rabbit intestine. Segments of isolated rabbit intestine with their sympathetic nerves intact were prepared by the method of Finkleman (1930). Rabbits were stunned and bled out, the abdomen opened and a segment of intestine with its mesentery was spread out. A ligature was placed around a suitable branch of mesenteric artery about 2 to 4 cm from the gut. A length of intestine 1.5 to 3 cm long which contained the branches of the selected mesenteric artery was excised and suspended in McEwen (1956) solution in a 70 ml. or a 100 ml. organ bath at 37° C. The bath was bubbled with 5% carbon dioxide in oxygen. In a few experiments Krebs bicarbonate solution or Tyrode solution was used. The artery together with its accompanying sympathetic nerves was passed through a channel containing bipolar platinum electrodes placed with the cathode nearest to the gut. The electrodes were arranged so that there was no interference with the movement of the intestinal segment in the bath. In some experiments the intestinal segments were arranged for transmural electrical stimulation (Paton, 1957). The lower end of the segment was tied over a glass tube so that the lumen enclosed a platinum wire 2 cm long, which was the internal electrode. The electrodes connected to the sympathetic nerve were joined together to form the external electrode. The periarterial sympathetic nerves were stimulated with 5 to 15 V square wave pulses from a constant voltage output electronic stimulator; other details of stimulation are given below in the section on RESULTS. The longitudinal contractions of the gut were recorded by a lever writing on smoked paper.

Vagotomy. The vagus nerves were divided by aseptic operation in rabbits anaesthetized with intravenous thiopentone sodium (20 mg/kg) and ether. Two methods of causing the degeneration of vagal fibres to the intestine were used: (a) the right vagus was divided in the neck, or (b) both vagi were divided in the abdomen at the point where they accompany the oesophagus immediately before it enters the stomach. Attempts were made to divide both vagi in the neck, but it was found that the rabbits survived for only 12 to 24 hr in either 1 or 2 stage operations. This has been reported previously (Lorber, 1939).

Cat atria. Isolated atria from young cats with their sympathetic nerves attached were prepared as described by Chang & Rand (1960); in addition the vagus nerves were attached (Burn & Rand, 1958a).

Drugs. The following drugs were used: guanethidine (2-(octahydro-1-azocinyl)-ethyl-guanidine sulphate), atropine sulphate, hyoscine hydrobromide, hexamethonium bromide, acetylcholine chloride, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate (serotonin), physostigmine salicylate, neostigmine methylsulphate, nicotine bitartrate, 2,6-xylyl ether of choline bromide (TM10), bretylium tosylate; the doses of these drugs are expressed as the salt. Reserpine, adrenaline and noradrenaline were used in doses given as the base, and doses of adenosine triphosphate are given as the acid.

RESULTS

Effect of guanethidine on stimulation of sympathetic nerves. Stimulation of the sympathetic nerves accompanying a mesenteric artery produced inhibition of the pendulum movements in the isolated segment of rabbit ileum. After the addition of guanethidine (1 μg/ml.) to the bath the inhibitory response was gradually lost and was usually replaced by a motor response. In a total of 35 segments of intestine from 27 rabbits the reversal of the response to sympathetic nerve stimulation from an inhibition to motor response has been observed in 25 segments. The exact nature of the motor response varied somewhat in various experiments (compare Figs. 1, 2, 3 and 5), but, in general, it resembled the response to a small dose of acetylcholine in that particular experiment. Atropine and hyoscine, in doses which abolished the response to acetylcholine, considerably reduced or abolished the motor response to sympathetic stimulation (Figs. 1, 2 and 3).

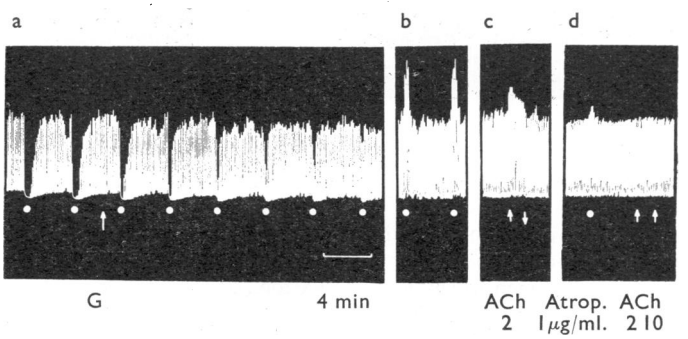

Fig. 1. Isolated rabbit ileum in 100 ml. bath. Periarterial sympathetic nerves stimulated at the white dots with 2 msec pulses for 14 sec every 4 min, in *a* at 50/sec, and in *b* and *d* at 10/sec. Guanethidine, 1 μg/ml. at G, blocked the inhibitory response. In *b*, 120 min later, stimulation produced a motor response. In *c*, 2 μg acetylcholine (ACh) produced a motor response. Atropine 1 μg/ml. in *d* blocked the motor responses to sympathetic stimulation and to acetylcholine.

In some experiments the movements of the intestine *in situ* were recorded. Stimulation of the periarterial sympathetic nerves at 20/sec and 50/sec produced inhibition. After an intravenous injection of 0.5 to 5 mg/kg guanethidine the inhibitory response was blocked and there was a pronounced motor response to sympathetic stimulation which was blocked by 10 mg/kg atropine.

Since the motor response to sympathetic stimulation after guanethidine appeared to be mediated by cholinergic fibres, attempts were made to enhance the response with anticholinesterases. The addition of eserine or neostigmine to the bath resulted

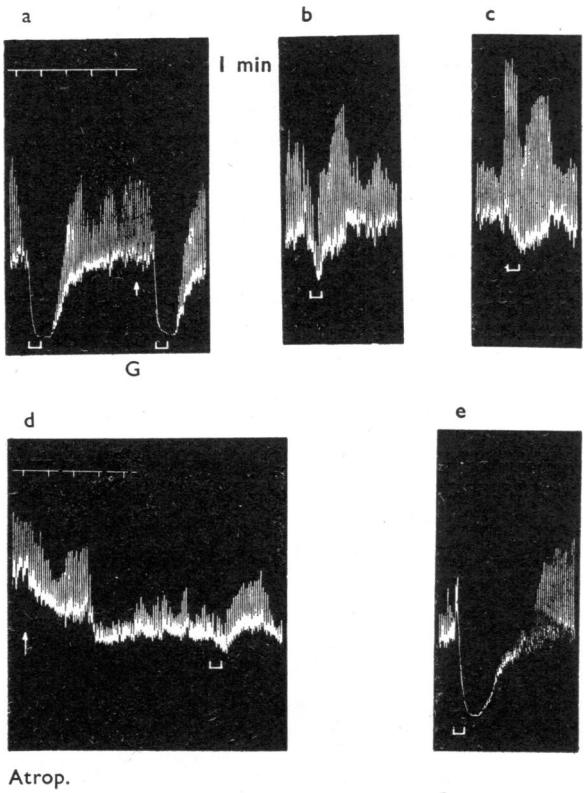

Fig. 2. Isolated rabbit ileum in Tyrode solution. The periarterial sympathetic nerves were stimulated with 0.5 msec pulses at 50/sec for 30 sec indicated by |__|. In *a*, 1 μg/ml. of guanethidine (G) was added to the bath. In *b*, 20 min later, the smaller inhibitory response was followed by a motor response. In *c*, 35 min after guanethidine, there was a pure motor response which was antagonized by atropine (Atrop., 2 μg in 130 ml. bath) in *d*. The bath was then washed repeatedly during 110 min which restored the inhibitory response in *e*.

in an increase in tone, and the intestinal segment was then insensitive to acetylcholine, noradrenaline and to sympathetic nerve stimulation. Burn (1952) illustrates an experiment in which eserine did not produce a rise in tone when glucose was absent from the bath fluid. Therefore we carried out an experiment in a glucose-free solution, but the effects of drugs and of sympathetic stimulation were then markedly reduced, and after eserine the response to acetylcholine was not increased.

The effect of hexamethonium on the motor response was somewhat variable. In the experiment shown in Fig. 3 a concentration of 1 mg/ml. did not affect the motor response, whereas in another experiment hexamethonium in a concentration of 10 μg/ml. reduced the motor response. Garry & Gillespie (1955) found that 50 μg/ml. hexamethonium always caused some block of the response of the colon to parasympathetic nerve stimulation, and 0.5 mg/ml. blocked it completely. Gillespie & MacKenna (1961) found that 0.2 mg/ml. hexamethonium blocked the

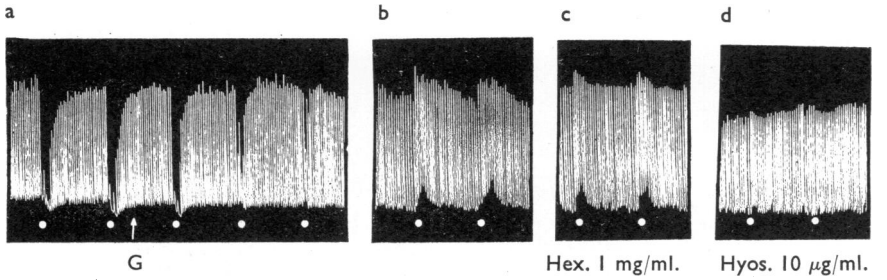

Fig. 3. Isolated rabbit ileum. The sympathetic nerve was stimulated with 2 msec pulses at 50/sec for 20 sec every 3 min at the white dots. In *a*, guanethidine 1 μg/ml. was added to the bath at G, and in *b*, 20 min later, a motor response was present. Hexamethonium 1 mg/ml. in *c* did not block this response, but 10 μg/ml. of hyoscine in *d* did.

motor response to sympathetic nerve stimulation seen in the isolated colon from reserpinized rabbits. They concluded that after reserpine sympathetic stimulation causes an activation of parasympathetic pathways. Since larger doses of hexamethonium than those used by Gillespie and his colleagues did not regularly block the motor responses to sympathetic stimulation revealed by guanethidine in the isolated ileum, it is unlikely that this motor response is due to parasympathetic nerves.

After guanethidine had caused an alteration of the response to sympathetic stimulation from inhibition to excitation it was difficult to restore the original effect by washing. One experiment in which the inhibitory response was successfully restored is shown in Fig. 2, in which the bath was washed 10 times at 5 min intervals; it is interesting to note that in this experiment the restored inhibition was preceded by a contraction.

The abolition of the motor response by atropine and by hyoscine is not a final proof that it is due to cholinergic nerves. The following experiments were undertaken to determine whether other substances may have caused the motor response.

Concentrations of mepyramine (0.5 μg/ml.) which completely blocked the response of the intestine to histamine (1 to 10 μg/ml.) did not affect the motor response to sympathetic stimulation revealed by guanethidine. Larger amounts of mepyramine reduced the motor responses both to acetylcholine and to sympathetic stimulation.

It is unlikely that the motor response to sympathetic nerve stimulation can be due to 5-hydroxytryptamine, since 50 times the concentration of atropine was needed to block it than to block an equiactive dose of acetylcholine on the rabbit intestine, and the antagonism of 5-hydroxytryptamine by atropine was only shown when atropine remained in the bath, whereas the atropine block of the motor responses to sympathetic stimulation and to acetylcholine was difficult to reverse by washing.

Adenosine triphosphate in concentrations of 1 to 10 μg/ml. produced only an inhibition of rabbit intestine, and smaller concentrations had no action.

Effect of guanethidine on responses to acetylcholine and noradrenaline. When the concentration of guanethidine in the bath was increased to 10 μg/ml. the motor response of the ileum to sympathetic stimulation was reduced or abolished. This

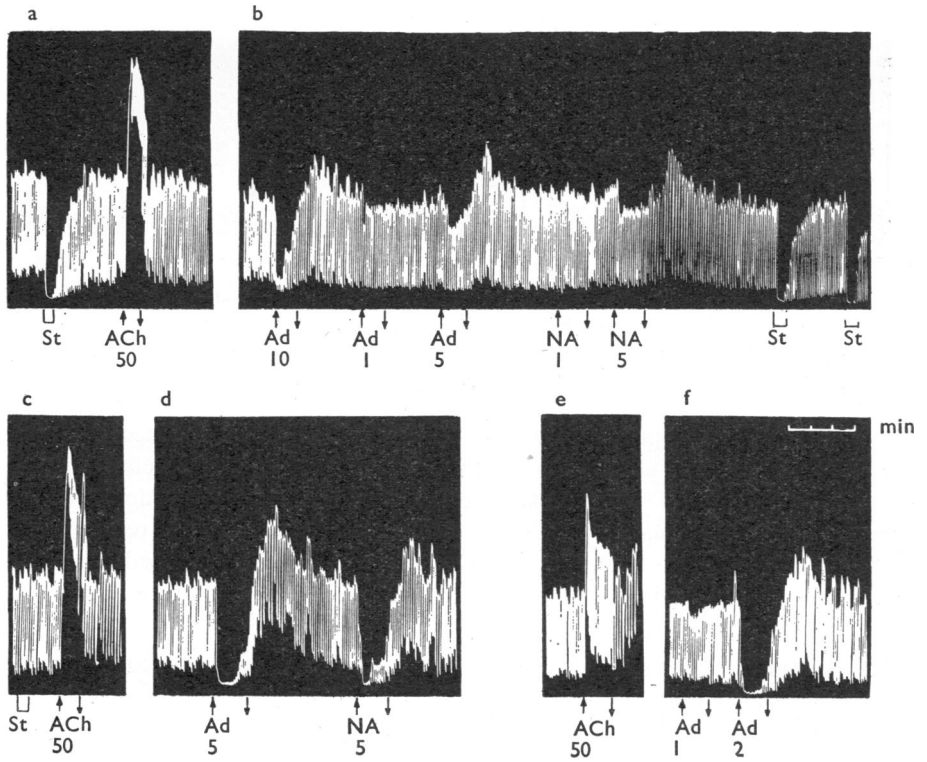

Fig. 4. Effect of guanethidine on response of ileum to adrenaline (Ad), noradrenaline (NA), acetylcholine (ACh), and sympathetic stimulation at 50/sec for 20 sec (St). Drugs were added to the bath at ↑ and washed out at ↓. The numerals refer to the doses in μg added to a 70 ml. bath. Observations were made before guanethidine in *a* and *b*, after 1 μg/ml. guanethidine in *c* and *d*, and after 10 μg/ml. guanethidine in *e* and *f*.

appears to be due to an anti-muscarinic action of guanethidine, since the motor response to acetylcholine was also diminished (Fig. 4e). The contraction of the colon in response to nicotine was diminished by guanethidine (Fig. 7). The inhibitor response to adrenaline and noradrenaline was potentiated by guanethidine. Fig. 4b shows that, before guanethidine, both adrenaline (5 μg) and noradrenaline (5 μg) produced a very slight decrease in amplitude of the pendulum movements, but after 1 μg/ml. of guanethidine, when the inhibitor response to sympathetic stimulation had been abolished, the same doses of adrenaline and noradrenaline now produced complete inhibition of movements and a decrease in tonus (Fig. 4d). After 10 μg/ml. of guanethidine the effects of adrenaline (Fig. 4f) and noradrenaline were still further potentiated.

Stimulus parameters. Garry & Gillespie (1955) have determined the stimulus parameters for optimum responses of the rabbit isolated colon to stimulation of the sympathetic and parasympathetic nerves. They found that the optimum frequency of sympathetic nerve stimulation for the inhibitory response was 100/sec and the optimum frequency for the motor response to parasympathetic nerve

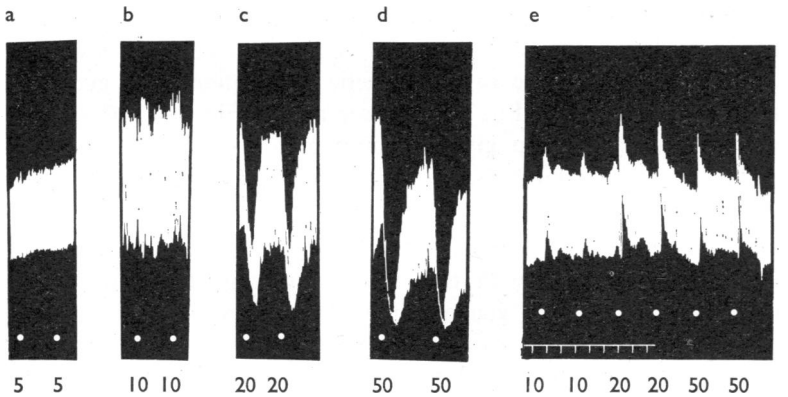

Fig. 5. Effect of rate of sympathetic nerve stimulation on response of isolated ileum before (*a–d*) and 20 min after guanethidine (*e*). Stimulation for 20 sec every 3 min with 2 msec pulses is indicated at the white dots; the frequency of stimulation in pulses/sec is given by the numeral. The time trace is in min.

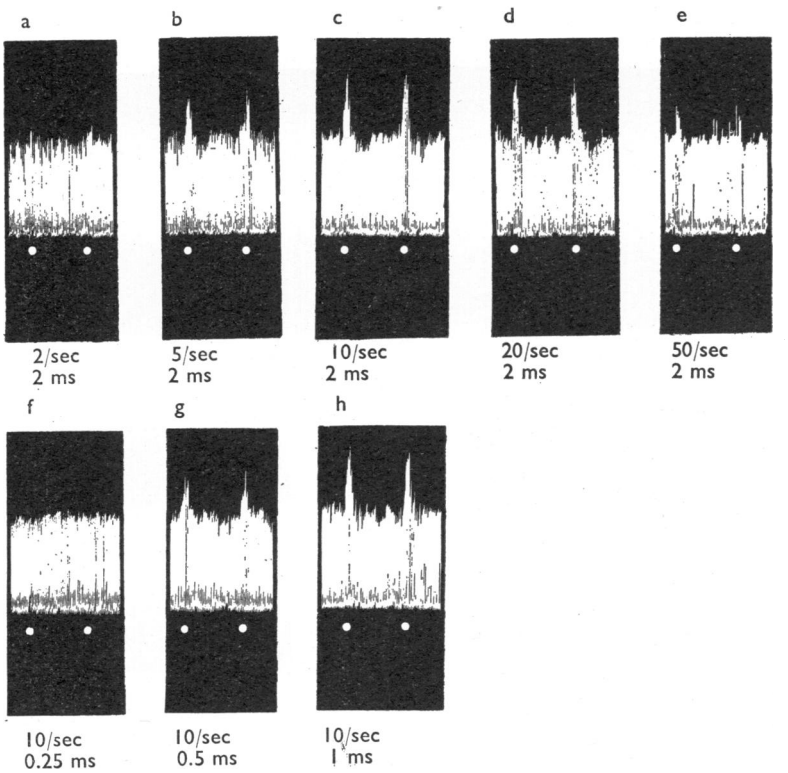

Fig. 6. Effect of varying frequency and pulse width on the motor response of ileum to sympathetic nerve stimulation after guanethidine (1 μg/ml.). Stimulation, at the white dots, was given for 25 sec every 4 min. The frequency in pulses/sec and the pulse width in msec (ms) is indicated in each panel.

stimulation was 10/sec. In our experiments with ileum the inhibitory response to sympathetic stimulation was well developed at 50/sec and less at lower frequencies (Fig. 5). The motor response to sympathetic stimulation after guanethidine was optimum at a lower rate; in Fig. 5 at 20/sec and in Fig. 6 at 10/sec. The pulse width of the square waves to give optimum motor responses was 1 to 2 msec (Fig. 6), which was the same pulse width which gave optimum inhibitor responses before guanethidine.

Transmural stimulation of segments of intestine with 2 msec, 8 V pulses at 50/sec gave an inhibition identical with that caused by sympathetic nerve stimulation. This inhibition was blocked by guanethidine. In order to produce a motor response by transmural stimulation it was necessary to increase either the frequency of stimulation or the width of the pulses. Thus 2 msec, 60 V pulses at 100/sec, or 5 msec, 60 V pulses at frequencies of 10 to 100/sec, were the minimum stimulus parameters necessary for a motor response with transmural stimulation; higher frequencies and longer pulse widths were more effective. Therefore it is unlikely that the motor response to sympathetic nerve stimulation after guanethidine was due to current spread from the electrodes.

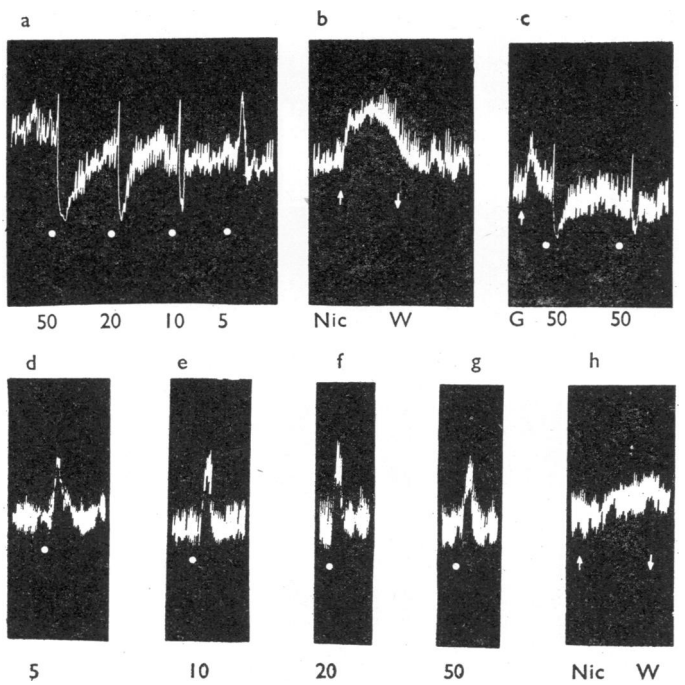

Fig. 7. Isolated rabbit colon. The periarterial sympathetic nerves were stimulated at the white dots with 2 msec pulses for 20 sec; the numerals refer to the frequency in pulses/sec. At Nic, nicotine bitartrate, 1 μg/ml., was added to the bath, and washed out at W. In panel *c*, guanethidine (G), 10 μg/ml., was added to the bath and remained in panels *d–h*, which were obtained 20 to 40 min later.

Region of intestine. Intestinal segments taken from duodenum and from various parts of the ileum and colon have given motor responses to sympathetic stimulation after guanethidine. In segments from the small intestine the inhibitor response was blocked by 1 μg/ml. guanethidine; segments from the large intestine were less sensitive and required 10 μg/ml. Fig. 7 is the record from a segment of colon. Stimulation at 5/sec before guanethidine resulted in a motor response; as the frequency of stimulation was increased the inhibitory component became more marked. After guanethidine stimulation at 5, 10, 20 and 50/sec produced approximately equal motor responses.

The effect of the age of rabbit on the responses. In the initial experiments it appeared that the motor response was more regularly observed in segments of ileum from young rabbits. In order to test whether age was a factor which affected the appearance of motor response to sympathetic stimulation, the observations recorded in Table 1 were made on ileal segments from litter-mate rabbits taken as close as possible to corresponding regions.

TABLE 1

GUANETHIDINE ON RESPONSE OF ILEUM TO SYMPATHETIC STIMULATION AT DIFFERENT AGES

Litter I				Litter II			
		Response to sympathetic stimulation				Response to sympathetic stimulation	
Age in days	Body wt. (kg)	Before guanethidine	After guanethidine	Age in days	Body wt. (kg)	Before guanethidine	After guanethidine
12	0·175	Motor response preceding inhibition	Motor response	12	0·155	Motor response preceding inhibition	Motor response
27	0·60	Motor response preceding inhibition	Motor response	27	0·45	Motor response preceding inhibition	Motor response
44	1·2	Motor response preceding inhibition	Motor response	44	0·7	Inhibition only	Motor response
61	1·6	Inhibition only	Motor response in $\frac{1}{3}$ segments tested	61	1·1	Inhibition only	Motor response in $\frac{1}{3}$ segments tested

In these experiments the response of segments of ileum was observed at various frequencies of stimulation. In the younger rabbits stimulation of the sympathetic at low frequencies (10 to 20/sec) produced an initial motor response followed by inhibition. With a higher frequency of stimulation (50/sec) a pure inhibitor response was obtained. After guanethidine the motor response was enhanced and the inhibition was abolished. In older rabbits inhibition only was seen at all frequencies. Finkleman (1930) described an initial motor response followed by inhibition after sympathetic stimulation of the nerves to the isolated rabbit intestine. He found

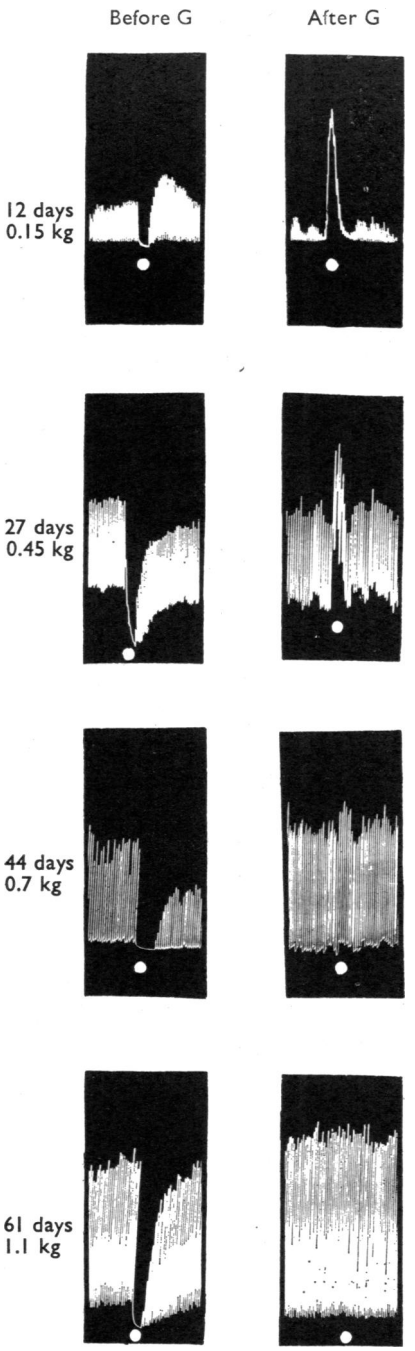

Fig. 8. Segments of isolated ileum from litter-mate rabbits of various ages and weights, indicated on the left. Sympathetic stimulation at the white dots was with 2 msec pulses at 50/sec for 20 sec. The left-hand column shows the responses before, and the right-hand column 20 to 30 min after, 1 μg/ml. guanethidine.

that these motor responses were seen only at low frequencies of stimulation and in the early part of the experiment, and were seen only in occasional rabbits. He made no comment on the age of the rabbits in which he saw a motor response.

Fig. 8 shows the responses of ileal segments of rabbits of various ages to sympathetic stimulation before and after the addition of guanethidine to the bath. In the 12-day-old rabbit the motor response is particularly well marked, but in the segment of ileum taken from a litter-mate killed at 61 days no motor response could be seen ; in this 61-day-old rabbit only one segment of three tested showed a motor response after guanethidine. In two litter-mates tested at intermediate ages the motor response was clear at 27 days and just detectable at 44 days.

In order to determine whether the increased motor responses in ileal segments from young rabbits was due to an increased sensitivity to acetylcholine, or to a decreased sensitivity to noradrenaline, experiments like that shown in Fig. 9 were

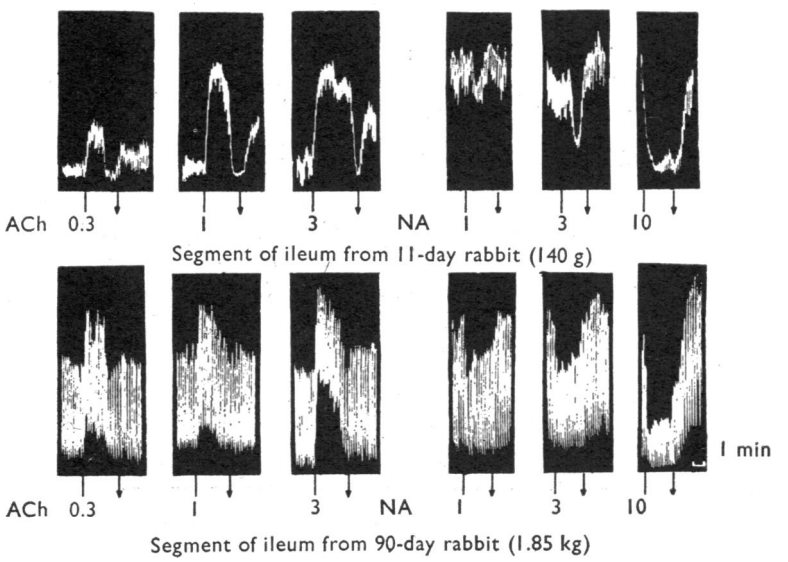

Fig. 9. Effect of acetylcholine (ACh) and noradrenaline (NA) on isolated ileum from young and adult rabbits. The figures beneath the vertical strokes indicate the dose of drug in μg in 70 ml. bath. The drugs were washed out at the arrow.

carried out. The nature of responses to both acetylcholine and noradrenaline were different in old and young rabbits. In young rabbits the amplitude of the rhythmic contractions was less and these drugs produced alterations in tone which exceeded in amplitude that of the rhythmic excursions. However, there was no age-dependent difference in sensitivity to either acetylcholine or noradrenaline.

Effect of degeneration of vagus nerves. The vagus nerves to the small intestine were degenerated either by cutting the right vagus in the neck or by cutting both vagi at the level of the abdominal portion of the oesophagus. Segments of jejunum were tested 8 to 20 days after nerve section. In 2 out of 5 rabbits, in which the

right vagus was sectioned in the neck, a motor response to sympathetic stimulation after guanethidine was seen in intestinal segments. In 2 out of 5 rabbits, in which both vagi were sectioned in the abdomen, motor responses of intestinal segments after guanethidine were convincingly demonstrated. Two other rabbits gave a slight indication of a motor response.

Xylocholine (TM10), bretylium and reserpine. In two experiments with xylocholine (5 μg/ml.) a motor response did not develop, and the inhibitor response to sympathetic stimulation was not completely blocked. In two experiments with bretylium (5 μg/ml.) the inhibitor response to sympathetic stimulation was com-

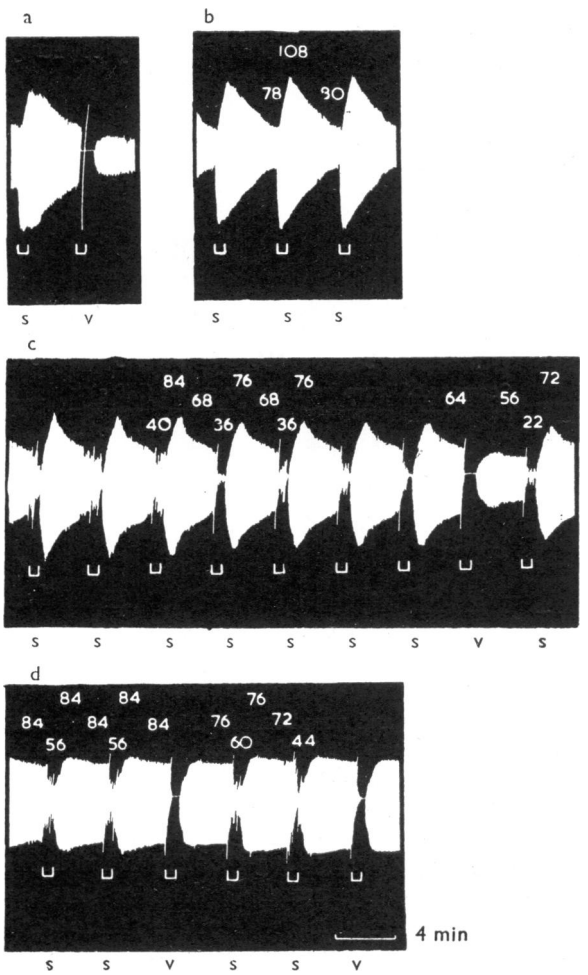

Fig. 10. Response of isolated cat atria to sympathetic stimulation at S and vagus stimulation at V. Stimulation, indicated by |__|, was with 2 msec pulses at 50/sec for 45 sec every 4 min. The numerals above the tracing refer to the rate of atrial contractions in beats/min. Control observations are shown in *a* and *b*; *c* is in the presence of 1 μg/ml. guanethidine, and *d*, 5 μg/ml. guanethidine.

pletely blocked, but no motor response was seen. A motor response to sympathetic stimulation was seen after pretreatment of rabbits with reserpine, thus confirming the observations of Gillespie & MacKenna (1961). When reserpine (1 μg/ml.) was added to the bath containing a normal segment of ileum, the inhibitor response was reduced after 3 to 4 hr but not abolished. A motor response was seen in 1 of 3 segments treated with reserpine in the bath. In 3 experiments with intestinal segments taken from rabbits previously injected daily with guanethidine, 10 mg/kg, for 4 days, the inhibitor response to sympathetic stimulation was no different from that of normal controls.

Cat atria. Responses of isolated cat atria to sympathetic and vagus nerve stimulation are shown in Fig. 10. When periods of sympathetic nerve stimulation were regularly repeated the pattern of the accelerator response and increase in amplitude of beating became constant and remained steady for long periods. After the addition of guanethidine (1 μg/ml.) to the bath the response to sympathetic nerve stimulation changed from uncomplicated increase in rate and amplitude to a mixed response consisting firstly of a decrease in rate and amplitude. This inhibitory phase, which resembled the response to submaximal vagus nerve stimulation, was followed by an increase in rate and amplitude which was similar to, but smaller than, the sympathetic response before guanethidine. After a larger dose of guanethidine (5 μg/ml.), only the first inhibitory phase of the response to sympathetic nerve stimulation was seen. At the same time the inhibition produced by vagal stimulation was less, and the record (Fig. 10d) shows that the responses to sympathetic nerve stimulation and vagus nerve stimulation are barely distinguishable.

DISCUSSION

The response of rabbit intestine to stimulation of extrinsic sympathetic and parasympathetic nerves has been recently studied and reviewed (Garry & Gillespie, 1955). It has generally been held that the occasional appearance of cholinergic responses to stimulation of sympathetic nerve trunks is due to parasympathetic fibres included in the sympathetic nerve trunk (Finkleman, 1930). Gillespie & MacKenna (1961) found that degeneration of the sacral parasympathetic nerves abolished the motor responses of the colon from the reserpinized rabbit to sympathetic stimulation. They suggested that reserpine might have the property of activating in some way the parasympathetic pathway, such that sympathetic nerve stimulation can then give rise to typically parasympathetic effects. We have three pieces of evidence that the parasympathomimetic effects that we have observed after stimulating the periarterial sympathetic nerve supply to the rabbit small intestine are not mediated entirely via parasympathetic nerves. Firstly, the motor response was not blocked by hexamethonium, although it was reduced in some experiments; secondly, the motor response was still present after section of the vagus nerves; and, thirdly, a motor response to sympathetic stimulation is more readily produced in young rabbits than in adults, and it is unlikely that the course followed by parasympathetic fibres should change during the first few months after birth. One possible explanation for the more pronounced motor response in young rabbits may be that the catecholamine content of the intestine is lower than in adults. In the adrenal glands from

several species the total catecholamine content increases with age (Hökfelt, 1951 ; West, Shepherd & Hunter, 1951). If this explanation is correct the motor response to sympathetic stimulation in intestine from young animals is due to cholinergic fibres whose presence is later concealed when greater stores of noradrenaline become laid down and the inhibitor response is consequently greater. The motor response of the intestine to sympathetic stimulation in reserpine-treated rabbits is presumably the reverse of this ; the stores of catecholamine are depleted, the inhibitor response less, and the motor response is thereby revealed.

Large doses of guanethidine have been shown to have the property, in common with reserpine, of depleting catecholamines from peripheral tissues (Bein, 1960 ; Cass, Kuntzman & Brodie, 1960). Maxwell *et al.* (1960) suggested that guanethidine acted by interfering either with the release, or the distribution subsequent to release, of transmitter substances from sympathetic nerve terminals, or in both these ways.

Cass, Kuntzman & Brodie (1960) showed that a single intravenous dose of 12.5 mg/kg of guanethidine depleted the noradrenaline in rabbit heart and spleen. More recently, Cass (personal communication) found that a single dose of 1 mg/kg of guanethidine did not reduce the noradrenaline content of rabbit tissues, but this dose of guanethidine is sufficient to abolish the usual response to sympathetic nerve stimulation *in situ*. In the organ bath 1 μg/ml. of guanethidine abolished the adrenergic response to sympathetic stimulation, but the noradrenaline content of segments of intestine taken from the organ bath at this time is the same as that of control segments (Cass, to be published).

The effect of guanethidine in altering the response to sympathetic nerve stimulation from an adrenergic to a cholinergic effect can be understood in the light of the hypothesis advanced by Burn & Rand (1959b, 1960a, b ; Burn, 1961) that the role of acetylcholine liberated from cholinergic sympathetic fibres is to release noradrenaline from a store in the vicinity of the nerve endings, so that nerve stimulation is ultimately adrenergic. We suggest that the action of guanethidine may be to prevent the release of noradrenaline by acetylcholine ; then the action of acetylcholine acting directly on muscarinic receptors is revealed.

In some patients treated with guanethidine hypotension is produced by exercise (Laurence & Rosenheim, 1960). This could be brought about if the effect of guanethidine were to abolish the adrenergic response to sympathetic nerve discharge and to replace it by a weak cholinergic response. McCubbin, Keneko & Page (1961), working with the dog perfused leg, found that guanethidine altered the response to sympathetic stimulation from vasoconstriction to dilatation which could be blocked by atropine.

The effect of guanethidine in altering the response of the cat isolated atria to sympathetic stimulation from tachycardia to bradycardia can explain the occurrence of bradycardia in patients treated with guanethidine (Page & Dustan, 1959). If during sympathetic blockade by guanethidine the sympathetic cholinergic nerves to the heart were incapable of releasing noradrenaline, then it is conceivable that the direct action of acetylcholine on the heart might be revealed.

We are grateful to the Wellcome Trust for the support of one of us (M. J. R.) and to Messrs May & Baker for giving the opportunity to M. D. D. to work on this subject.

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